IJBM

International Journal of

BIOMEDICINE

ISSN 2158-0510

Available online at
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International Journal of Biomedicine (ISSN 2158-0510) is published four times a year by International Medical Research and Development Corp. (IMRDC), 6308, 12 Avenue, Brooklyn, NY 11219 USA

Customer Service: International Journal of Biomedicine, 6308, 12 Avenue, Brooklyn, NY 11219 USA; Tel: 1-917-740-3053; E-mail: editor@ijbm.org

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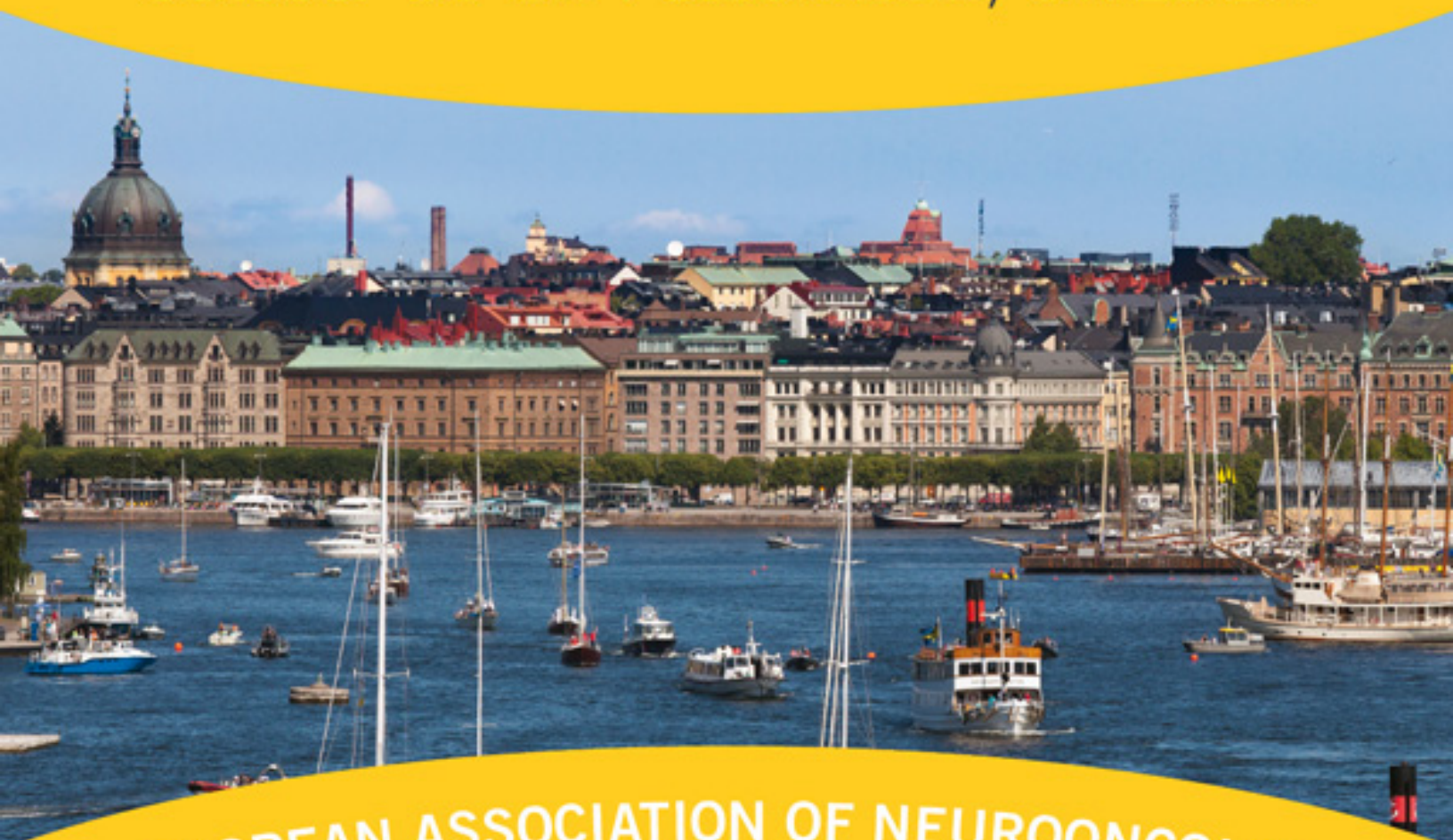

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FROM THE EDITOR

INTERNATIONAL JOURNAL OF BIOMEDICINE

Genetic Diversity, Epigenetic Reprogramming and Environmental Factors: Leading Directions in the Study of the Complex Human Diseases

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Abstract

The article discusses the main issues of the complex interactions between genetic, epigenetic and environmental factors in the development of the complex human diseases.**(International Journal of Biomedicine. 2017;7(4):269-275.)**

Key Words: genetic diversity • epigenetic reprogramming • environmental factors • complex human diseases

Complex human diseases originate from Paleolithic caves, where Homo sapiens (HS) started. The oldest human alleles originated in Africa, in parallel with the development of our species, millions of years before people first migrated out of Africa 50,000 to 60,000 years ago.[1,2] These ancient polymorphisms are shared by all human populations; they account for approximately 90% of human variation and reflect the evolution of the human genome.[3] The genetic diversity of man dates back to an ancestral African population that lived about 200,000 years ago. In the course of their many thousands of years of development, modern humans have faced the global challenges of the external environment. The colonization of other continents and other climatic zones, which started about 70-40 thousand years ago and lasted for 40-45 thousand years with the waves of hybridization (interbreeding of HS with the Neanderthal man and Denisovan man), was accompanied by global environmental challenges and required adaptation to new habitat conditions.

The Neolithic revolution (the transition from the appropriative economy to a producing economy, 12,000-9,000 years ago) with the development of agriculture, cultural farming and cattle breeding, led to a rapid expansion of the population and a drastic change in diet, necessitating changes to adapt to the changed structure of nutrition and metabolism. Therefore, some genetic changes are associated with differences in types of nutrition. The most known among them is hypolactasia—lactose intolerance. Lack of lactase in adults is the initial ancestral sign for HS. However, most adult Europeans produce lactase and can drink milk without harm to health. These people are carriers of a mutation in the DNA region that regulates the synthesis of lactase. The mutation spread after the appearance of dairy cattle breeding about 9-10 thousand years ago and is found mainly among European peoples. The traditional diet acts as a selection factor and leads to a change in the frequencies of alleles and the spread in the population of genetic variants that are most adaptive with this diet. In another example, the encounter between the ancient civilization and Scythians in the Northern Black Sea region in the 4th century BC, was accompanied by "global food stress." Nutrition conflict gave rise to catastrophic consequences. The first wave of settler Greeks died from the cold and unusual food 5-10 years after relocation. At the genetic level, a conflict of enzymes began. For example, Greek wine became a catastrophe for nomads due to the lack of the enzyme alcohol dehydrogenase. "Drunk as a Scythian" (Herodotus: The History). Biologically, HS and their immediate ancestors for hundreds of thousands of years adapted to the lifestyle of hunter-gatherers. Then came the transition from gathering to agriculture, with its accompanying changes in diet and lifestyle, which continued for tens and hundreds of generations.

Adaptation of the population to dietary factors and lifestyle is accompanied by genetic changes with an increase in the frequency of adaptive alleles in the population. However, the human genome, as a relatively slowly evolving system, is not able to change under the pressure of environmental factors in a small number of generations and quickly respond to the challenges of the environment.

With the development of the producing economy, along with infectious diseases, many common diseases that rarely occurred in ancient hunter-gatherers, or were in general unknown, appeared. For example, the low-cholesterol diet of hunter-gatherers made them adaptive for the ability to intensively absorb cholesterol, which under the new conditions became a risk factor for atherosclerosis. Epochal

studies conducted 100 years ago in Egypt by Sir Mark Armond Raffer, allowed the signs of atherosclerosis to be revealed histologically in the aorta and other large arteries of numerous Egyptian mummies, that were 3,000 years old.[4] The presence of the signs of atherosclerosis in the aorta and other large arteries in 76 Egyptian mummies was confirmed by whole-body CT scanning study by AH Allam and colleagues.[5]

Having passed the line of modern chronology (A.D.), mankind again began to move around the planet: the Great Migration of Peoples (3rd-7th centuries), with mass migration of the population, habitat change, assimilation of peoples, the destruction of some and the formation of new ethnic groups. Great geographical discoveries (15th-16th centuries) and the "Columbus exchange" (plants, animals, food, peoples, and infectious diseases moving around the planet) are considered by scientists as a stage of the "First Globalization." Together with migrations, infections such as smallpox, leprosy, plague, tuberculosis, malaria, and syphilis were rampant on the planet. Epidemics—a characteristic sign of the Middle Ages—were the result of the growth of cities, terrible famine, climate change, unsanitary conditions, mass migration of a large number of people, and crusades.

The first pandemic of the plague (Justinian Plague), which was preceded by the pandemic of leprosy and smallpox, which originated in the Eastern Roman Empire in the 6th century and covered almost the entire territory of the civilized world, killed more than 100 million people for several years. The second pandemic, widely known as the Black Death or the Great Plague, originated in China in 1334 and spread along the great trade routes to Constantinople and then to Europe, where it claimed an estimated 60% of the European population.[6]

Epidemics made the problem of resistance to infections urgent. Epidemics, often associated with wars, can change the frequencies of alleles throughout the lifespan of one generation due to a sharp decline in the population.

The first studied example of resistance is found in the spread of sickle cell anemia, a hereditary disease in the tropical and subtropical zones caused by a mutation in the hemoglobin gene. Mutation carriers were resistant to malaria. Researchers found that in the areas with a high incidence of malaria, the heterozygous condition is most "advantageous," since homozygous carriers of mutant hemoglobin die from anemia, homozygous carriers of the normal gene are affected by malaria, and in heterozygous carriers anemia appears in a mild form, but these carriers are protected from malaria.

A very interesting story of positive selection in the human genome is the 32-bp deletion in the chemokine receptor CCR5, a variant that confers resistance to AIDS. C-C chemokine receptor type 5 (CCR5) is a type of chemokine defined as small proteins having diverse functions that include immune surveillance and immune cell recruitment. In 1996, it was discovered that CCR5 behaves as a co-receptor for entry of HIV into cells.[7] CCR5 receptors are not expressed on the cell surfaces in homozygous individuals having the CCR5-Δ32 variant due to an incomplete protein providing high level protection against HIV infection.[8] Studies have found that although heterozygous individuals carrying the CCR5-Δ32 allele can be infected by HIV, the disease progression rate is slower in these people.[9] About 1% of Caucasian people have inherited two copies of CCR5-Δ32 genes leading to virtual immunity to HIV infection. On the other hand, 20% of Caucasians carry only one copy of the CCR5-Δ32 allele, which gives some protection against infection, thus making the disease less severe when infection occurs.[10] This variant was postulated to be a relatively recent response to plague or smallpox. New research shows that the frequency of CCR5-Delta32 in Bronze Age samples is similar to that seen today, pushing the observed age of the allele back to at least 3,000 and possibly 5,000 years ago.[11]

A powerful global challenge for modern humans was the Great Industrial Revolution (the period from the end of the 18th century to the beginning of the 20th century) with the development of industry, urbanization, and pollution of the environment with a large number of new chemicals that did not exist in nature before. The tremendous leap in the development of science, technology, and medical care have increased the human life expectancy with a shift in the vector of morbidity. Information globalization (from the 20th century to the present), adopting the baton of the Great Industrial Revolution, defined a new level in the development of civilization, with fundamentally new opportunities and previously unknown problems of human development, with changing traditional stereotypes of behavior and lifestyle, and with the changing population age structure and signs of a demographic revolution. The rapidity of change and the contraction of historical time—the basic characteristics of the modern period of globalization—have become a new and powerful challenge for modern HS.

Exponential population growth, fueled by the development of agriculture in the past 10,000 years and of urbanization in the past 700 years, has resulted in a vast number of new alleles.[3] Collectively, these alleles have generated an immense degree of genetic variation. Given the size of the present-day human population, every point mutation compatible with life is likely present in someone, somewhere.[3]

Genetic variations as disease markers

Human genetic variations—the differences in DNA sequence within the genome of individuals in populations—can take many forms, including single nucleotide changes or substitutions, tandem repeats, insertions and deletions (indels), additions or deletions that change the copies number of a larger segment of DNA sequence (that is, copy number variations (CNVs)), other chromosomal rearrangements such as inversions and translocations (copy neutral variations), loss of heterozygosity (LOH), copy neutral LOH (acquired uniparental disomy).[12]

Single nucleotide polymorphisms (SNPs) are the most abundant type of genetic variation in the human genome. This is approximately equivalent to 3 million SNPs being carried by each individual genome. Therefore, the DNA sequence of any two genomes is estimated to be about 99.9% identical, and the 0.1% genetic variations that are mainly comprised of SNPs are believed to be responsible for the phenotypic differences, such as physical traits (for example, height, and hair and eye colors), disease susceptibility, and drug responses, among

individuals in populations.[12]

The completion of the Human Genome Project is a major scientific development in human genomics and biomedical sciences. The reference DNA sequence has provided the basis for studying genetic variations in the human genome among individuals in populations. Information on the complete sequence of the human genome is freely available *(http://genome.ucsc.edu; http://www.ensemble.org; http:/www.ncbi.nlm.nih.Gov/genome/quide/human)*. Currently, after the completion of the Human Genome Project, more than 17 million SNPs in human genome have been documented in the dbSNP.

Genome-wide association studies (GWAS) designed to discover SNPs that are associated with a complex trait with the genotyped SNPs have reported thousands of SNPs that are robustly associated with one or more complex traits, including quantitative traits and common diseases.[13,14] Typically, the associated SNPs in total only explain a small proportion of the genetic variation in the population, and this observation has led to the perceived problem of "missing heritability."[15-17] Most of the risk alleles that have been identified by GWAS are common (allele frequency >5%) and confer small effect sizes (odds ratio <1.5).[18,19] The vast majority of such variants have no established biological relevance to disease or clinical utility for prognosis or treatment.[3] For example, a recently published 12-year follow-up study of cardiovascular disease (CVD) in more than 19,000 women found that the 101 SNPs identified by GWAS as risk variants for CVD did not predict cardiovascular outcomes.[20] However, there are more examples of how common variants make a major contribution to disease. Sickle cell anemia and the thalassemias are caused by multiple mutations in hemoglobin genes that persist at polymorphic frequencies in malarial endemic regions worldwide.[21] Autoimmune conditions, such as systemic lupus erythematosis, multiple sclerosis, type I diabetes, and rheumatoid arthritis, are strongly influenced by common polymorphic variations at the MHC loci. Alzheimer's disease is strongly influenced by an allele of APOE4 that occurs at polymorphic frequencies in most populations.[23] Lactose intolerance (or lactase persistence) is caused by the effect of any one of several different alleles in noncoding enhancers of the lactase promoter; different regulatory alleles are common in different populations.[24]

The recognition that rare alleles are important contributors to common complex human diseases is a major paradigm shift in human genetics.[3] For example, Liddle's syndrome—a very rare form of hypertension[25] influenced by rare genetic variations—and familial breast cancer induced by BRCA1 and BRCA2 mutations implicate multiple, highly penetrant, yet very rare, variations and yet both hypertension and breast cancer have more common forms for which GWA studies and related strategies have been, and should be, pursued.

The genetic architecture of complex diseases remains elusive. How much each type of genetic variation contributes to inherited risk and the relative proportion of rare versus common variants is unclear.[12] A good source to impute missing genotypes for previous GWAS data became the 1000 Genomes Project, which created the largest public catalogue of human variation and genotype data. The goal of the 1000 Genomes Project was to find most genetic variants with frequencies of at least 1% in the populations studied. Data from the 1000 Genomes Project is available to the worldwide scientific community through freely accessible public databases.

Epigenetic Reprogramming

The ultimate goal of gene discovery in a complex disease is to identify and characterize biological pathways and processes critical to the disorder. Key pathways may be disrupted via many different causes—genetic, epigenetic, and environmental.[3] The traditional view that interactions between genes and the environment control disease susceptibility can now be expanded to include epigenetic reprogramming as a key determinant of the origins of human disease.[26] Currently, epigenetics is defined as heritable changes in gene expression that do not alter DNA sequence but are mitotically and transgenerationally inheritable.

The term "epigenetic" was coined by Waddington[27] to refer to heritable alterations in gene expression that are not due to changes in DNA sequence. Epigenetic modification, such as DNA methylation and histone modification, alter DNA accessibility and chromatin structure, thereby regulating patterns of gene expression. Molecular mechanisms of epigenetic reprogramming include DNA methylation, histone modification, non-coding RNA, nucleosome positioning and many others. These molecular changes are able to maintain in various tissues and organs those features of gene expression that give them all the necessary properties and distinguish certain tissues and organs from others. The entire sum of epigenetic transformations is understood as an epigenom. An epigenom can be considered as a connecting link between the static genome and the constantly changing environment. [28,29] The activity of the epigenome defines the programs of gene expression that act in certain types of cells at the right time. It can be said that the phenotype is the result of a cooperative interaction of the genotype (a specific sequence of DNA) and the epigenotype. Thus, epigenetic reprogramming is the process by which an organism's genotype interacts with the environment to produce its phenotype and provides a framework for explaining individual variations and the uniqueness of cells, tissues, or organs despite identical genetic information.[26]

Given its central importance in non-genomic inheritance and earlier discovery, DNA methylation has been named the "prima donna" of epigenetics.[30] DNA methylation is a heritable epigenetic mark involving the covalent transfer of a methyl group to the C-5 position of the cytosine ring of DNA by DNA methyltransferases.[31]

This chemical modification occurs predominantly on CG dinucleotides in mammalian genomes. However, recent studies have revealed that non-CG methylation (mCH) is abundant and nonrandomly distributed in the genomes of pluripotent cells (embryonic stem cells) and brain cells, and is present at lower levels in many other human cells and tissues.[32] M.D. Schultz with colleagues[33] identified widespread tissue-specific differential CG methylation (mCG), partially methylated domains, allele-specific methylation and transcription, and the unexpected presence of mCH in almost all human tissues.

DNA methylation is typically considered a potentially enduring epigenetic modification (particularly among postmitotic cells). In contrast, posttranslational modifications to the N-terminus tails of histone proteins are highly varied and dynamic and include acetylation, methylation, phosphorylation, and ubiquitination. These epigenetic processes are not independent (i.e., DNA methylation can influence histone modification and vice versa) and collectively influence the accessibility of DNA to transcription factors and RNA polymerase. DNA methylation is typically associated with reduced transcription, and histone acetylation is typically associated with increased gene expression.[34-36]

Most DNA methylation is essential for normal development, and it plays a very important role in a number of key processes, including genomic imprinting, X-chromosome inactivation, and suppression of repetitive element transcription and transposition.[37] DNA methylation can be affected by both inherited DNA sequence variation and a broad range of environmental factors, such as nutrition, exposure to toxic pollutants and social environment.[38-41]

The state of genome instability in connection with the violation of DNA methylation processes appears to be the fundamental basis for the manifestation and/or maintenance of the pathological process in carcinogenesis, atherosclerosis, autoimmune process. [42-45]

The phenomenon of total hypomethylation, which is the most important indicator of genome instability, is defined quite clearly in three acute conditions of the organism: at the very beginning of embryonic development, at the final stages of ontogeny of the organism in the process of its aging and, finally, in the process of malignant transformation. Generally, during the aging process, global hypomethylation of DNA occurs in a repetitive sequence pattern that may promote genomic instability.[46] Not only is aging correlated with hypomethylation of proto-oncogenes, but also with hypermethylation of tumor suppressor genes, potentially leading to increased risk of cancer and other diseases.[47]

Epigenetic changes in disease are not always focal, but can be global and encompass large chromosomal regions.(48) For example, the aberrant expression of micro RNAs has been linked to various age-related diseases, such as Alzheimer's disease, cardiac disease and many cancers, including leukemia and lymphoma.[49-53]

Hypomethylation in atherosclerosis, as well as in cancer, contributes to the pathogenesis of the disease, inducing chromosomal instability, affecting such specific genes related to the development of the disease as the genes of 15-lipoxygenase and extracellular superoxide dismutase.[54] Atherosclerosis is also marked by the hypermethylation of individual genes. This concerns such an important gene for the development of atherosclerosis and aging in general, as the estrogen receptor-α gene on smooth muscle cells.[55] It is the presence of these receptors on cells that allows the antiproliferative effect of estrogens in smooth muscle cells (SMCs), thereby providing cardiovascular protection; suppressing the expression of the receptor gene will promote the induction of the proliferation of SMCs, thereby contributing to the development of atherosclerosis.[56] In addition, the activated estrogen receptors increase the expression/activity of NO synthase, and hence the production of NO itself, which also has the ability to inhibit the proliferation of SMCs in the vessels.[57] Recent studies have shown that differences in DNA methylation exist between major ethnic groups,[58-61] highlighting the potential contribution of epigenetic modifications to human phenotypic variation.

Maud Fagny and colleagues [61] have found that the current habitat and historical lifestyle of a population have similarly critical impacts on the methylome, but the biological functions affected differ strongly. Specifically, the methylation variation associated with recent changes in habitat mostly concerns immune and cellular functions, whereas the variation associated with historical lifestyle affects developmental processes. Furthermore, methylation variation—particularly that correlated with historical lifestyle—shows strong associations with nearby genetic variants that, moreover, are enriched in signals of natural selection. Maud Fagny and colleagues have suggested that populations can initially respond to environmental challenges via epigenetic changes, uncoupled from variation in the DNA sequence, with the adaptive phenotype increasingly being achieved via genetic changes as time passes.[61]

It has also been suggested that epigenetic changes may account for the missing heritability determinants of complex diseases, such as atherosclerosis, hypertension, metabolic syndrome, and diabetes.[42-45, 62]

Hyperhomocysteinemia in atherosclerosis is perhaps one of the mechanisms of the phenomenon of total DNA hypomethylation, characterizing, apparently, both the onset and progression of the disease. Homocysteine (Hcy) is biochemically linked to the principal epigenetic tag found in DNA. Hcy plays a crucial role in methyl-donor biosynthesis.[63] The methyl-group responsible for DNA and histone methylation originates from S-adenosyl methionine (AdoMet), via Met biosynthesis through folate-dependent or -independent pathways of Hcy remethylation. Following the transfer of the methyl group, AdoMet is converted into S-adenosyl homocysteine (AdoHcy), which inhibits the majority of AdoMet-dependent methyltransferases. If Hcy accumulates, AdoHcy will accumulate as well, potentially inhibiting transmethylation reactions. Thus, increased Hcy may be regarded as a global DNA hypomethylation effector via AdoHcy accumulation. Mechanisms of HCy-dependent accumulation of AdoHcy are highlighted in a review by Diane E. Handy.[62] The inverse relationship between Hcy plasma concentrations and DNA methylation patterns has been confirmed in many other reports.[64-67] Several studies support the concept that DNA hypomethylation may be responsible, in part, for vascular complications associated with increased circulating levels of Hcy.[68-73]

Growing evidence suggests that chromatin factors are involved in the incidence of T2DM and may mediate the complex interaction between genetic variants, environment, and gene expression.[74] Barrès et al. [75] identified mCH as one of the critical factors involved in diabetes. They examined the methylation patterns in muscle tissue from a cohort of T2DM, impaired glucose-tolerant, and normal glucose-tolerant individuals. They discovered that the T2DM and impaired glucose-tolerant subjects had significantly higher mCH in the

promoter of the PGC-1α gene compared with normal glucose-tolerant subjects, whereas the number of CG sites was limited in the promoter regions and their methylation states were not significantly altered. Barrès et al.[75] found that in human primary myocytes, the mCH in the PGC-1α promoter can be induced by a high concentration of free fatty acids, such as palmitate and oleate or TNF-α, all of which were present at high levels in diabetic individuals. PGC-1α is a critical gene involved in the regulation of mitochondria biogenesis.[76] The mCH of its promoter was accompanied by lower transcription and a dramatic reduction of mitochondrial DNA content. Furthermore, several recent studies have begun to demonstrate the association between mCH and Rett syndrome. [77-79]

During the course of human life, there is constant interaction between the external and internal environments—interaction that is required for normal development and health maintenance. Exposure to pharmaceutical and toxic chemicals, diet, stress, lifestyle choices, and other environmental factors may result in conflict with the programmed adaptive changes made during early development, and explain the alarming increases in some diseases.[26,48] The plasticity of certain epigenetic modifications can be followed throughout development and differentiation and in response to environmental stimuli. It seems possible that such epigenetic modifications may be amenable to pharmacological interventions.[62] Advances in epigenetic technology may soon allow repair of defective epigenetic modifications by a variety of therapeutics.[48] For example, the drug azacitidine, the first FDA-approved epigenomic drug, treats leukemia by reactivating tumor suppressor genes, and similar drugs are now in development.[80,81]

Thus, despite the risk presented by inherited genes and mutations, epigenetic factors play a decisive role in the actual development of disease. Investigation of epigenetic profiling can help in determining the risk of developing a specific disease in an individual with a particular type of genotype. Also the same epigenetic profile, along with knowledge of the genomic sequence, can help to determine which medications or alternative medicine approaches would be effective in preventing or curing a particular disease.

There is a long road between understanding the intimate mechanisms of the genome-epigenome interactions in the development of disease and the targeted therapeutic approaches. The ancient Greeks said that knowledge is the radius of a circle, and ignorance is the circumference of a circle. With the definition of the structure of DNA, the radius of understanding has grown, but at the same time the circumference of the circle has also grown, but we believe that "Viam supervadet vadens" (Lucius Seneca).

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International Journal of Biomedicine 7(4) (2017) 277-281
http://dx.doi.org/10.21103/Article7(4)_RA1

REVIEW ARTICLE

Neurology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Genetic Polymorphism of Cytokines as a Predictor of Phenotypic Development of Chronic Pain Syndrome in Cancer Patients

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Abstract

The authors present a literature review using materials provided in the following databases: “MedLine”, “PubMed”, “Wiley Online Library”, “Web of Science”, “Oxford University Press”, “SAGE Premier”, dated 1995–2016. The paper describes the results of current international studies on the role of cytokine genes polymorphisms in the genesis of chronic cancer pain. We emphasize the role of inter-individual differences based on genetic polymorphism of cytokines and their receptors in personalized anesthetic care and accompanying therapy in oncology. **(International Journal of Biomedicine. 2017;7(4):277-281.)**

Key Words: chronic pain • oncology • analgesic therapy • pharmacogenetics • nociception • cytokine receptors

Abbreviations

CB2, cannabinoid receptor 2; **CX3CL1**, chemokine (C-X3-C motif) ligand-1; **CXCR3**, CXC chemokine receptor 3; **CCL**, CC chemokine ligand; **COX-2**, cyclooxygenase 2; **ERK–kinase**, extracellular signal-regulated kinase; **IL**, interleukin; **IFN-γ**, interferon gamma; **MCP-1**, monocytic chemotactic protein-1; **NGF**, nerve growth factor; **NOS3**, nitric oxide synthase 3; **PG**, prostaglandin; **SNPs**, single nucleotide polymorphisms. **TGF**, transforming growth factor, **XC**, XC chemokines.

Introduction

A high prevalence of chronic pain syndrome in oncological patients represents a challenging issue of palliative care. The influence of different factors on chronic pain progress and drug resistance to analgesic therapy is associated with an understanding of the complex pathogenic mechanisms of this pain.[1] Mechanical damage to peripheral neurons by cancer cells activates a cascade of pathophysiological processes in the nociceptive system. Cancer cells are also known to produce algogenes.[2] Nociceptive receptors are excited in response to inflammatory and tumor-induced algogenes, which leads to an increase in pain syndrome severity.[3] Prostaglandins and a number of biologically active substances (endothelin, TNFα, IL-1, IL-6, EGF, PDGF, extracellular hydrogen ions H+, ET-1) ensure primary sensitization of nociceptors.[4,5] Moreover, cancer cell activity causes a metabolic acidosis that impairs sensory neurons. Therefore, excitation of nociceptive receptors is influenced by both mechanical and proinflammatory factors. Proteolytic damage to nociceptors predetermines the genesis of inflammatory cancer pain and the progression of cancer.[6]

Immune competent cells, glial cells and cancer cells produce damaged cytokines.[7,8] Proinflammatory cytokines (TNF-α, IL-1, IFN-γ, and IL-6) and anti-inflammatory cytokines (IL-10, IL-4, and TNF-β) are known as markers of tissue damage.[9] IFN-γ is a key modulator of CB2 receptors.[7] Activation of CB2 receptors located in glial cells contributes to neuropathic pain. Cytokines might participate

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in the proliferation and angiogenesis of cancer cells.[9] IL-1β, TNF-α and IL-6 are early cytokines synthesized in response to neuron damage and are potent COX-2 activators.[10] COX-2 upregulation leads to tissue alteration and an increase in neuron sensitization.[7,11] In cases of cancer pain, cytokines promote pain sensitivity through direct cellular interaction or nociceptor activity modulation.[1,9] A total of 50 known cytokine ligands ensure their interaction with different cells of the organism. Ligands are conditionally subdivided into CCL, CXC, CX3C, and XC.[12,13] Cytokine receptors are widely represented in leukocytes, neurons and glial cells.[14] However, ligands are not strictly specific and might mediate pain signals through interaction with many types of receptors.[15]

Therefore, cytokine regulation of receptors and ion channels accounts for nociceptive and neuropathic pain components.[12,16] The main mechanism serving as the basis for development of neuropathic and nociceptive pain components is MCP-1 cytokine induction. MCP-1 only acts through CCR2 receptors and ensures the neuropathic pain mechanism. The CX3CL1 chemokine participates in the pathophysiology of neuropathic pain by induction of IL-1 and IL-6. The cytokine IL-1β participates in the genesis of neuropathic pain.[17] CXCR3 is of critical importance in the development of bone cancer pain through Akt-kinase and intracellular ERK-kinase signaling pathways.[18] The known spliced variants of CXCR3 may account for phenotypic variations, but it is a subject of discussion at the present day.[18]

The purpose of this study was to explore the results of pharmacogenetic research on the association between cytokine gene polymorphism and chronic pain syndrome in oncological patients.

Materials and Methods

We have analyzed recent full-text publications in such international databases as MedLine, PubMed, Wiley Online Library, Web of Science, Oxford University Press, and SAGE Premier. The analyzed period comprised 10 years (from 1996 to 2016). The search for publications was conducted using the following keywords: "cytokine," "pharmacogenetics," "single nucleotide polymorphisms" (SNPs) and "tumor."

Results and Discussion

Localization of the analyzed cytokine genes and their receptors is presented in Table 1. Summarized data on the association between SNP cytokine genes and the risk of cancer development are presented in Table 2.

Contemporary research shows that cytokines might exert influence upon inter-individual differences in the oncological pain syndrome by hosting different SNP interleukin genes. Persistent neuropathic pain is also associated with depressed mood and deterioration in oncological patients' quality of life.[19] At the present time, studies on SNPs associations in cytokine-coding genes are few.[20] Thus, the following SNPs of the TNF-A gene, which code TNF-α, were found to be associated with the risk of cancer development: *1031* T>C, *863* C>A, *857* C>A, *851* C>T, *419 G>C*, *376 G>A*,*308 G>A*, *238 G>A*, *162 G>A* и 49 *G>A*.[21-23] These SNPs increase the risk of cancer development due to a higher level of TNF-α.[24] Currently, SNP *308G>A* (rs1800629) of the TNF-α gene is known to predetermine a higher pain syndrome intensity and a lower response to opioids in lung cancer patients,[9,25] being associated with the syndrome of cancer cachexia and fatigue. The CC genotype (*837 T>C*, rs5275) of the TNF-α gene carriers with lung cancer have a lower risk of severe pain development as compared to carriers of SNP *308G>A* (rs1800629) and *50 C>T* (rs8904) of the *NFKBIA-EX6* gene. Moreover, a protective genotype carrier state reduces the risk of severe pain development by 38%. Therefore, investigations into the role of inflammatory gene polymorphism in modulating pain severity is critical in oncopharmacology.[26]

Table 1.

Chromosome localization of cytokine genes and their receptors [31, 12, 42, 43]

Genes	Localization
Genes of cytokines	
IL-1L	2q13
IL-1B	2q13-21
ΦНО-α	6p23-q12
IL-10	1q31-q32
IL-6	7p15.3
IL-4	5q23-31
IFNg	12q24.1
IL-18	11q22.2_q22.3
Genes of cytokine receptors	
IFNgR1	6p23-24
IFNgR2	21q22.1
IL-6RB	17
IL-4RA	16p12.1-11.2
IL-10RA	11q23
IL-8RA IL-8RB	2q35
Genes of cytokine receptor antagonist	
IL-1RN	2q13

Polymorphism of immune response genes (*PTGS2, TNF-A, NFKBIA, IL6, IL8)* is associated with the degree of cancer pain intensity in lung cancer patients.[27] In particular, SNPs rs5277 and rs1799964 of genes *LTA* and *PTGS2* respectively are associated with pain syndrome severity.[26] Polymorphism of the *IL1β* gene also predetermines differences in pain perception and changes in morphine consumption during the postoperative period,[10] and in the development of depression[28] and fatigue.[10,28] An increase in the expression of the *IL1*β gene causes peripheral hyperalgesia, and vice versa: administration of the IL-1 receptor antagonist leads to suppression of the nociceptive reaction.[5,28,29] SNPs of the following genes are reported to be associated with the burden of pain and with the development of depression and fatigue in patients with lung malignancy *NOS3* (*1474 T>A*, rs1800783), *IL1B* (allele *31C*, rs1143627), *TNFR2* (Met196Arg, rs1061622), *PTGS2* (*837T>C*, rs5275), *IL10RB*

(Lys47Glu, rs2834167)[30] against the background of existing non-genetic factors (the patient's sex, the stage of cancer).[31,32] SNP *174G>C* (rs1800795) of the promoter of the *IL-6* gene is associated with alterations in the serum level of the cognominal cytokine IL-6. Homozygous carriers of the GG genotype have a higher level of IL-6 expression in comparison with homozygous carriers of the CC genotype. The majority of African Americans (83.6%) and Latin Americans (70.5%) have the homozygous GG genotype, and Europeans have the heterozygous GC genotype. Cancer patients with the G allele (genotypes CG and GG) tend to have a shorter life expectancy than patients with the CC genotype. Additionally, SNP *174G*>C of the IL-6 gene is associated with pain syndrome severity in patients with non-small-cell lung cancer and with daily dosage of morphine (the CC genotype patients require higher doses of morphine in comparison with the CG or GG genotype patients).[33] The homozygous GG genotype patients tend to have lower survivability in cases of sepsis, colorectal adenoma and stomach cancer.[34] Moreover, SNPs of *IL6* (rs2069845), *IL13* (rs1295686) and *TNF-A* genes (rs18800610) are reported to be associated with pain, fatigue, sleep disturbance and depression in patients with breast cancer.[19,35]

The results of the current studies show the role of *IL-1* and *IL-8* in the variability of pain response, the consumption of postoperative morphine, and the development of depression and fatigue in lung cancer patients[20] as well as associations of severe pain with polymorphisms of genes *IL1B* (rs1143627),[36] *IL8* (rs4073),[37] and *TNF-A* (rs1800629).[20] Research suggests that there are associations between polymorphisms of genes *IL1R1* (rs2110726) and *IL13* (rs1295686) and postoperative pain control for breast cancer patients, and that patients with breast cancer who are carriers of the C allele in the SNP-marker rs11674595 of the *IL1R2* gene, have the risk of developing severe, persisting pain syndrome in the mammary glands.[38,39]

Associative dependencies reported in the current studies determine the relevance of investigations into the role of these genes in the genesis of chronic pain syndrome in oncopharmacology. Moreover, genetic polymorphism is described as a possible prognostic marker for the development of adverse reactions;[40] in particular, SNP rs1799964 of the *TNF-A* gene is a prognostic factor for the development of oral mucositis against the background of chemotherapy in esophageal cancer patients.[41]

Conclusion

The presented literature review attests to a possible influence exerted by the genetic polymorphism of interleukins on the severity and control of cancer-origin chronic pain (nociceptive, neuropathic). The association between interleukin genes polymorphism and chronic pain syndrome control, as well as the pattern of disease progression, is of prognostic value and determines the interest in exploration of targeted influence on the main targets in nociceptive processing: cytokines and their receptors. Individual differences of severe pain, cancer-related weakness, depression, and neurotoxicity of opioids in oncological patients give rise to clinical interest in developing a patient-specific approach to analgesics and accompanying therapy aimed at maintaining quality of life in patients with malignant tumors.

Table 2.

Associations between genetic polymorphism of interleukins and the risk of cancer development

IL	SNPs	Functional role
TNF-α	308 G>A (rs1800629)	Protective effect over T-cell lymphoma [44] Lack of association between polymorphism and the risk of hepatocellular carcinoma development in people of the Asian race [25] The risk of hepatocellular carcinoma development in the population of China [45] The risk of esophageal squamous cell cancer and esophageal acinic cell carcinoma [21] Homozygous (AA) and heterozygous (AG) subjects have a high risk of colorectal cancer development in the Mexican population [46] The risk of oral cavity cancer [22] The risk of esophageal cancer in the Chinese population[23]
TNF-α	308 G>A (rs180062 857 C>T (rs1799724) 863 C>A (rs1800630) 1031T>C (rs1799964) 252 A>G	The risk of oral cavity cancer [21]
TNF-α	ERCC1-8092	The risk of hepatocellular cancer development in the Chinese population [45]
IL10	1082 GG (rs1800896) 819 C>T	The protective effect in relation to T-cell lymphoma [44] The risk of stomach cancer [27] The risk of urologic cancer for the Asian population [47]
	592 C>A	The risk of colorectal and urologic cancer in the population of Eastern Asia [47,48,49]
	1082A>G (rs1800896) 592 C>A	No association with the risk of non-small cell lung cancer [24,26]
IL18	607C (rs1946518) 251 A>T (rs4073)	The risk of non-small cell lung cancer in the Chinese population [32] The risk of pancreatic cancer [50]
IL4	590 C>T (rs2243250)	The risk of prostate cancer [47]
IL-4R	rs2243228 rs2227284 rs2070874	The risk of pancreatic cancer and cervical cancer [51]
IL6	174 G>C (rs1800795)	Decrease in survivability of homozygous GG genotype patients with colorectal tumor and stomach cancer [52]
IL-1RN	2018 T>C (rs419598)	The risk of colorectal cancer [46]
IL-10	CTLA4 / CT60-AA CTLA4 / A49G-AA	The risk of renal cell carcinoma [49]

Competing interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 7(4) (2017) 282-285
http://dx.doi.org/10.21103/Article7(4)_OA1

ORIGINAL ARTICLE

Neurology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Role of the Immunological Markers in the Pathogenesis of Symptomatic Epilepsy in Children

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Abstract

The objective of our study was to determine the profile of the spontaneous and mitogen-induced cytokine production in children with symptomatic epilepsy of different etiologies.

Materials and Methods: We examined 38 patients (aged between 3 and 18 years) with consequences of strokes (18.4%), neuroinfections (36.8%), neonatal brain injury (28.9%), and brain development anomalies (15.8%). The levels of spontaneous and mitogen-induced production of the main cytokines (IL-2, IL-6, IL-1β, TNFα) were determined by ELISA.

Results: We traced the decrease and/or inhibition in the production of the main regulatory cytokines of monocyte-macrophage profile (TNFα, IL-1β, IL-6) and adaptive immunity (IL-2) in children with symptomatic epilepsy; and we also revealed the insufficiency of the innate immunity factors in children, which was expressed in the suppression of the regulatory cytokine production, even with *in vitro* stimulation. the chronic course of the studied neurological conditions is a reflection of the condition of secondary immunodeficiency. **(International Journal of Biomedicine. 2017;7(4):282-285.)**

Key Words: children • symptomatic epilepsy • innate immunity • pro-inflammatory cytokines

Introduction

Approximately 50 million people worldwide have epilepsy, making it one of the most common neurological diseases globally.[1] The social significance of epilepsy is determined by its prevalence, the young age of patients, the most severe consequences and complications, and inefficiency of treatment in 20%-25% of cases. The disease significantly limits life activity, and about 50% of patients eventually become disabled.[2]

In the pathogenesis of various diseases of the nervous system, an important role is assigned to immune mechanisms. Most researchers believe that the pathology of the central nervous system (CNS) and immune system can be viewed as the pathology of a single functional system that reflects signals from both the external and internal environments. It has been demonstrated[3,4] that neuroimmune disorders are an important component in the pathogenesis of epilepsy. In particular, the role of inflammatory mediators in the development and progression of epilepsy is being actively studied. There are numerous experimental and clinical data on the role of proinflammatory factors in the pathogenesis of epilepsy, in particular, on the association of elevated levels of proinflammatory cytokines and acute-phase proteins with the risk of developing convulsive seizures.[3,5-9] In epilepsy, a chronic inflammatory process promotes the activation of microglia and astrogliosis, which is accompanied by damage to neurons,[7] resulting in disrupted cytoplasmectonics of the hippocampus with the development of local neurodegeneration.[5] The involvement of the inflammation process in the pathogenesis of epilepsy is vividly confirmed by the effectiveness of various anti-inflammatory drugs (corticosteroids, neurosteroids, immunoglobulins, etc.) in the treatment of forms of epilepsy that are resistant to traditional anticonvulsants.[6]

It is known that regulatory cytokines play an important role in the development, progression and prognosis of immunopathological processes. IL-1β is the most potent pro-inflammatory cytokine that is crucial in host-defense responses

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to infection and injury.[10] This cytokine is an important mediator of the inflammatory response, and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis.[11] IL-1β is produced by activated macrophages, endothelial cells, B cells, and fibroblasts. IL-1β is responsible for a broad spectrum of immune and inflammatory responses, induces T-cell and B-cell activation, and consequently the synthesis of other pro-inflammatory cytokines (such as IFN-γ, IL-6 and TNF), and antibody production. This cytokine also induces the expression of itself in newly-arriving monocytes, thus reinforcing the overall process.[12]

In recent years, studies have shown that epileptic seizures can induce the production of cytokines, which in turn influence the pathogenesis and course of epilepsies.[13,14] Since the etiology of symptomatic epilepsy varies, the question arises whether the etiology of the disease is important in increasing the levels of pro-convulsant cytokines, or whether the changes are nonspecific.

The objective of our study was to determine the profile of the spontaneous and mitogen-induced cytokine production in children with symptomatic epilepsy of different etiologies.

Materials and Methods

We examined 38 patients (aged between 3 and 18 years) with consequences of strokes (18.4%), neuroinfections (36.8%), neonatal brain injury (28.9%), and brain development anomalies (15.8%). The diagnosis of symptomatic epilepsy was made based on anamnestic data, the results of a brain MRI, and electroencephalographic data. Exclusion criteria were idiopathic forms of epilepsy, progressive and pharmacoresistant forms of epilepsy. Study of pro- and anti-inflammatory cytokine profiles was performed in blood plasma upon the patients' admission to the hospital and before treatment. The control group included 8 health children of the same ages as the study group.

The study was approved by the Tashkent Medical Pediatric Institute Ethics Committee. Written informed consent was obtained from the child's parents.

We determined the levels of spontaneous and mitogen-induced production of the main cytokines (IL-2, IL-6, IL-1β, and TNFα) by ELISA using an IFA-analyzer and the commercial test system Vector-Best (Novosibirsk, Russia) in the department of immunopathology and immunopharmacology of Republican Scientific Center of Immunology of RUz (2016-2017). For assessment of the mitogen-induced cytokine production, we used Cytokine Stimulus kit, the main components of which are a sterile medium and a complex mitogen (a mixture of lyophilized polyclonal activators that are used to induce cytokines *in vitro*).

Statistical analysis was performed using the statistical software «Statistica». (v6.0, StatSoft, USA) and Microsoft Excel 2007. Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±SEM for continuous variables. The Mann-Whitney U Test was used to compare the differences between the two independent groups. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

As is known, the formation of the clinical stage of neuroimmunology was preceded by the accumulation of fundamental knowledge in this field, the study of immunopathogenesis of neurological diseases, and the achievements in the creation of new immunocorrecting drugs. In the study of immunopathogenesis of neurogenic diseases, an important role is assigned to the immune factors in the formation, development and progression of pathological processes. The immunopathological mechanism also plays a significant role in the chronicization of a number of diseases. In this connection, the role of immune mechanisms in the development of CNS diseases has been the subject of numerous studies. [6,7,9,15,16] Of considerable interest are studies of the cytokine profile with the evaluation of the balance of pro- and anti-inflammatory cytokines as well as the production of mitogen-induced cytokine. This approach allows assessing the potential ability of the body to develop and progress the disease and has prognostic potential. In general, the study of cytokines with assessment of the cytokine-producing capacity of cells is an important criterion for assessment of nonspecific immunity. The main results of our studies are presented in Table 1.

Table 1.

The profile of the spontaneous and mitogen-induced cytokine production in children with symptomatic epilepsy of different etiologies

Cytokine	Control group (n-8)	Neuro-infection (n=14)	Stroke (n=7)	Neonatal brain injury (n=11)	Brain development anomalies (n=6)
IL-2 spontaneous production	17.2 ±0.9	9.5 ±1.1*	8.0 ±1.3*	8.0 ±0.8*	13.3 ±0.8*
IL-2 mitogen-induced production	24.4 ±2.4	12.8 ±1.9*	14.7 ±4.6*	12.4 ±1.9*	19.4 ±1.1*
Il-6 spontaneous production	14.5 ±4.4	20.2 ±5.2	16.5 ±6.4	26.5 ±7.4*	27.5 ±8.7*
IL-6 mitogen-induced production	195 ±0.6	148.3 ±10.7*	163.3 ±13.6*	164.7 ±7.5*	123.5 ±17.6*
IL-1β spontaneous production	20.2 ±9.3	8.0 ±2.3*	4.3 ±0.2*	5.1 ±1.1*	4.9 ±0.3*
IL-1β mitogen-induced production	69.0 ±10.2	47.2 ±4.1*	49.5 ±3.0*	56.7 ±6.5	41.0 ±9.6*
TNF-a spontaneous production	25.8 ±5.6	23.1 ±5.1	17.8 ±2.4*	29.2 ±7.6	34.3 ±3.1

** ($P<0.05$) - compared to control group*

Thus, spontaneous and mitogen-induced production of IL-1b was significantly reduced in all groups of children with various neurological nosologies. The lowest value of

spontaneous production of IL-1β was detected in the group of children with brain development anomalies. Moreover, spontaneous production of IL-1β was significantly decreased in all groups compared to control values, which indicates a certain deficiency of innate immune response. The analysis of the mitogen-induced production of IL-1β also revealed a significant reduction in IL-1β production *in vitro*, which also indicates the depletion of potential reserves of innate immunity, which in turn can be manifested by the functional insufficiency of the immune response.

One of the most important and well-studied lymphokines involved in the process of developing and enhancing the immune response is IL-2. Stimulation and production of IL-2 in the body supports the growth of T-lymphocytes. Therefore, it is called the T-cell growth factor. The spectrum of its biological action is wide. IL-2 induces proliferation of B-lymphocytes, activates cytotoxic T-lymphocytes, and stimulates natural killers (i.e. stimulates the cellular components of the immune system). IL-2 is an important pro-inflammatory cytokine that stimulates the proliferation and differentiation of activated T-lymphocytes into the effector Th-lymphocytes or cytotoxic T-cells. The main producers of IL-2 are T-helpers. An important result of the action of IL-2 on resting or stimulated antigen or mitogen cells is to ensure their proliferation. This biological activity of IL-2 defines it as a typical growth factor of cells of the lymphoid-myeloid complex.

Our results showed that spontaneous and mitogen-induced IL-2 production in all groups of children was significantly decreased in comparison with the control data. The lowest value of spontaneous and induced production of IL-2 was found in the group of children with birth trauma and with acute disorders of cerebral circulation. Thus, the low values of spontaneous and mitogen-induced IL-2 production indicate T-cell immunodeficiency.

Analysis of the TNFα level showed that spontaneous production of this cytokine was decreased in groups of children with inflammatory diseases of the brain and with acute impairment of cerebral circulation, but, at the same time, it was slightly increased in children with birth traumas and brain development abnormalities. It is known that an elevated TNFα value indicates the severity of tissue damage on the background of the inflammatory reaction. In pathological conditions, microglia release large amounts of TNFα; this *de novo* production of TNFα is an important component of the so-called neuroinflammatory response.[17,18] In such situations, there is a need for a clinical interpretation of the pathological process.

The study of IL-6 level showed that spontaneous production of this cytokine was significantly increased in all groups of patients. Moreover, the greatest value of IL-6 was found in a group of children with birth trauma and brain development abnormalities, which also indicated the presence of severe damage to brain tissue. At the same time, the induced production of IL-6 was reduced, with the lowest values in the group of children with developmental anomalies, which indicates innate changes in cytokine regulation. It should be noted that the actions of IL-6 are extremely diverse. On the spectrum of biological action, Il-6 is close to IL-1β and TNFα; it is involved in the development of inflammation, in immune reactions, and in the regulation of hematopoiesis. It also serves as a growth factor for plasma cells, and participates in intersystem interactions. IL-6 refers to cytokines that complete the development of the inflammatory reaction.[3,6] In the immune system, the main targets of IL-6 are B-lymphocytes. IL-6 performs a certain, but unclear, role in the interaction of the immune and neuroendocrine systems.[14]

Thus, in the pathogenesis of symptomatic epilepsy, we traced the decrease and/or inhibition in the production of the main regulatory cytokines of monocyte-macrophage profile (TNFα, IL-1β, IL-6) and adaptive immunity (IL-2) in children; and we also revealed the insufficiency of the innate immunity factors in children, which was expressed in the suppression of the regulatory cytokine production, even with *in vitro* stimulation. These immunological disorders were accompanied by the suppression of IL-2 production. Thus, the chronic course of the studied neurological conditions is a reflection of the condition of secondary immunodeficiency. The results obtained on the role of immunological disorders in the pathogenesis of the disease can be used to optimize epilepsy treatment.

Competing interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 7(4) (2017) 286-288
http://dx.doi.org/10.21103/Article7(4)_OA2

ORIGINAL ARTICLE

Neurology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Serum Level of Homocysteine and Perinatal CNS Lesions in Infants

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Abstract

This study reports an assessment of adaptive possibilities of the central nervous system (CNS) in infants. The study shows how the homocysteine level depends on the severity of neurological deficit (ND) in infants, and how the serum homocysteine level changes during the various treatment regimens. **(International Journal of Biomedicine. 2017;7(4):286-288.)**

Key Words: neurological deficit • homocysteine • CNS • infants

Introduction

A number of perinatal pathological factors, primarily hypoxic and ischemic, are important causes of CNS damage in early childhood.[1] A sensitive indicator of brain damage is homocysteine (Hcy).[2] Hcy is a non-protein-forming, sulfur-containing amino acid that functions as a key intermediate in methionine metabolism. Met is first demethylated to form Hcy, which is then metabolized through two pathways: transsulfuration to cysteine and remethylation to methionine. Deficiency in Hcy metabolic enzymes could lead to abnormal Hcy level. Hyperhomocysteinemia is defined as a medical condition characterized by an abnormally high level (above 15 μmol/L) of homocysteine in the blood.[3]

Elevated Hcy is associated with an increased risk of occlusive vascular disease, birth defects (neural tube defects), complications during pregnancy, and psychiatric disorders.[4,5]

Hcy influences the fetus in the early stages, through disordered brain structure development and function, and/or through placenta vascularization disorders, which reduces the oxygen feed to the fetus.[6]

Hyperhomocysteinemia may arise from genetic defects of enzymes involved in homocysteine metabolism. The enzymes involved can be 5, 10-methylene tetrahydrofolate reductase, methionine synthase, and cystathionine-β-synthase. Hyperhomocysteinemia can also arise from nutritional deficiencies of folate, vitamin B_6, and vitamin B_{12}.[7]

Based on the blood homocysteine level, we aimed to generate the criteria for evaluating ND development and outcome in infants after different treatment approaches.

Materials and Methods

The study included 419 patients (52% boys and 48% girls) aged from 0 to 6 months. The main group (Group 1) included 336 patients in the first year of life who received inpatient treatment because of perinatal CNS damage of a different degree of severity. The control group (Group 2) included 83 apparently healthy children. Children in the control group passed standard clinical examinations in specified periods of observation at the stage of outpatient services.

There were several obligatory criteria for patients to be included in the control group: absence of neurological symptoms, absence of a neurologist's supervision, and pharmacotherapy of neurological deviations during the first year of life.

Groups 1 and 2 were divided into two subgroups according to age: Group 1a (n=163) and Group 2a (n=43) between the ages of 1 to 3 months; Group 1b (n=173) and Group 2b (n=40) between the ages of 4 to 6 months. In accordance with the ND severity, the main group was also divided into subgroups: mild degree (I, n=122), moderate

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degree (II, n=118), and severe degree (III, n=96).

All of the children underwent a somatic and neurological examination. To assess the damage to the nervous system, YA Yakunin's classification (1979) was used. ND was evaluated by a quantitative method based on the 3 points according to the severity (0 - norm, 1 - mild, 2 - moderate, and 3 – severe ND).

The serum level of Hcy was determined by enzyme immunoassay using «Axis-Shield» test kit.

In accordance with a treatment regimen, the main group was also divided into 2 subgroups: subgroup A (n=170), patients who received therapy depending on a general clinical manifestation; and subgroup B (n=166), patients, who received therapy depending on a dominant syndrome (increased intracranial pressure – ICP**)**. The treatment regimens are presented in Table 1.

Table 1.

The prescribed schemes of therapy in the studied groups

Age (months)	Mild degree of ND (I)		Moderate degree of ND (II)		Severe degree of ND (III)	
	Subgroup					
	A	B	A	B	A	B
1-3	Scheme D (n=30)	Scheme B (n=29)	Scheme D (n=30)	Scheme A (n=29)	Scheme D (n=24)	Scheme C (n=21)
4-6	Scheme D (n=31)	Scheme B (n=32)	Scheme D (n=30)	Scheme A (n=29)	Scheme D (n=25)	Scheme C (n=26)

Scheme A: neuroprotector cortexin + drugs for correction of increased ICP

Scheme B: vasoactive agent vinpocetine + vitamin therapy (folic acid + B-group vitamins) + drugs for correction of increased ICP

Scheme C: nootropic and vasoactive drugs, central muscle relaxants drugs for correction of increased ICP, vitamins and biogenic stimulators

During treatment, we used therapeutic agents with various degrees of nootropic effect, and vasoactive agents. For correction of spasticity, drugs with muscle relaxant effect were used (sirdalud, baclofen, mydocalm). For correction of increased ICP, we used diacarb, triampur and magnesium sulfate. The study was approved by the Voronezh State Medical University Ethics Committee. Written informed consent was obtained from the child's parents.

Statistical analysis was performed using StatSoft Statistica v6.0. Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±SD for continuous variables. Student's unpaired and paired t-tests were used to compare two groups for data with normal distribution. Multiple comparisons were performed with one-way ANOVA and post-hoc Tukey HSD test. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

Analyzing the frequency of clinical neurological manifestations in the main group, we found that the dominant neurological syndrome was increased ICP (91%).

In the control group, the serum level of Hcy (Table 2) did not differ significantly in Group 2a and Group 2b. In all subgroups of Group 1, the average Hcy level significantly increased compared to the control group. However, a more pronounced increase of the Hcy level was found in Group 1b.

ANOVA showed a main effect of the ND severity ($P<0.000$) in both age groups. In fact, post-hoc comparisons between groups revealed a decrease in the Hcy levels with the severity of ND. These results reflect the association between Hcy level and the ND severity.

In patients with mild clinical manifestations of ND in Group 1a, the serum level of Hcy showed statistically significant positive dynamics during treatment, both in subgroups A and B, but serum level of Hcy reached the control level of healthy infants only in subgroup A (Table 2).

Table 2.

The serum level of Hcy (µmol/ml) in the studied groups

Group 1 (n=336)	ND severity									Group 2 (n=83)
	I			II			III			
	Before treatment	After treatment		Before treatment	After treatment		Before treatment	After treatment		
	$P_{1a\text{-}1b}$= 0.0001	A (n=61)	B (n=61)	$P_{1a\text{-}1b}$= 0.0275	A (n=60)	B (n=58)	$P_{1a\text{-}1b}$= 0.0255	A (n=49)	B (n=47)	
Group 1a (n=163)	5.633±0.486 (n=59)	4.853±0.791 ^ (n=30)	5.057±0.017 **^ (n=29)	7.532±1.412 (n=59)	6.162±0.954 **,^ (n=30)	6.164±0.959 **,^ (n=29)	9.879±1.018 (n=45)	7.05±0.93 **,^ (n=24)	7.945±0.734 **,^ (n=21)	Group 2a (n=43) 4.598±0.549▫
Group 1b (n=173)	6.014±0.567 (n=63)	5.809±0.423 ** (n=31)	5.871±0.422 ** (n=32)	8.049±1.082 (n=59)	7.614±0.617 **,# (n=30)	7.047±0.612 ** ^ (n=29)	9.021±2.347 (n=51)	8.01±0.871 **# (n=25)	9.01±1.871 ** (n=26)	Group 2b (n=40) 4.598±0.549▫

***-P=0.000 compared to Group 2 (a/b); #- P<0.05 compared before treatment in each ND group;*
^-P=0.000 compared before treatment in each ND group;
▫ -P=0.0000 (F=274.9314 and F=112.7278) compared to Group 1a (I,II,III) and Group 1b (I,II,III) before treatment;
Group 1a: F=210.5429, P=0.0000 (differences between I, II, III) before treatment
Group 1b: F=63.9604, P=0.0000 (differences between I, II, III) before treatment.

In patients with mild clinical manifestations of ND in Group 1b, the serum level of Hcy showed only a positive trend, both in subgroups A and B, and serum level of Hcy did not reach the control level of healthy infants. Thus, we found an age-related effect on the Hcy level during treatment.

In patients with moderate clinical manifestations of ND in all age subgroups of Group 1, the decrease of serum Hcy level was statistically significant during provided therapy, both in subgroups A and B. However, a more pronounced decrease of the Hcy level was found in Group 1a, but serum level of Hcy did not reach the control level of healthy infants. Thus, with age and intensification of ND severity, the positive effect on the serum Hcy level decreases regardless of the treatment scheme.

The minimal changes in the serum Hcy level were found in patients with severe clinical manifestations of ND in Group 1b. The serum Hcy level did not change significantly during treatment in subgroup B.

Thus, the character of the dynamics of the serum Hcy level during ND therapy in children of the first year of life can reflect the age-related features of the pathological process and the adequacy of therapy.

Competing interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 7(4) (2017) 289-292
http://dx.doi.org/10.21103/Article7(4)_OA3

ORIGINAL ARTICLE

General Surgery

INTERNATIONAL JOURNAL OF BIOMEDICINE

The Efficacy of Platelet-Rich Donor Plasma for the Topical Treatment of Venous Trophic Ulcers

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Abstract

The purpose of the study was to evaluate the efficacy of platelet concentrate obtained from donor plasma in the local treatment of venous trophic ulcers. The study included 106 patients (92 women and 14 men) aged from 38 to 79 years with varicose disease of the lower extremities with the sixth stage of chronic venous insufficiency in accordance with the international clinical part of the CEAP classification. The main group included 51 patients who received donor plasma enriched with platelets (10^6 in 1 μl) in the local treatment. The control group consisted of 55 patients who, in the local treatment, were given only modern dressings in strict accordance with the modern concept of treatment of chronic wounds. The results of the treatment were evaluated after 10 days of medical intervention. The use of the platelet-rich donor plasma in the regional treatment of trophic ulcers of venous etiology stimulates the regeneration processes and initiates the formation of granulation tissue, epithelialization, and angiogenesis. A wide range of local therapeutic effects, stimulating the regeneration, allows reducing the treatment time, improving the quality of life of patients and reducing the cost of treatment. **(International Journal of Biomedicine. 2017;7(4):289-292.)**

Key Words: venous trophic ulcers • local treatment • regeneration • platelet-rich donor plasma

Introduction

Venous trophic ulcers (VTUs) are a big medical, social and economic problem. Treatment of patients with nonhealing wounds on the background of chronic venous insufficiency (CVI) requires significant material costs, patience and high professionalism of doctors.[1,2] The formation of granulation tissue and epithelialization in ulcers is usually stimulated by local products. In conditions of impaired nutrition, the synthesis of tissue elements in the wound is slow.[3] Therefore, it seems appropriate to use whatever means are available to stimulate regeneration.

Platelet-rich plasma (PRP) is increasingly being used as a new alternative approach in various fields of medicine. The curative properties of PRP rely on the fact that platelets are a physiological reservoir of a variety of growth factors, with healing function which have an active role in tissue regeneration.[4,5]

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The major platelet growth factors are: PD-EGF - epidermal growth factor of platelets, PDGF - platelet derived growth factor, BMP - bone morphogenetic protein, TGF - transforming growth factor, IGF - insulin-like growth factor, VEGF - vascular endothelial growth factor vessels, ECGF, a growth factor for endothelial cells, bFGF - basic fibroblast growth factor. The intracellular amount of endogenous growth factors is determined at the genetic level and is in a certain ratio.[6] By acting on the receptors of the membranes of stem cells, growth factors cause their proliferation. Growth factors have an oligopeptide structure and affect cell membrane receptors type I and II, and promote the growth and differentiation of healthy progenitor cells.[7]

The commonality of biological responses in different tissues of the body to damage or the generic mechanism of the action of growth factors has provided a wide application of PRP in cosmetic, general, plastic and facial surgery, as well as in dermatology, ophthalmology, dentistry, traumatology, orthopedics, and sports medicine.[8-12] The efficacy of autologous PRP for the restoration of damaged tissues with low healing potential has been proven. Preparation of autologous PRP requires a procedure room for blood sampling, a laboratory,

trained medical staff and time to directly obtain PRP, which is inconvenient in outpatient centers.[13] We believe that process optimization is possible using platelet concentrates obtained from donor plasma.

The purpose of the study: to evaluate the efficacy of platelet concentrate obtained from donor plasma in the local treatment of VTUs.

Materials and methods

The study included 106 patients (92 women and 14 men) aged from 38 to 79 years (average age of 64.2±16.4 years) with varicose disease of the lower extremities with the sixth stage of CVI in accordance with the international clinical part of the CEAP classification.

The study protocol was reviewed and approved by the Ethics Committee of Voronezh State Medical University named after N. N. Burdenko, All participants provided the written informed consent.

The inclusion criteria were men or women aged 20-80 years, venous trophic ulcer of the lower extremities (area less 20 cm^2), the phase 2 of chronic wound process, ankle brachial index (ABI) ≥ 0.8 and ≤1.2, informed consent form signed.

The exclusion criteria were chronic infectious diseases, treatment with radiotherapy or chemotherapy, history of cancer, ABI <0.8 or >1.2, the phase 1 of chronic wound process, low compliance of patients, diabetes, decompensated heart failure, systemic diseases of connective tissue, hormone therapy, lymphedema, pregnancy.

The duration of existence of trophic ulcers was 5.3±1.4 years (from 4 months to 8 years). The period of time for open sores was from 3 months to 6 years, an average of 1.8±0.36 years. The average size of VTU was 14.6±3.2 cm^2 (from 8.0 to 20 cm^2). The non-healing ulcers were first debrided to remove any necrotic tissues and fibrin. In all patients, clinical examination showed that the wound was consistent with the phase 2 of chronic wound process: at the bottom of the wounds there were sluggish single granulations, areas covered with fibrin, and the epithelialization edge was not expressed. Bacteriological monitoring revealed mainly S. aureus, S. epidermidis, and E. coli bacterial contamination not higher than 10^3CFU/g. Cytological examination was consistent with an inflammatory type of cytogram. Patients randomly were divided into main and control groups.

The main group (MG) included 51 patients who received donor plasma enriched with platelets (10^6 in 1 μl) in the local treatment. PRP was obtained with the Trima Accel Automated Blood Collection System Version 6.0. The main requirements for alloplatelets are infectious and immunological safety, which are achieved by:

- screening of blood-borne infections
- a reduction of immunological risks
- maintenance of an adequate stock of platelet concentrate
- timely delivery of the corresponding platelet concentrates
- monitoring and prevention of side effects

Immediately before using the platelet-rich donor plasma (PRDP), a 10% calcium chloride solution (2 ml per 20 ml of PRDP) was added to it to activate platelets. The composition was mixed slowly until a thick, uniform mass was formed, which was injected into the bottom and edges of the wound. The wound was closed with a bandage supporting the moist environment in the wound.

The control group consisted of 55 patients who, in the local treatment, were given only modern dressings in strict accordance with the modern concept of treatment of chronic wounds.

In all patients before the study and in the dynamics, the size of the ulcer was evaluated with the help of the vector-raster editor Spotlight Pro 10 (CSoft), the condition of the skin around the ulcer was assessed, and bacteriological and cytological studies were performed. All patients underwent ultrasonic duplex scanning, ultrasound examination of soft tissues, computed tomography (CT) scanning, magnetic resonance imaging (MRI), and trans-cutaneous oxygen measurement.

The results of the treatment were evaluated after 10 days of medical intervention. During the registration, processing and analysis of digital material, we used Microsoft Excel and Microsoft Word. All values are presented as mean ± standard deviation or as number (percentage). The inter-group comparisons were performed using Student's t-test. A probability value of $P<0.05$ was considered statistically significant.

Results

In the study group, after 10 days treatment, 43(84%) patients reported reduction in pain, heavy legs, swelling, wound pain, and cramps in the calf muscles. A maceration of the skin around venous ulcers was observed in 5(9.8%) patients. In 41(80%) patients, bright juicy granulations appeared, covering almost the entire bottom and forming a distinct cushion of boundary epithelialization; on average, the area of trophic ulcers for the group decreased by 4.3cm^2 (*P<0.05*) due to the boundary epithelialization (Fig.1).

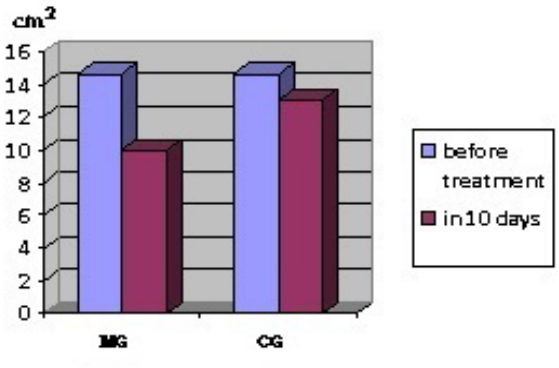

Fig. 1. *Dynamics of the area of wounds*

In 10(19.6%) patients, significant dynamics in the area and the state of wounds was not marked, granulation was sluggish, and epithelialization was slow. Microbiological monitoring revealed complete bacterial elimination in 44(86%)

of patients. In 7(14%) patients we found opportunistic strains in the wound, bacterial contamination of which did not exceed 10^2-10^3 microbial cells. A cytological study revealed the regenerative type of cytogram in 41(80%) patients and the inflammatory type in 10(20%) cases.

In the control group, after 10 days treatment, 39(71%) of patients reported a reduction in pain and in the burning sensation in the wound area, and painless dressings. In the objective assessment, 32(58%) patients showed granulation and marginal epithelialization. Skin maceration and perifocal inflammation were observed in 11(20%) patients. In this group, the area of trophic ulcers decreased by 1.6 cm^2 ($P<0.05$) due to the boundary epithelialization (Fig.1). In 23(42%) of the patients, there was a marked increase in the exudation from the wound, and we took measures to protect the skin around ulcers by using an absorbent wound dressing; the wounds were still clean and actively granulated, but changes in the depth and area of the wounds were not statistically significant. During microbiological monitoring, bacterial elimination was observed in 31(56%) patients, in 21(38%) cases the wound showed pathogenic strains—10^2-10^3 microbial cells; we observed Pseudomonas aeruginosa in 10^5CFU/g in 3 patients. A cytological study revealed the regenerative type of cytogram in 32(58%) patients, the inflammatory type in 19(35%) cases, and the degenerative-inflammatory type in 4(7%) cases.

Discussion

Autologous PRP treatment is used for managing various types of lesions including corneal, vasculitic, neuropathic and diabetic foot ulcers, among others.[14-16] Several studies using this technique have demonstrated adecrease in healing time compared to conventional treatments (in particular, the currently recommended MWC). Releasing cytokines and hemostatic factors into the tissue of the growth factors, after the destruction of platelets, induces chemotaxis of the major cell types participating in the repair processes. Histamine and serotonin, isolated by platelets, activate macrophages and increase vascular permeability, which opens access to the focus of inflammation. In the process of wound reparation, the local concentration of growth factors, cytokines and other biologically active substances plays an important role.[17,18]

The clinical efficacy of PRDP was obvious in our study; the bright juicy granulations, which filled out almost the entire bottom of the wounds with the formation of a pronounced boundary cushion epithelialization, were identified in 80% of patients of the main group and only in 58% of the control group. The investigation of the wound area in dynamics revealed that the application of PRDP led to a decrease in the area of the wound surface by 29%, compared with 11% in the control group. Data from cytological studies confirmed a more rapid transition to the regenerative type of cytogram under using PRDP.

The results of the microbiological monitoring confirm that the antibacterial and fungicidal activity contained in platelets by proteases such as metalloprotease-4 is able to prevent infection.[19]

We believe it is important to note also a positive dynamics in the subjective sensations of patients: a reduction in pain, heavy legs, swelling, wound pain, and cramps in the calf muscles.

Conclusion

Thus, the use of PRDP in the regional treatment of trophic ulcers of venous etiology stimulates the regeneration processes and initiates the formation of granulation tissue, epithelialization, and angiogenesis. A wide range of local therapeutic effects, stimulating the regeneration, allows reducing the treatment time, improving the quality of life of patients and reducing the cost of treatment.

Competing interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 7(4) (2017) 293-297
http://dx.doi.org/10.21103/Article7(4)_OA4

ORIGINAL ARTICLE

Experimental Surgery

INTERNATIONAL JOURNAL OF BIOMEDICINE

Interleukin Expression in the Area damaged by the Development of Abdominal Cavity Adhesions

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Abstract

Background: This study sought to determine the dynamics of IL gene expression during serous membrane damage using an animal model of aseptic peritoneal injury.

Methods: In our study, we used 35 male Wistar rats. Macroscopic and microscopic studies were conducted between 6 hours and 30 days after peritoneal damage was induced. In the damaged peritoneal area, we assessed IL gene expression across the experimental timeframe.

Results: We found that the majority of the studied genes had three characteristic peaks in expression: at 6 hours, on day 3, and on day 14. These effects were observed for chemokine (CXC motif) ligands 1 and 3, IL1b, and IL6. Two peaks of increased expression (on days 3 and 14) were noted for CXCL1, CXCL5, INFγ, IL2, IL4, IL10, TNF, and CD40LG.

Conclusion: We hypothesize that the absence of attention to the changes that occur in the peritoneum after aseptic damage has prevented research from focusing on the important stage of the formation of the richly vascularized adhesions that are unable to regress. Based on the results of our study, we conclude that it is critically important to influence the last wave of IL expression activation (2 weeks after aseptic peritoneum damage) to effectively prevent adhesion formation.**(International Journal of Biomedicine. 2017;7(4):293-297.)**

Key Words: interleukins • adhesions • peritoneal cavity • chemokines

Abbreviations

CXCL, C-X-C motif chemokine ligand; **CD40LG**, CD40 ligand; **IFNγ**, interferon gamma; **IL**, interleukin; **TNF**, tumor necrosis factor.

Introduction

Postoperative abdominal adhesions and fibrosis are major complications of surgery and often result in infertility, abdominopelvic pain, and small bowel obstruction. Adhesions are likely the result of inflammatory responses to surgery-derived tissue trauma, bacterial infection, or foreign substances in the peritoneal cavity. However, the molecular mechanisms that underlie adhesion formation remain unknown.[1]

The major proinflammatory cytokines secreted due to peritoneal trauma are TNFα, IL1β, IL6 and IL8. TNFα and IL1β are the early regulators of the immune response and induce the release of secondary cytokines such as IL6 and IL8.[2,3] Currently, the data on the correlation between the concentration of cytokines in plasma and peritoneal fluid are contradictory. Most researchers have observed an increase in cytokine concentration in the zone of damage compared with the concentration of serum cytokines.[4] However, Florence Riché and colleagues did not find such a correlation in their study.[5] F. Fredriksson and colleagues established that the levels of IL-6, IL-1β, TNF-α in the peritoneal fluid increased 6 hours post-injury, whereas only the level of IL-6 increased in the plasma at this time point.[6]

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According to various researchers, the peak concentrations of proinflammatory cytokines were observed during the first 24 hours after peritoneal damage; these levels stabilized on the third day and decreased by the seventh day post-injury.[4,7-9] G.Wang and colleagues operated on the abdominal organs of patients and experimental models and found that the peritoneal fluid concentrations of IFN-γ and IL-17 increased 6-12 hours after surgery and reached their peak at this time, whereas TGF-β1 concentrations had two peaks at 2 and 72-96 hours after injury.[10]

The inconsistent data regarding cytokine dynamics after serous membrane damage increase the importance of studying cytokine gene expression. Receiving new knowledge on this issue will allow us to investigate the mechanisms involved in the formation of adhesions and develop methods to prevent their formation.

Objective: To study the dynamics of IL gene expression during serous membrane damage using the peritoneal aseptic inflammation model.

Materials and Methods

In our study, we used 35 nine-month-old male Wistar rats weighing 220-250 g. The rats were sedated using ketamine 50 mg/kg, droperidol 2.5 mg/kg and atropine 0.4 mg/kg. An aseptic inflammatory process in the abdominal cavity was simulated by opening the serous-muscular layer of the cecum with a 1cm incision, followed by closing the wound using Schmieden sutures and the scarification of the abdominal peritoneum of the right side channel in a 1.5 cm × 1.5 cm region.[11,12] The animals were housed in keeping with the rules for good laboratory practice. The experiments were performed in accordance with the norms for the humane treatment of animals, which are regulated by the International Guidelines of the Association for the Assessment and Accreditation of Laboratory Animal Care in accordance with the protocol approved by the Institutional Animal Care and Use Committee of the Irkutsk Scientific Center of Surgery and Traumatology. Animals were sacrificed and tissues were collected under ketamine anesthesia at 7 time points, ranging from 6 hours to 30 days. The severity of the adhesions was visually assessed in accordance with the developed protocol (Table 1).

Tissues were fixed with FineFix (Milestone S.r.l., Sorisole (BG), Italy) and then embedded in paraffin for histological investigation. We used Hematoxylin & Eosin staining and the Van Gisone method to detect collagen fibers. We used a Nikon 80i microscope to visualize the slides.

To study cytokine gene expression, tissue was taken from the damaged zone of the cecum and placed in RNAlater solution (Ambion, Canada, Cat #7020). After exposure to the solution at 4°C for 12 hours, the material was placed in storage at -20°C. The study of the serous-muscular layer of the cecum in intact animals served as a control (n=5). To isolate total RNA, the RNeasy Mini Kit was used (Qiagen GmbH, Germany, Cat. No.74104). For RNA DNase clearing, Rnase-Free DNase (Qiagen GmbH, Germany, Cat. No. 79254, Lot No.139294845) was used. After incubation, the samples were cleared using the RNeasy Mini Kit (Qiagen GmbH, Germany, Cat. No.74104). To obtain cDNA, a cDNA-RT2 First Strand Kit (Qiagen GmbH, Cat No. 330401, Lot No. DC08-8) was used. The gene expressions of the ILs were determined using the RT²-Profiler™ polymerase chain reaction (PCR) Array Rat Wound Healing Kit (Qiagen GmbH, Cat. No. 330503).

Statistical analyses of the results were performed using the provided online software obtained with the RT2_Profiler PCR Array® kit (http://www.qiagen.com/Products/Genes and Pathways/Data Analysis Center Overview Page/RT2 Profiler PCR Arrays Data Analysis Center).

Table 1.

The macroscopic scale for assessing severity of adhesions in the abdominal cavity

Score	Number of commissures	Morphology	Extension	Intestinal deformation
0	Absent	NA	NA	NA
1	solitaire	membranous	1 anatomic region	Light, w/o luminal narrowing
2	2 commissures (interviscerale or viscera-parietal)	Loose, non-vascularized	1 abdomen level	Medium deformation w/o luminal narrowing
3	>2 commissures	Dense, non-vascularized	2 abdomen levels	Deformations and luminal narrowing up to ½
4	Conglomerate	Dense, vascularized	Totally	Severe deformations and luminal narrowing >½

Results

Marked peritoneal hyperemia with visible fibrin overlays was observed 6 hours after simulating peritoneal damage under aseptic conditions. A microscopic examination showed hemorrhage, moderate neutrophilic infiltration of the submucosal layer, areas not covered by mesothelium, thickening and neutrophilic infiltration of the peritoneum in the areas adjacent to the damaged area, and neutrophilic infiltration in the sutured intestinal tract. Within 24 hours of injury, the animals showed moderate hyperemia of the peritoneum and fibrinous deposits on the peritoneum.

Formations of intestine-omentum, intestine-abdominal wall, and intestine-intestine adhesions were observed in 80% of the cases. Significant neutrophilic infiltration of the peritoneum and subserous layers was observed in the region of adhesion formation and the zone of the sutured intestinal tract.

Three days after injury, the mild hyperemia of the peritoneum remained, and the fibrinous deposits persisted. The formation of adhesions was observable in 80% of the cases. A microscopic analysis of the adhesion sites showed a proliferation of granulation tissue, characterized by a large area of adhesions, increased density in the connective tissue, and significant inflammation surrounding this junction. On day 7, all of the animals had formed adhesions, with 80% having

formed a conglomerate of adhesions and intestine-intestine type adhesions. All cases showed multiple adhesions, with 3-4 recorded adhesions per animal on average. A microscopic analysis revealed long, indurated adhesions without significant vascularization; the formation of a capsule surrounding the sutures was also noted. On day 14, the adhesive process was observed in all of the animals, and multiple intestine-intestine, intestine-omentum, and intestine-abdominal wall adhesions were observed. These adhesions led to severe deformity and narrowing of the intestinal tube as well as swelling of the superjacent segments in 60% of all cases. Microscopy revealed thick, vascularized adhesions and a proliferation of connective tissue surrounding the sutures.

On day 30, the adhesions in the abdominal cavity of the animals were most severe. In all cases, multiple adhesions were formed, including the most prognostically unfavorable type, the intestine-intestine adhesion. These adhesions led to severe deformity and the narrowing of the intestinal tube and the swelling of the superjacent segments in 60% of all cases. Microscopic examination revealed extensive adhesions with a high density of collagen fibers and rich vascularization. The scoring system for estimating the severity of adhesions [12] revealed that the severity of adhesions was moderate between 6 hours and 1 day. Further observation showed that the intensity of the adhesion process progressed with time, reaching its maximum at 30 days post-operation (Figure 1).

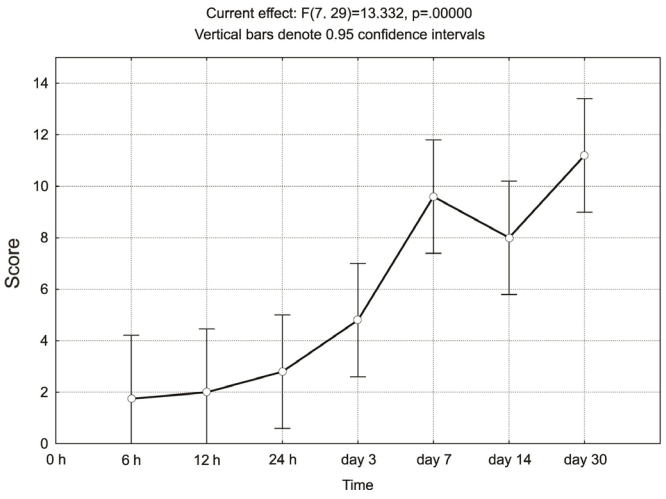

Fig. 1. *Intensity of the adhesion process in the abdominal cavity.*

We then evaluated the expression of interleukin genes in the serous-muscular layer of the damaged cecums. The results were compared with the gene expression in intact animals.

We found an increased expression of proinflammatory ILs 6 hours after the peritoneal injury (Figures 2 and 3). Specifically, increased expressions of CXCL1 (26.7-fold increase), CXCL3 (93.1-fold increase), IL1b (9.0-fold increase) and IL6 (25.7-fold increase), one of the major mediators of the acute phase of inflammation, were observed. The level of IL6 was significantly higher than in intact animals (*P=0.035*).

The maximum expression of CXCL1 (59.3-fold increase) as well as significant increases in CXCL3 (59.6-fold increase), CXCL5 (21.6-fold increase), IFNγ (121.4-fold increase), IL2 (108.1-fold increase), IL4 (26.7-fold increase), IL6 (11.3-fold increase), IL10 (12.6-fold increase), TNF (11.1-fold increase), and CD40LG (15.3-fold increase) were observed on the third day.

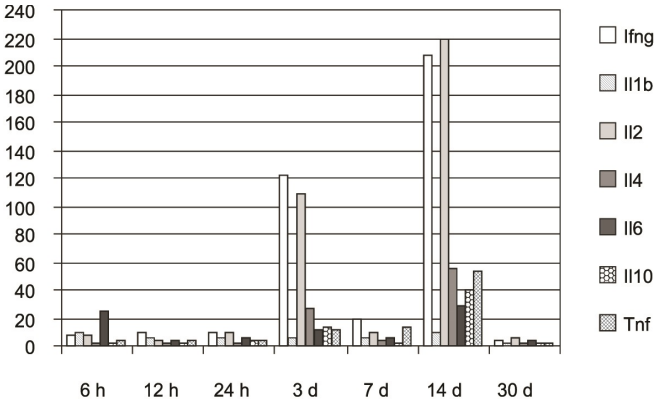

Fig. 2. *Change in IL gene expression in the zone of damage.*

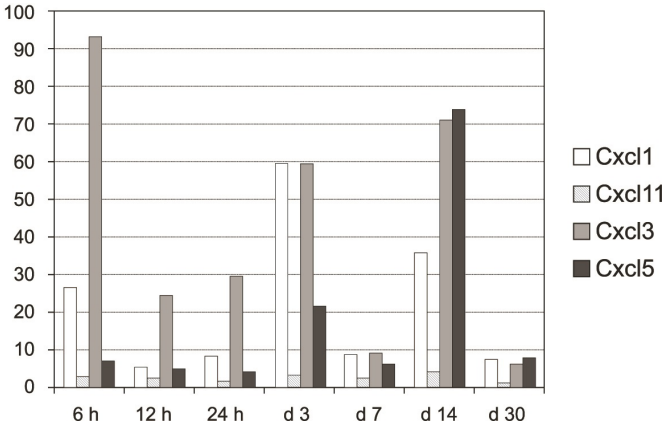

Fig. 3. *Change in chemokine gene expression in the zone of damage.*

By day 7, the expression level of the majority of the ILs had decreased. By day 14, however, repeated increases in the expressions of CXCL1 (35.8-fold increase), CXC3 (71.0-fold increase), CXCL5 (74.2-fold increase), IFNγ (207.0-fold increase), IL10 (39.6-fold increase), IL2 (219.3-fold increase) IL4 (55.7-fold increase), IL6 (29.0-fold increase), and CD40LG (29.1-fold increase) were observed. TNF reached its maximum level (52.8-fold increase) at this time point, as did CXC5, IFNγ, IL10, IL2, IL4, and CD40LG.

By day 30, the expression levels of the IL genes approached those of intact animals.

Discussion

We found that the majority of the studied IL genes were characterized by the presence of three peaks of increased activity: at 6 hours, on day 3 and on day 14 (for CXCL1, CXCL3, IL1b, and IL6). Two peaks of increased expression

(on day 3 and on day 14) were noted for CXCL11, CXCL5, IFNγ, IL2, IL4, IL10, and CD40LG (Table 2). We believe that the first peak at 6 hours was associated with the initiation of the inflammatory response that is naturally accompanied by the increased expression of proinflammatory ILs.[2,3] This observation coincides with the opinion of other authors concerning the key role that injury plays in the production of proinflammatory cytokines during the early period after peritoneal damage.[6,10]

Table 2.

Expression of interleukines genes in the zone of damage in 6 hours, on day 3 and day 14 of the pathological process (degree of increase compared to intact animals)

Time	6 hours	3 days	14 days
Cxcl1	26.7	59.3	35.8
Cxcl11	2.6	3.2	4.2
Cxcl3	93.1	59.6	71.0
Cxcl5	6.8	21.6	74.2
Ifng	8.0	121.4	207.0
IL10	1.9	12.6	39.6
Il1b	9.0	5.1	9.5
Il2	8.0	108.1	219.3
Il4	2.3	26.7	55.7
Il6	25.7	11.3	29.0
Il6st	0.3	0.7	1.9
Tnf	3.1	11.1	52.8
Cd40lg	0.6	15.3	29.1

The second peak most likely reflects the work of the key elements of the reparative process; specifically, day 3 is the start of the fibroblastic phase of inflammation during aseptic wound healing.

The most unusual peak (in terms of both time and maximum intensity) was the late peak of cytokine expression on the 14th day. This peak appears to reflect the intensity of the proliferative process during aseptic peritoneal damage and the restructuring of tissue associated with this proliferation. This peak was preceded by the maximum rise in intensity of the adhesion process in the abdominal cavity based on the observed morphological changes between the 14th and 30th day post-injury. We believe that the increase in CXCL5, which stimulated angiogenesis on the 14th day, is an appropriate response to peritoneal injury and indirectly reflects the intensity of the adhesion formation process and the formation of richly vascularized adhesions.

Almost all of the previous research on the dynamics of the concentration of cytokines after peritoneal injuries has been limited to observations on the first, third, and seventh days after surgery. The absence of attention to the changes in the peritoneum that occur long after aseptic damage precludes researching the important stage of the adhesion process, when richly vascularized adhesions incapable of involution are formed. Based on the results of our study, we believe that influencing the later wave of IL expression and activation (i.e., 2 weeks after the aseptic abdominal injury) is particularly important to prevent adhesions. This is especially important because it has been demonstrated that the neutralization of IFNγ, IL-17 and TGF-β1 during the maximum concentration of these cytokines significantly reduces the formation of adhesions in the abdominal cavity,[10] and the risk of adhesions re-forming after adhesiolysis is correlated with high levels of IL-6 and IL-1 in the abdominal cavity.[13]

At the same time, peritoneal fibroblasts should be viewed as a target to influence the adhesion formation process because they are the most significant pool of cells in the locus of adhesion formation. Previous studies have shown that fibroblasts isolated from the region of adhesions have significantly higher levels of IL-6, IL-10 and TNF-α mRNA than those from the abdominal cavity isolated outside of the adhesion zone.[14,15]

Competing interests

The authors declare that they have no competing interests.

Sources of Funding

The study was supported by the Federal Agency for Scientific Organizations (Project No. 0543-2014-0003) and the Ministry of Industry and Trade of the Russian Federation (Project No. 12411.1008799.13).

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International Journal of Biomedicine 7(4) (2017) 298-301
http://dx.doi.org/10.21103/Article7(4)_OA5

ORIGINAL ARTICLE

Experimental Surgery

INTERNATIONAL JOURNAL OF BIOMEDICINE

Experimental Justification of Using Aseptisorb-A and Platelet-Rich Plasma in Endoscopic Treatment of Mold Bleeding Stomach Defects

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Abstract

The aim of this study was to investigate the possibility of applying the biologically active draining sorbent Aseptisorb-A in combination with platelet-rich plasma **(**PRP) to arrest bleeding of mold stomach defects in dogs.

Methods and Results: The experimental study was done on 12 outbred dogs (both sexes). Fibrogastroduodenoscopy (FGDS) was performed on all animals under intravenous anesthesia. During FGDS, two ulcerative defects (pilot and control) were made in the antrum of the stomach at 4-5 cm distant from each other. Endoscopic hemostasis in pilot ulcers was achieved with the help of pneumatic insufflation of powder-like Aseptisorb-A (0.3 mg) on the bleeding defect with further application of platelet-rich autologous plasma from the animal. Endoscopic treatment of control ulcers was not done; such ulcers were used to estimate the time of spontaneous hemostasis. It was determined that in pilot ulcers after described interventions, bleeding arrest occurred in 3.0|2.5|4.0 sec (Me|upper quartile|lower quartile) ($P<0.01$). It was noted that in all pilot ulcers, hemostasis was definitive and there was no recurrence of bleeding. In the control ulcers, bleeding arrest occurred in 29.0|27.5|30.5 sec ($P<0.01$). In endoscopic gastroscopy, two cases of the reinitiation of haemorrhages in the form of hematin on ulcers were fixed. The reparative process in pilot ulcers treated with Aseptisorb-A and PRP occurred quicker and more efficiently. Complete healing of pilot ulcers occurred in 8.0|8.0|8.5 days ($P<0.01$) with formation of a slight sword-cut, which did not destroy the wall of the organ. Complete healing of control ulcers was identified in 15.0|15.0|16.0 days ($P<0.01$) with formation of a rough scar, which deformed the organ's wall.

Conclusion: Using the biologically active draining sorbent Aseptisorb-A in combination with PRP in endoscopic treatment of mold bleeding in the defects of stomachs accelerates the reparative process, reduces the time of healing in experimental ulcers, improves the quality of healing and does not damage stomach tissue. **(International Journal of Biomedicine. 2017;7(4):298-301.)**

Key Words: mold bleeding • endoscopic hemostasis • Aseptisorb-A • platelet-rich plasma • reparative process

Introduction

The problem of treating bleeding gastroduodenal ulcers is one of the most challenging in emergency abdominal surgery. Currently, in the treatment of patients with ulcerative gastroduodenal bleeding (GDB), endoscopic hemostasis (EH) is accepted as a first-line treatment modality.[1]

However, the results of using already available methods of EH do not fully satisfy surgeons due to the continuing growth of the number of rebleeding cases. Development of new methods of EH is a topical problem of modern clinical practice.[2-5]

In the Voronezh City Center for the treatment of patients with GDB, the endoscopic pneumatic insufflation of granular sorbents has been successfully used for more than 24 years in complex treatment. One of the biologically active drainage sorbents of the new generation is Aseptisorb-A. This sorbent has been successfully used in the treatment of GDB because of its pronounced hydrophilic, cytoprotective, hemostatic, anti-inflammatory, analgesic, and antibacterial properties. However, it has been noted that granular sorbents are not endowed with pronounced hemostatic properties and when applied in the form of monotherapy, bleeding recurrences are possible.[6-12]

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A number of authors have noted that it is important not only to stop peptic ulcer bleeding, but also to create the condition for the fast and quality healing of gastroduodenal ulcers.[13-16] The possibility of combined use of biologically active draining sorbents with platelet-rich plasma (PRP) in the treatment of peptic ulcer GDB seems very promising.

The aim of this study was to investigate the possibility of applying the biologically active draining sorbent Aseptisorb-A in combination with PRP to arrest bleeding of mold stomach defects in dogs.

Material and Methods

The experimental study was done on 12 outbred dogs (both sexes). The weight of the dogs was from 10 kg to 15 kg. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Fibrogastroduodenoscopy (FGDS) was performed on all animals under intravenous anesthesia (Zoletil 100: 7.5 mg/kg body weight). During FGDS, two ulcerative defects (pilot and control) were made in the antrum of the stomach at 4-5 cm distant from each other. A 3.0 ml of 96% alcohol was injected in the submucosal layer of the stomach. At the same time, the animal was intravenously injected with vincristine (0.01 mg/kg). In 3-4 days, all animals had 2 acute ulcerative stomach defects (10 mm in diameter).[17]

In examination of bioptic tissue in pilot and control groups before treatment, the anatomic picture was the same and corresponded to an acute stomach ulcer: nidal mixed cell infiltration of the stroma was noted in the area of the defect. On the surface of the tissue, over a placement of fibrin, the impurity of leucocytes and erythrocytes was evident.

There was a layer of the mucous membrane with nidal edema, vessels and fine-focal haemorrhage. Glands were normal but in some areas, cystophorous ectasia was noted; interferruterous stroma had nidal mixed cell infiltration and edema. Neutral glycoproteins were located irregularly in the superficial layers of the mucous membrane. In order to cause bleeding in pilot and control ulcers, the bottom of the ulcer defect was damaged with the help of kelectome.[18]

EH in pilot ulcers was achieved with the help of pneumatic insufflation of powder-like Aseptisorb-A (0.3 mg) on the bleeding defect with further application of platelet-rich autologous plasma from the animal. Endoscopic treatment of control ulcers was not done; such ulcers were used to estimate the time of spontaneous hemostasis. Time of final hemostasis was measured with a seconds counter.

Endoscopic observations of changes in experimental and control defects were performed every other day. Results of the research were estimated according to time of hemostasis, restoration of recurrent hemorrhage, and to time of healing of ulcer defects. Histological sections were stained with hematoxylin and eosin. Collagenous fibers were identified with the help of fuchsin according to Van-Gizon's method, neutral glycoproteids in the periodic acid–Schiff (PAS) reaction, and argyrophilic fibers using silver nitrate. The stained preparations were examined using a light microscope (×100, ×400 magnification).

The statistical analysis was performed using the statistical software Microsoft Excel. The results are presented as median (Me), upper quartile (UQ) and lower quartile (LQ). The Mann-Whitney U-test was used to compare the differences between the two independent groups. The Wilcoxon criterion was used to compare the differences between the paired samples. A probability value of $P<0.05$ was considered statistically significant.

Results

It was determined that in pilot ulcers after described interventions, bleeding arrest occurred in 3.0|2.5|4.0 sec (Me|UQ|LQ) ($P<0.01$). Sorbent was turned into a hydrogel haemostatic medical composition. It fixed firmly in the area of the defect and protected it from negative factors of gastric juice. It was noted that in all pilot ulcers, hemostasis was definitive and there was no recurrence of bleeding.

In the control ulcers, bleeding arrest occurred in 29.0|27.5|30.5 sec ($P<0.01$). In that case, a scarlet quaggy clot was formed. The clot was easily washed with fluid. In endoscopic gastroscopy, two cases of the reinitiation of haemorrhages in the form of hematin on ulcers were fixed. It was not necessary to interfere, bleeding had stopped. In further gastroscopy to control the defects of ulcers, two more episodes of the continuation of haemorrhage in the form of hematin on ulcers were fixed. It was not necessary to interfere, bleeding had stopped.

Studying of reparative actions in the pilot ulcers showed that the reparative process in these ulcers treated with Aseptisorb-A and PRP occurred quicker and more efficiently.

In 3.0|3.0|4.0 days ($P<0.01$) after endoscopic treatment with Aseptisorb-A and PRP, the defect at the bottom of the ulcer was purified. Pilot ulcers diminished in size to 7.0|6.0|8.0 mm, and inflammatory conditions in form of edema and hyperemia on the edges of the ulcer were less obvious. The inflammatory process in pilot ulcers remitted completely in 3.5|3.0|4.0 days ($P<0.01$). First signs of regenerative process in the area of the bottom of pilot ulcers in the form of pieces of granulation tissue were identified in 3.2|3.0|4.0 days ($P<0.01$). Cystophorous glands with signs of papillary proliferation were identified on microslides of the pilot ulcers. In some microslides, there were a large number of plethorical thin-walled vessels with focal accumulation of lymphocytes and neutrophils in mucous and superficial parts of submucosal layer. This anatomical picture proves the presence of granulation tissue in pilot ulcers.

On microslides of the control ulcers, there were signs of ulcerative injury in the form of desquamation. There were areas of fibrin accumulation with impurity of hemolyzed erythrocytes and leucocytes on the surface of control ulcers. The increased number of neutrophils in connective stroma confirmed an acute inflammatory process. Complete healing of pilot ulcers occurred in 8.0|8.0|8.5 days ($P<0.01$) with formation of a slight sword-cut, which did not destroy the wall of the organ.

In pilot ulcers, the anatomical picture of bioptic tissue

did not have any pathological changes. Research of cellular and fiberlite elements revealed the signs of reparative processes, which were characterized by enlargement of the absolute and relative number of fibroblasts and enlargement of tender reticular fibers in the subepithelial layer. Cells rich in neutral glycoproteins were in both superficial and deep layers of the mucous membrane. There was a large number of cystophorous glands in the control ulcers. Their epithelium was partially desquamated and filled with heavy pink masses similar to mucous. Proliferation of connective fibers was determined in the submucosal layer.

These results prove a high rate and quality of reparative regeneration in treatment of mold ulcers with Aseptisorb-A in combination with PRP. L. Aruin considers the high rate and quality of reparative regeneration in treatment of mold ulcers to be very important. [19]

Purification of the control ulcers occurred in 6.0|6.0|6.0 days (*P<0.01*); the appearance of granulation in the area of the bottom ulcer defect was detected in 6.0|6.0|7.0 days (*P<0.01*). Sizes of control ulcers diminished to 8.5|8.0|9.5 mm (P>0.05); the edges of the ulcer were still irritated. In 9.0|9.0|10.0 days, the inflammatory process decreased, ulcers diminished in sizes to 4.0|3.5|5.0 мм (*P<0.01*), and the first signs of epithelialization were revealed. Complete healing of control ulcers was identified in 15.0|15.0|16.0 days (*P<0.01*) with formation of a rough scar, which deformed the organ's wall. In 15 to 17 days, in microslides of control ulcers, there was fibrous degeneration of the mucous membrane in the submucosal layer. This fact indicates formation of a rough cicatricle defect in the healing area.

The effectiveness of treatment with granulated sorbent in combination with PRP of experimental bleeding defects is presented in Table 1.

Table 1.

The effectiveness of treatment with Aseptisorb-A in combination with PRP of experimental bleeding defects

Variable	Pilot ulcers			Control ulcers			P
	Me	UQ	LQ	Me	UQ	LQ	
Hemostasis, sec	3.0	2.5	4.0	29.0	27.5	30.5	<0.01
Purification of ulcer defect, day	3.0	3.0	4.0	6.0	6.0	6.0	<0.01
Appearance of granulation, day	3.2	3.0	4.0	6.0	6.0	7.0	<0.01
Disappearance of inflammation, day	3.5	3.0	4.0	9.0	9.0	10.0	<0.01
Onset of epithelialization, day	4.0	3.5	5.0	9.0	9.0	10.0	<0.01
Complete healing, day	8.0	8.0	8.5	15.0	15.0	16.0	<0.01

Conclusion

Using the biologically active draining sorbent Aseptisorb-A in combination with PRP allows us to perform reliable hemostasis of mold bleeding in the defects in dogs' stomachs without signs of recurrent bleeding. Use of Aseptisorb-A in combination with PRP in endoscopic treatment of mold bleeding in the defects of stomachs accelerates the reparative process, reduces the time of healing in experimental ulcers, improves the quality of healing and does not damage stomach tissue.

Competing interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 7(4) (2017) 302-306
http://dx.doi.org/10.21103/Article7(4)_OA6

ORIGINAL ARTICLE

Histology & Cytology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Effects of Fibroblast Transplantation on the Content of Macrophages and the Morphology of Regenerating Ischemic Cutaneous Wounds

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Abstract

Background: The study of the morphological structure and the determination of macrophagal fraction (MF) in the newly formed epidermis and dermis on the 19th day after the transplantation of auto- and heterofibroblasts and a dermal equivalent with heterofibroblasts will allow determining the optimal method for ischemic wound healing.

Materials and Methods: The study was performed on 28 white mature mice of the C57/B1 line aged between 5 and 7 months. In an ischemic cutaneous wound, 0.4 ml of fibroblast suspension (1.33 million cells) and a dermal equivalent were transplanted. The biopsy material was embedded in paraffin and stained with H&E by the Weigert-Van Gieson method to visualize the elastic and collagen fibers. Macrophages were determined by monoclonal antibodies to CD68. On the 19th day of the healing of ischemic cutaneous wound, the wound healing process goes through the transition from the stage of proliferation with GT formation into the stage of differentiation or fibrosis. The most positive for regenerative histogenesis and inflammation is the introduction of autofibroblasts. The most differentiated epidermis is formed after transplantation into the wound of the dermal equivalent with heterofibroblasts due to the presence of hairpieces in the form of formed hair follicles. The favorable effect of the dermal equivalent with heterofibroblasts differs from the influence of the autofibroblast suspension only by several percent: the thickness of the epidermis by 4.29%, the area of collagen fibers by 2.66%, and the area of the blood vessels by 4.04%. The most positive treatment for regenerative histogenesis and inflammation is the introduction of autofibroblasts. The most differentiated epidermis is formed after transplantation into the wound of the dermal equivalent with heterofibroblasts, due to the presence of pieces hair in the form of formed hair follicles.

Conclusion: The favorable effect of the dermal equivalent with heterofibroblasts differs from the influence of the autofibroblast suspension by only several percent: the thickness of the epidermis by 4.29%, the area of collagen fibers by 2.66%, and the area of the blood vessels by 4.04%. **(International Journal of Biomedicine. 2017;7(4):302-306.)**

Key Words: ischemic cutaneous wound • regenerative histogenesis • fibroblast • dermal equivalent • macrophage

Abbreviations

EG, experimental group; **GT**, granulation tissue; **H&E**, hematoxylin and eosin; **MF**, macrophagal fraction.

Introduction

A defect of the skin that persists for a long time against the background of reduced blood circulation remains a problem of modern surgery.[1] There are various causes of loss of the skin, such as violation of the blood supply and innervation of the skin, trauma (including gunshot), local effects of high and low temperatures,[2] ionizing radiation, and others. The problem of skin repair has still not been solved, although many methods have been proposed and applied, from pharmacological methods to surgical plastic closure of the wound.[3] Currently, the world's achievements in molecular cell biology have created the basis for the application of cellular technologies in the treatment of long-term wound defects.[4] One such treatment, now widely used, is the introduction of

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autologous and heterologous fibroblasts into the wound, which significantly shortens the healing time.[5,6] However, despite a number of works on this subject,[7,8] the reorganization of the tissue components of the ischemic wound at different healing periods remains poorly understood.

The objective of our research was to study the morphological structure, collagen formation and angiogenesis in the biopsies of the newly formed epidermis and dermis on the 19th day of their recovery, in the ischemic wound model after the introduction of auto- and heterofibroblasts, and after dermal equivalent transplantation with heterofibroblasts.

Materials and Methods

Design of the experiment

The study was performed on 28 white mature mice of the C57/B1 line aged between 5 and 7 months. The animals were divided into the control group (CG), consisting of 7 individuals, and 3 experimental groups (EG1, EG2, and EG3) with 7 animals in each group. The animals were housed in keeping with the rules for good laboratory practice (GLP). The experiments were performed in accordance with the norms for the humane treatment of animals, which are regulated by Directive 86/609/EEC on the protection of animals used for experimental and other scientific purpose. In all groups, we performed an operation for modeling a cutaneous wound in the scapular region after intraperitoneal administration of a 2.5% solution of Avertin (0.3-0.4 ml). The skin was resected in the form of a circle with a diameter of 12 mm. A silicone ring with an external diameter of 12 mm was fixed to the edges of the wound with a skin-fascial nodular suture with atraumatic suture material Polypropylene 5-0 to exclude the possibility of epithelialization of the wound and the closure of its mobile skin area.[9] The ischemia of the wound was carried out by superimposing the suture seam by use of Polypropylene 5-0 at a distance of 1.0 cm from the outer diameter of the wound, which disrupts blood circulation in the system near the scapular arteries. Arterial anastomosis around the scapula is formed by a. Circularisscapula (the branch of artery axillaris) and *Ramus descendens* (the branch of the transverse cervical artery originating from the *Truncus thyrocervicalis*). From the excised skin of the mice, fibroblasts were isolated under sterile box conditions with a laminar flow of air. After enzymatic removal of the epidermis, pieces of skin were placed in a DMEM-F12 medium (Lonza) and crushed with vascular scissors to a size of 1-2 mm. Then equal volumes of solutions of type I collagenase (200 U/ml, Sigma) and dyspase (30 U/ml) (Gibco) were added to the pieces of tissue. The resulting mixture was incubated for 1 hour at 37°C with constant stirring. After filtering the suspension through a 0.40 μm diameter filter and centrifuging for 7 minutes at 1000 rpm, the fibroblasts were re-suspended and cultured in the DMEM-F12 medium supplemented with 10% calf serum (HyClone) and 50 U/ml penicillin-streptomycin (PanEco) in Petri dishes. The Petri dishes were placed in an incubator at 37°C and 5% CO_2 to reach 100% confluence. Trypsin(0.25%)–EDTA(0.02%) solution was used to transfer the cells.

In EG1 and EG2, 0.4 ml of fibroblast suspension of the first or second passage in the growth medium DMEM-F12 in the amount of 1.33 million cells was intraoperatively injected into the bottom of the wound and around it. In EG1, we used heterofibroblasts, in EG2 - autofibroblasts. In EG3, a dermal equivalent with heterofibroblasts, prepared on the bases of type I collagen from rat tails, was transplanted into the wound. A sterile 0.34 M solution of NaOH was combined with a concentrated (x10) Medium 199 in a 1:1 ratio. The resulting mixture was combined with a cooled solution of collagen, after which a suspension of fibroblasts was added to the DMEM-F12 culture medium, containing 10% fetal serum (HyClone). The resulting mixture was incubated at 37°C until the gel was completely polymerized.[10]

Morphological examination of scars

On the 19th day after the operation, in all groups, the formed scar was intraoperatively excised and fixed in a 10% buffered formalin solution for morphological examination. The material was embedded in paraffin and stained with H&E and by the Weigert-Van Gieson method to visualize the elastic and collagen fibers. Morphological examination of histological preparations was carried out with a OLIMPUS SX-31 light-optical microscope. The thickness of the epidermis, the number of microvessels in the sections, the area of collagen fibers and microvessels in the dermis of the scars were determined using an image analysis program (ImageJ 1.46r, National Institutes of Health, USA). We use a 10X ocular lens and a 40X objective lens (a total magnification of 400X). Fifty measurements were performed in each group. The obtained digital data (expressed in pixels) were *converted* into μm by using special coefficients: 6379251 for a 10X lens and 98911797 for a 40X lens.

Immunohistochemical identification of macrophages

The presence of macrophages was determined by an immunohistochemical method after dewaxing and rehydrating paraffin sections. To restore the antigenic properties of the cells of the regenerate tissue after fixation in formalin, heat-induced epitope retrieval was performed. The primary antibodies were CD68-polyclonal antibodies (Gene Tex Inc., USA) in a 1:100 dilution. Secondary antibodies containing a large number of horseradish peroxidase molecules were applied to histological sections and incubated in a humid chamber for 30 minutes with washing in Tris-buffer solution between each stage for 10 minutes. To detect and visualize the reaction, from 1 to 3 drops of 3,3-diaminobenzidine (DAB Chromogen/Substrate) (Gene Tex, USA) were added to each section. To adequately represent the structure of the tissue and cell nuclei, the sections were additionally stained with Meyer's hematoxylin for 3 minutes. The sections were dehydrated and placed in Aquatex gel (aqueous mounting agent; Andwin Scientific, France) under cover glasses. In addition, a control study was performed to exclude pseudo-positive and pseudo-negative results.

Macrophagal fraction (MF) was determined by counting the number of CD68-positive cells per 100 cells during microscopic examination (x1350 magnification), followed by calculating the average percentage, based on the results of the studied sections of each biopsy specimen in CG and EGs.

Statistical analysis was performed using the statistical software «Statistica». (v6.0, StatSoft, USA) and Microsoft

Excel 2007. Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±SEM for continuous variables. The Mann-Whitney (U Test) was used to compare the differences between the two independent groups. A probability value of P=0.05 was considered statistically significant.

Results

During the healing of an ischemic cutaneous wound, the silicone ring fell away spontaneously due to the gradual epithelialization of the wound from the edges to the center and the eruption of the ring-retaining seams, which was regarded as an important sign that the regenerative processes were active. In CG, the silicone ring fell away on day 12.4±0.10 after the operation to create an ischemic cutaneous wound. Under the thick remains of the scab, we found the complete wound epithelialization. In CG, on the 19th day after the operation, the epidermis of the biopsy specimen was formed by a multilayer epithelium with a thickness of 55.24±0.11μm. We found a basal layer and several rows of spiny cells; a granular layer was fragmentarily visible. The stratum corneum was thin and at the initial stages of cell differentiation (Fig.1).

In EG1, epithelialization of the wound and falling away of the silicone ring took place one day earlier than in CG, namely, on day 11.4±0.06 after the operation and transplantation of the suspension of heterofibroblasts in growth medium DMEM-F12. The thickness of the epidermis (64.29±0.20 μm) was higher by 14.08% (P=0.05) than in CG. The epidermis was formed and consisted of four layers: basal, prickly, granular and horny. The granular layer was seen by sections.

In EG2, epithelialization of the wound and falling away of the silicone ring were recorded even earlier than in CG and EG1—by day 11.00±0.01 after operation. On the 19th day of regenerative histogenesis, the tissue defect of the skin was eliminated most significantly. The thickness of the epidermis was higher than in CG by 50.52% (P=0.05). Layers of the epidermis were significantly more differentiated. A pronounced stratum corneum was visible on the surface. The epidermis extended into the underlying granulation tissue (GT), forming papillae and hair follicles.

In EG3, the silicone ring fell away on day 12.20±0.11 after the operation. On the 19th day after transplantation of the dermal equivalent with heterofibroblasts, the wound was covered with a thick layer of epidermis. The epidermis looked more differentiated than in the previous groups. All layers of the epidermis were present and well developed, including the granular layer. The thickness of the epidermis was 102.74±1.13μm, which was greater by 43.87% (P=0.05) than in CG. The epidermis formed the outgrowths into the underlying GT, which were the basic islets for hair development and formation of the papillary layer of the dermis.

In the biopsy specimens of CG and three EGs, under the epidermis there was GT filling the wound cavity. In CG, the boundary between the epidermis and the future dermis was clear, but no full papillae were formed. GT was formed by interlacing collagen fibers without a clear orientation, between which there were cells represented mainly by functionally active fibroblasts. Collagen fibers occupied 33.76±0.22% of the dermis area. We did not find elastic fibers. A few blood capillaries and venules were widened, and their area constituted 1.02±0.01% of the dermis area. Vertically arranged capillaries of various diameters were identified. The internal surface of the vessels was lined with a single layer of endothelial cells. CD68-positive cells, which are identified as macrophages, were localized mainly around blood vessels or in the lumen of the vessels, which confirms their hematogenous origin (Fig. 2).

In EG1, GT did not differ morphologically from control, but collagen fibers occupied an average of 55.44±0.17% of the dermis area. CD68-positive cells colored in brown were present near the blood vessels. In EG2, GT was characterized by a significant increase in the processes of angiogenesis and collagen formation. The collagen fibers acquired a regular orientation parallel to the epidermis, especially noticeable in the deep layers; their area was 63.14±0.12% of GT area, and the area of vessels was 1.73±0.01%. (Fig. 3) We did not find leukocyte infiltration, but we did find a small number of macrophages in the deep layers of the dermis (Fig.4). Cellular elements of the fibroblastic series were represented by the large and elongated adventitious cells, which indicated their functional activity. Elastic fibers were absent in all parts of the dermis. In EG3, we found the formed hair tabs in GT of the dermis. The area occupied by collagen fibers was 60.15+0.37%, and the area of the vessels was 1.68±0.01% of GT area. Thin and weakly oriented bundles of collagen fibers filled out the entire dermis. We found a small amount of CD68-positive cells near the blood vessels. Table 1 shows the change in the area of vessels, collagen fibers, and MF in GT of skin biopsy specimens in CG and EGs.

Table 1.

Change in the area of vessels, collagen fibers, and MF in GT of skin biopsy specimens in CG and EGs

Group	Change in the area of vessels by relative to CG, in %	Change in the area of collagen fibers relative to CG, in %	MF	Change in MF relative to CG, in %
CG	0	0	17.21±0.02	0
EG1	+14.29	+39.11	15.14±0.02	-10.03
EG2	+43.33	+46.53	9.75±0.03	-43.35
EG3	+39.29	+43.87	10.66±0.02	-38.06

Thus, angiogenesis was most active against the background of autofibroblast transplantation, when the area occupied by the vessels in the dermis is the largest and the percentage of its growth in comparison with CG is maximal. In this case, collagen formation was also most active. At the same time, after transplanting the dermal equivalent with heterofibroblasts into the experimental wound, we observed a pronounced favorable course of the wound process: the percentage increase in the area of the vessels and collagen fibers was less than in EG2 by only 2.66% and 4.04%, respectively, and epidermal differentiation was most advanced. MF was the smallest in EG2 and this parameter was lower than in CG.

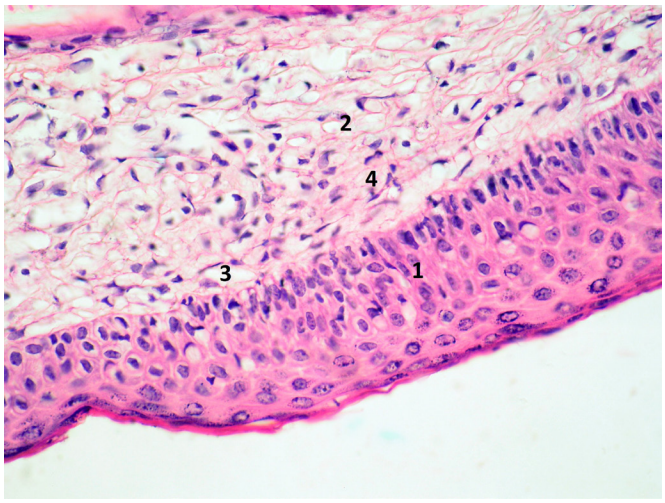

Fig. 1. *Mouse skin biopsy. Control group. H&E staining. 1 - epidermis; 2 - granulation tissue; 3 - vessel; 4 - collagen fibers. Magnification: x200*

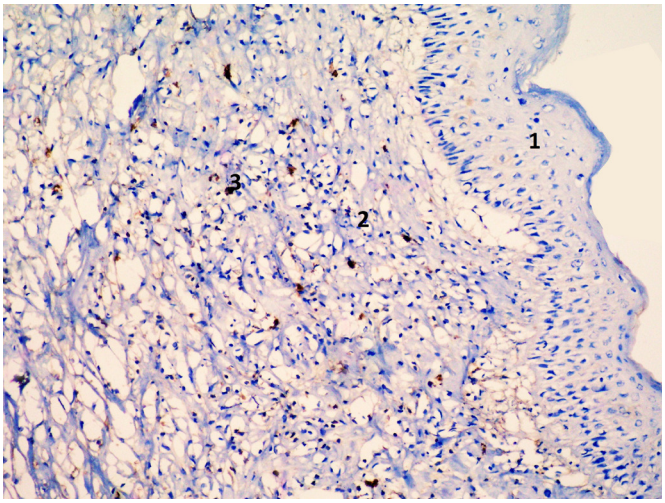

Fig. 2. *Mouse skin biopsy. Control group. Immunohistochemical identification of macrophages. 1 - epidermis; 2 - granulation tissue; 3 - CD68-positive cell (macrophage). Magnification: x100.*

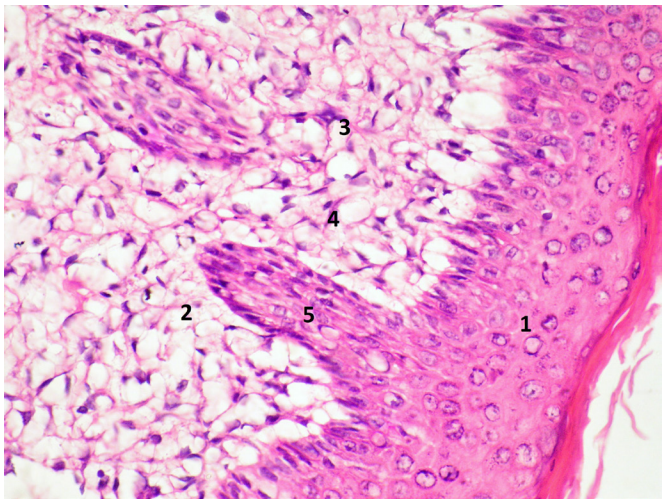

Fig. 3. *Mouse skin biopsy (EG2): after autofibroblast transplantation. H&E staining. 1 - epidermis; 2 - granulation tissue; 3 - vessel; 4 - collagen fibers. Magnification: x200.*

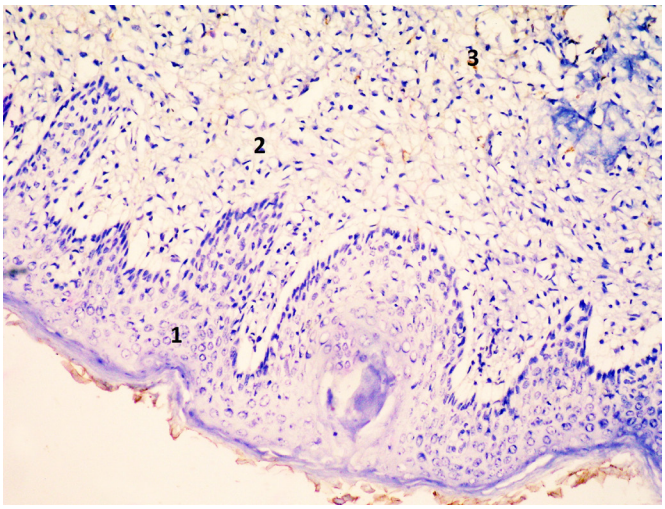

Fig. 4. *Mouse skin biopsy (EG2): after autofibroblast transplantation. Immunohistochemical identification of macrophages. 1 - epidermis; 2 - granulation tissue; 3 - CD68-positive cell (macrophage). Magnification: x100.*

Discussion

The process of skin wound healing includes three stages: inflammation, proliferation with GT formation, and remodeling or fibrosis.[11] With the normal healing of wounds, an influx of inflammatory cells to the wound site occurs until the fourth to sixth day, followed by a proliferative phase, during which the inflammatory cells are replaced by fibroblasts. In long-term, non-healing ischemic wounds, the elements of all three phases can simultaneously be present, but inflammation is predominant.[12] Reduction of inflammation promotes an activation of GT development with subsequent fibrosis. Macrophages can serve as a marker of inflammatory activity due to their ability to excrete pro-inflammatory cytokines. A low MF in GT indicates the abatement of the inflammatory phase of wound repair and the active formation of GT, a temporary matrix, which performs mechanical and regulatory functions. In the search for approaches to reducing the time of inflammation, it was shown that dermal fibroblasts are the source of adiponectin, which acts as an active anti-inflammatory cytokine and induces the production of anti-inflammatory factors such as IL-10 and IL-1RA.[13] According to our data, autofibroblasts have the most pronounced anti-inflammatory effect, which significantly shortens the healing time of a cutaneous ischemic wound.

Conclusion

Thus, **o**n the 19th day of the healing of an ischemic cutaneous wound, the wound healing process goes through the transition from the stage of proliferation with GT formation into the stage of differentiation or fibrosis. The most positive

treatment for regenerative histogenesis and inflammation is the introduction of autofibroblasts. The most differentiated epidermis is formed after transplantation into the wound of the dermal equivalent with heterofibroblasts, due to the presence of pieces hair in the form of formed hair follicles. The favorable effect of the dermal equivalent with heterofibroblasts differs from the influence of the autofibroblast suspension by only several percent: the thickness of the epidermis by 4.29%, the area of collagen fibers by 2.66%, and the area of the blood vessels by 4.04%.

Competing interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 7(4) (2017) 307-309
http://dx.doi.org/10.21103/Article7(4)_OA7

ORIGINAL ARTICLE

Acarology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Prevalence of Tick-Borne Pathogens in Hard Ticks That Attacked Human Hosts in Eastern Siberia

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Abstract

The aim of this study was to evaluate the risk of tick-borne infections in humans. The prevalence of 4 tick-borne pathogens was studied in the population of Ixodid ticks attacking human hosts in Irkutsk city and neighbouring territories from 2007 to 2017.

***Methods and Results*:** In total, 46,357 tick specimens detached from bitten people were analyzed. The antigen of tick-borne encephalitis virus (TBEV) was detected in each tick individually by ELISA assay using a commercial kit for the envelope protein E of TBEV. Total RNA and DNA were extracted from ticks using a RiboPrep kit. Reverse transcription was performed using a Reverta-L kit and RNA\DNA of TBEV; *B. burgdorferi sensu lato*, *A. phagocytophylum* and *Ehrlichia muris\E. chaffeensis* were detected using a real-time multiplex PCR kit**.** In total, during 8 years of observations, *I. persulcatus* caused approximately 86% of bites, *Dermacentor sp.* 13.95 %, and *H. concinna* 0.05 %. The most prevalent tick-borne pathogen in *I. persulcatus* ticks was Lyme disease agent *B. burgdorferi sensu lato*, which was detected in 12±6.5% of specimens annually. *A. phagocytophilum* and *Ehrlichia sp*. were detected in 7.8±2.7% and 4.6±1.5% of specimens, respectively. TBEV was present in 1±0.7% of *I. persulcatus*.

***Conclusion*:** *I. persulcatus* remains the most important vector of tick-borne diseases to humans in Eastern Siberia. *D. nuttalli* and *D. silvarum* are much less aggressive to humans and are less infected with major tick-borne pathogens. *H. concinna* does not play any significant role as a disease vector. However, a rigorous analysis of TBEV spread in the *Dermacentor sp*. population is necessary. **(International Journal of Biomedicine. 2017;7(4):307-309.)**

Key Words: Tick-borne encephalitis virus • *Borrelia burgdorferi sensu lato* • *Anaplasma phagocytophilum* • *Erlichia muris*

Introduction

Ixodid ticks are the vectors of several dangerous human pathogens. The most important of them are tick-borne encephalitis virus (TBEV) and *Borrelia burgdorferi sensu lato* (*Spirochaetaseae*). TBEV causes about 3,000 cases of tick-borne encephalitis (TBE) in Europe and up to 10,000 cases in the Russian Federation annually.[1,2] The agent of Lyme disease, *B. burgdorferi sensu lato*, is spread worldwide and causes approximately 85,500 cases of disease annually, including up to 9,000 cases per year in the Russian Federation.[1,3] Several "emerging" tick-borne diseases (TBDs) have been described in recent decades, including human granulocytic anaplasmosis and human monocytic ehrlichiosis caused by *Anaplasma phagocytophilum* and *Ehrlichia chaffeensis,* respectively.[4]

In the Irkutsk region (Eastern Siberia, Russia), TBDs pose serious threats to the health of local residents and visitors. To reduce the risk of infection, the Centre for Diagnosis and Prevention of Tick-Borne Diseases (hereinafter, the Centre) was established at our institution. According to sanitary rule SP3.1.3.2352-08, each tick is routinely tested for infection with TBEV and *B. burgdorferi sensu lato*, and if the pathogen is detected in the tick, the patient receives the treatment with anti-TBEV immunoglobulins and/or antibiotics to prevent the disease according to modern Russian healthcare regulations. [5] Approximately 7,000 bite victims attend the Centre after tick bites annually, and more than half of them deliver the causative tick specimen.[6] In this study, we evaluated the prevalence of tick-borne pathogens in the population of Ixodid ticks that

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attacked humans in Irkutsk city and neighbouring territories during 11 consecutive seasons of tick activity between 2007 and 2017.

Materials and Methods

Ticks

The study was performed in Irkutsk city and neighbouring territories within a range of about 100 km. This area is situated in Eastern Siberia with landscapes formed by typical taiga forests and harsh continental (borderline subarctic) climate. Ticks were delivered to the Centre by bitten people between March 25, 2007, and October 17, 2017. Each patient was interviewed to collect the following information: the time, geographic location and circumstances of the tick attack; the age, gender and occupation of the patient; the history of vaccination against TBE; the history of tick bites and TBDs in the past; and, finally, the current state of the patient's health. The study was approved by the Ethics Committee of Scientific Center for Family Health and Human Reproduction Problems. Written informed consent was obtained from each patient.

Tick species identification

Each tick was washed twice in 70% ethanol and once in distilled water, then dried on filter paper. Its life stage and species were identified, and then it was immediately dissected for pathogen detection. The developmental stage and identification of tick species was done using key guides to tick fauna of Russia and adjacent countries.[7-9] Due to time restrictions, the *D. nuttalli* and *D. silvarum* tick species were identified only to the genus level and are hereafter designated as *Dermacentor sp*.

Detection of tick-borne pathogens

The agents of Lyme disease were detected by light microscopy according to the standard procedure.[10] The antigen of TBEV was detected in each tick individually by ELISA assay using a commercial kit for the envelope protein E of TBEV (Vector-Best, Novosibirsk). Total RNA and DNA were extracted from ticks using a RiboPrep kit (AmpliSens, Moscow). Reverse transcription was performed using a Reverta-L kit (Amplisens, Moscow) and RNA\DNA of TBEV; *B. burgdorferi sensu lato*, *A. phagocytophylum* and *Ehrlichia muris\E. chaffeensis* (hereinafter, *Ehrlichia sp.*) were detected using a real-time multiplex PCR kit "AmpliSens® TBEV, *B.burgdorferi sensu lato*, *A. phagocytophilum*, *E. chaffeensis*/*E. muris* - FL" (Amplisens, Moscow).

Results were presented as mean values, where appropriate. To evaluate the variability of the data, we estimated the standard deviations of a mean. The long-term trends of the dynamics of tick attack rates were evaluated using linear approximation. Calculations were performed using the MSOffice EXCEL software package.

Results

In total, 46,357 tick specimens detached from bitten people were analyzed. The most abundant species was *I. persulcatus* with a mean incidence of 3,620±473 attacks per year. Two closely related *Dermacentor* species provided a mean incidence of 591±133 attacks per year, whereas another endemic species, *H. concinna*, appeared to be less aggressive to humans with only 2.3±1.5 attacks per year. In total, during 11 years of observations, *I. persulcatus* caused approximately 86% of bites, *Dermacentor sp.* 13.95%, and *H. concinna* 0.05%. The 11-year dynamics of tick attachment to human hosts is present in Fig.1. The trend toward an increase of the attack rate was observed for *I. persulcatus*, though the reliability of approximation is low (R^2= 0.4309).

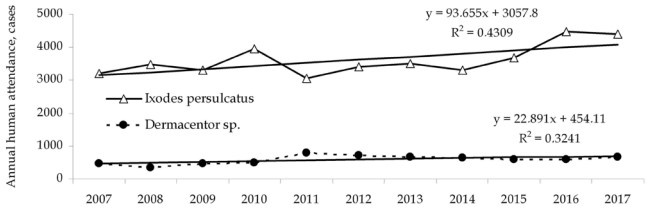

Fig. 1. *The 11-year dynamics (2007-2014) of tick attachment to human hosts.*

The most prevalent tick-borne pathogen in *I. persulcatus* ticks was Lyme disease agent *B. burgdorferi sensu lato*, which was detected in 12±6.5% of specimens annually. *A. phagocytophilum* and *Ehrlichia sp*. were detected in 7.8±2.7% and 4.6±1.5% of specimens, respectively. TBEV was present in 1±0.7% of *I. persulcatus*. In *Dermacentor sp*., the prevalence of every infection was below 5%, with the highest rate for *A. phagocytophilum* (4.2±3.7%). The 11-year dynamics indicate some increase of infection rates of TBEV and *B. burgdorferi sensu lato* in *I. persulcatus* (Fig. 2A, B); however, the changes in *Dermaventor sp*. are not obvious. In 2014, the prevalence of *A. phagocytophilum* and *Ehrlichia sp*. was unusually high among both *I. persulcatus* and among *Dermacetor sp*, but it has decreased during last three years (Fig. 2 C, D). None of the infections were detected in *H. concinna* ticks.

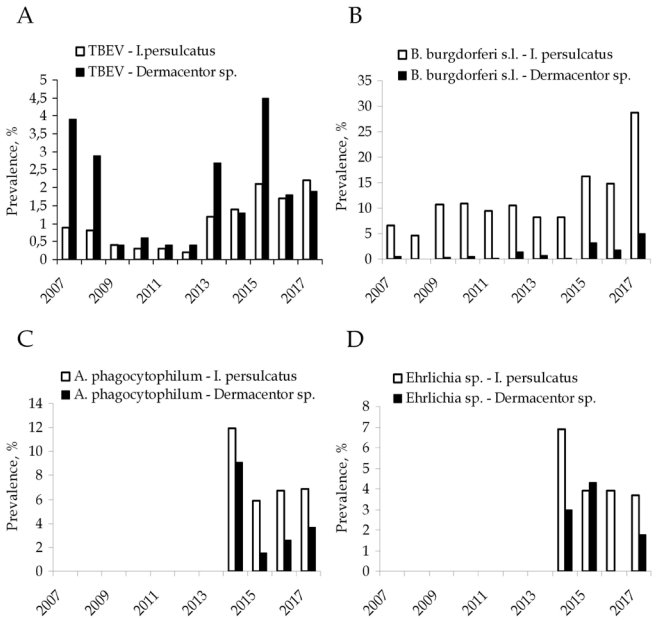

Fig. 2. *Annual prevalence of infections in Ixodes persulcatus and Dermacentor sp. for TBEV (A), B. burgdorferi sensu lato (B), A. phagocytophilum (C), and Ehrlichia sp. (D).*

Discussion

As usual, *I. persulcatus* remains the most important vector of TBDs to humans in Eastern Siberia. *D. nuttalli* and *D. silvarum* are much less aggressive to humans, whereas *H. concinna* does not play any significant role as a disease vector, though its abundance in Eastern Siberian landscapes may reach 14 ticks per one kilometre.[11] This is probably because of the mosaic distribution of *H. concinna* caused by the relatively rare natural conditions suitable for these ticks, such as wet deciduous forests and shrub meadows.

The dynamics of TBEV prevalence indicate a trend toward a significant increase in the infection rate during 2014–2017. This increase was more characteristic of *I. persulcatus*, whereas the data for *Dermacentor sp*. ticks were not as clear and exhibited high variation between consecutive years. Unpredictable variations of data in *Dermacentor sp*. ticks, in combination with a relatively small sample volume, indicate the need for rigorous analysis of TBEV spread among these ticks. For the Lyme disease agent, a similar increase was observable in the *I. persulcatus* population; however, this pathogen is only sporadically present in *Dermacentor sp*. ticks.

Thus, about 86% of ticks were identified as *Ixodes persulcatus*; the *Dermacentor sp*. caused about 14% of bites, whereas *Haemaphysalis concinna* caused 1–5 bites per year. The mean prevalence of infection in *I. persulcatus* equalled 12%, 7.8%, 4.6% and 1% for *B. burgdorferi sensu lato*, *A. phagocytophylum*, *Ehrlichia sp*., and TBEV, respectively.

I. persulcatus remains the most important vector of tick-borne diseases to humans in Eastern Siberia, with the highest attack rate and with about 25% of ticks being infected with at least one of four pathogens. *D. nuttalli* and *D. silvarum* are much less aggressive to humans and are less infected with major tick-borne pathogens. *H. concinna* does not play any significant role as a disease vector. However, a rigorous analysis of TBEV spread in the *Dermacentor sp*. population is necessary.

Competing interests

The authors declare that they have no competing interests.

Acknowledgments

The study was partially supported by the Russian Fund for Basic Research (Project No.15-47-04348).

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International Journal of Biomedicine 7(4) (2017) 310-314
http://dx.doi.org/10.21103/Article7(4)_OA8

ORIGINAL ARTICLE

Biotechnology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Stability of Hyaluronan-Pectic Gel Particles in the Conditions of the Artificial Gastrointestinal Environment

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Abstract

Spherical hyaluronan-pectic gel particles (HPGPs) from hyaluronic acid (HA) and low-methyl esterified pectins of callus cultures of tansy, duckweed, campion and commercial apple pectin were obtained by the method of ionotropic gelation in the presence of calcium ions. We investigated the morphology, swelling and degradation of the obtained HPGPs in the conditions of a simulated gastrointestinal environment and established that the greatest stability in the artificial environment of the digestive tract is achieved with HPGPs obtained from the pectin of tansy callus cultures. HPGPs can be used as potential carriers for drug delivery systems in parts of the small and large intestine.**(International Journal of Biomedicine. 2017;7(4):310-314.)**

Key Words: hyaluronic acid • pectin • callus culture • gel particles • gastrointestinal environment

Abbreviations

DDS, drug delivery systems; **GIT**, gastrointestinal tract; **GLC**, gas-liquid chromatography; **HA**, hyaluronic acid; **HPGPs**, hyaluronan-pectic gel particles.

Introduction

Pectins are natural water-soluble, non-toxic, biodegradable and biocompatible polysaccharides of plant origin exhibiting high physiological activity. Pectic polysaccharides, the main carbohydrate chain of which is represented by 1,4-linked residues of α-D-galactopyranosyluronic acid, are capable of gelling. In recent years, the pectins in the form of spherical particles have been studied for use in drug delivery systems in the body (DDS - drug delivery systems).[1-3] Calcium-pectic gel particles delay drug release in the upper gastrointestinal tract and release it as a result of the degradation of particles by pectic enzymes of the colon.[4] HA is a biocompatible immunoneutral mucopolysaccharide whose macromolecules consist of disaccharide units, the components of which are N-acetyl-D-glucosamine and D-glucuronic acid, interconnected by β-1→4 and β-1→3 bonds. HA in the form of salts, polyelectrolyte complexes or mixtures with other substances of polymeric or other natures has been used more widely in recent years in aesthetic medicine, the treatment of joint diseases, and tissue engineering, as an adhesion barrier to prevent the formation of adhesions in surgery.[5,6] However, the biomedical use of HA is hampered by its short life span and insufficient mechanical strength in the aquatic environment.

To increase the strength and efficiency of natural polymers as carriers of drugs, they can be conjugated.[7] Thus, some reports have demonstrated the preparation of gel particles from HA and chitosan.[8] We have obtained the complex hydrogel particles from HA and pectins of callus cultures for the first time.

The purpose of this work was to obtain HPGPs and to study their morphology and the process of their degradation

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under conditions of an artificial gastroenteric environment as potential systems for the targeted delivery of drugs to the small and large intestines.

Materials and Methods

Objects of the study and conditions of cultivation of callus cultures

In this work, we used HA from cock-crests (Sigma-Aldrich, United Kingdom) with the molecular mass of >300 kDa, commercial apple pectin AU-701 with a degree of methoxylation of 36%-44% and with the molecular weight of 406 kDa (AP, Herbstreith & Fox KG, Germany, low-methyl esterified pectins (6%-22%) from callus cultures of tansy *Tanacetum vulgare* L. (TVC), duckweed *Lemna minor* L. (LMC), campion *Silene vulgaris (M.) G.* (SVC) with a molecular mass of >300 kDa, and calcium chloride ($CaCl_2$, Sigma, USA).

Callus cultures of the bladder campion, tansy and common duckweed were grown on modified Murashige and Skoog agar medium.[9] The bladder campion and common duckweed CC were cultivated with 1.0 mg/l of 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.5 mg/l of 6-benzylaminopurine (BAP) added to the medium. The tansy callus was cultivated with the addition of 2,4-D (1.5 mg/l)+BAP (0.5 mg/l). The calluses were subcultured with an interval of 21 days (campion) and 28 days (tansy and duckweed) at the temperature of 26±1°C in the dark. The callus tissue was frozen at the end of the cultivation.[10,11]

Isolation of polysaccharides

Before the isolation of polysaccharides, the biomass was degraded by a single freeze-thaw cycle. The extraction of biomass was carried out with distilled water at 50°C for 6 hours, the raw material:solution ratio was 1:10. The biomass was then separated from the extract and treated with HA solution (pH 4.0) with a 1:10 ratio of raw material:solution at 50°C for 3 hours to make the pectin substances water soluble. Then, the pectins were extracted by an 0.7% aqueous solution of ammonium oxalate with a 1:10 ratio of raw material:solution at 68-70°C for 6 hours. The extraction was carried out in the digester VK-V-100 (Russia).

The resultant extract was separated from the plant mass by centrifugation (5°C, 9500 rpm, 2 h) with a continuous-flow centrifuge (Beckman Coulter Avanti J-25I with cooling and a flow-through rotor JCF-Z) and sequentially concentrated with simultaneous dialysis using the ultrafiltration system Vladisart (Russia); the pore sizes of the membrane filters were 300 kDa and 100 kDa. The separation continued until a complete absence of carbohydrates, controlled by the phenol-sulfuric acid method.[12] The concentrated solution was frozen at the temperature of -40°C for 10-20 min (Chirst CB 18-40) and freeze-dried at the temperature of -30-40°C (Chirst BETA 2-8 LO plus). The yields of the fractions were estimated as a percentage of the weight of the dry callus biomass. The pectin fractions with molecular weights of more than 300 kD were used in the work.

General experimental conditions

The total content of carbohydrates in the extracts was determined by the reaction with phenol in the presence of concentrated H_2SO_4;[12] the content of glycuronic acids was determined by its reaction with 3,5-dimethylphenol in the presence of concentrated H_2SO_4[13] (the calibration curve was plotted for D-galacturonic acid; photocolorimetry was carried out at two wavelengths 400 nm and 450 nm). The protein content was determined by the Lowry protein assay[14] (the calibration curve of bovine serum albumin; photocolorimetry at 750 nm). Spectrophotometric measurements were performed with a SF-103 spectrophotometer (Aquilon, Russia).

Total acid hydrolysis

A 0.5 ml of 2M trifluoroacetic acid (TFA) containing myo-inositol (0.5 mg/ml) was added to a sample (1-2.5 mg). The mixture was thermostated for 4 hours at 100°C. Excess acid was removed by repeatedly evaporating the hydrolyzate to dryness with methanol. Neutral monosaccharides were identified by the GLC method in the form of the corresponding polyol acetates.

GLC was performed on a chromatograph Hewlett-Packard 4890A (USA) with a flame ionization detector and an integrator HP-3395A on a capillary column RTX-1 (0.25 mm Ø ×30 m; Restek, USA); helium was a carrier gas. The temperature program ranged from 175°C (1 min) to 250°C (2 min) with the temperature of 3°C/min. The percentage of monosaccharides of the total mass of the sample was calculated from the peak areas using the detector response coefficients.[15] Myo-inositol was an internal standard.

Preparation of HPGPs

HPGPs were obtained in the presence of calcium ions by the ionotropic gelling method 9 (Fig. 1).[4,16]

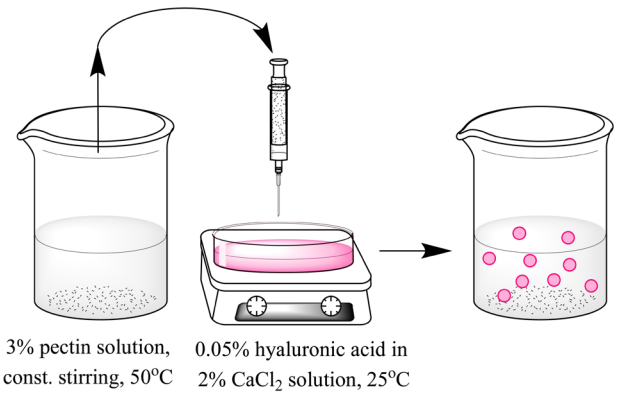

Fig. 1. *Scheme for the preparation of HPGPs.*

Pectins (30 mg or 50 mg) were dissolved in distilled water (1 ml) by slowly stirring with a magnetic stirrer MM-5 (Russia) for 2-5 hours at room temperature until complete dissolution.

Gel particles of a spherical shape were prepared by squeezing out a solution of pectin (3% or 5%) from a syringe through a needle with a hole diameter of 0.6 mm at a distance of 4-5 cm in a slowly stirred 0.05% solution of HA containing calcium chloride (2%) and further mixed for 20 minutes at room temperature. The resulting spherical gel particles were

then washed three times in distilled water, with stirring, for 5 minutes and dried for 10-14 hours at 37°C. Then, the diameter, density and volume of the HPGPs were determined using an optical microscope (Altami, Russia) with a camera and an image analysis program (ImageJ 1.46r, National Institutes of Health, USA). For calibration, a linear scale was used; one pixel corresponded to 0.024 mm.

The study of the swelling and degradation of HPGPs

To study the swelling and degradation of HPGPs under conditions simulating the gastroenteral environment, we used an artificial gastric medium (SGF solution, pH 1.25), a medium of the small intestine (SIF solution, pH 7.0) and a medium of the large intestine (SIF solution, pH 7.0+pectinase (1.18 U/mg; Sigma), as described previously.[17] Ten mg of dry gel particles of each pectin type were sequentially incubated in 5ml of SGF (2 h), SIF (4 h) and SIF+pectinase (0.5, 15, 18 h), with shaking (Titramax 1000, Heidolph, Germany), at 100 rpm and at 37°C. At certain intervals, the diameter, density and volume of 100 randomly selected gel particles of each pectin type were determined as described above. The experiments were performed in triplicate

The degree of swelling of the gel (SD, %) was determined by the equation;

$SD=(D_1-D_0)/D_0\times100\%$, *where*

D_1 - particle diameter (mm) after a certain incubation time in the medium,

D_0 - initial particle diameter (mm).[18]

The statistical analysis was performed using the statistical software BioStat (version 4.03) and Microsoft Office Excel 2007. The mean (M) and standard deviation (SD) were calculated. Multiple comparisons were performed with one-way ANOVA and Tukey's HSD test. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

Pectin polysaccharides with molecular weights of more than 300 kDa were isolated from callus cultures of TVC, LMC, SVC; their total chemical characteristic was described.

The fraction of pectin with molecular mass of more than 300 kDa from the callus of campion had the largest yield (2.8%). The yield of SVC>300 was on average 2.5 times higher than the yields of LMC>300 and TVC>300.

The study of the pectin monosaccharide composition revealed that residues of D-galacturonic acid, arabinose and rhamnose were the dominant constituents of all the pectins obtained. The residues of glucose, xylose, mannose and apiose were also found in the composition of fractions in a smaller amount.

Fractions of pectin with a molecular weight of more than 300 kDa from all the cultures had a close content of D-galacturonic acid residues (75-84%), but the ratio of galactose:arabinose was different: 1:1.2; 1:1.4 and 1:1.5 for LMC>300, TVC>300 and SVC>300, respectively. The content of galactose and arabinose residues was similar in the pectins of campion and tansy, whereas the duckweed pectin had high content of these monosaccharide residues. The sum of neutral monosaccharide residues in LMC>300 (33%) was higher than in SVC>300 (6%) and TVC>300 (5%). The protein content of the pectin fractions was 3.0% (SVC>300), 4.5% (TVC>300) and 7.2% (LMC>300)

Spherical HPGPs are formed as a result of gelling, in which intermolecular cross-links arise between divalent calcium ions and negatively charged carboxyl groups of pectic macromolecules (Fig. 2) and, probably, with HA molecules.[19,20]

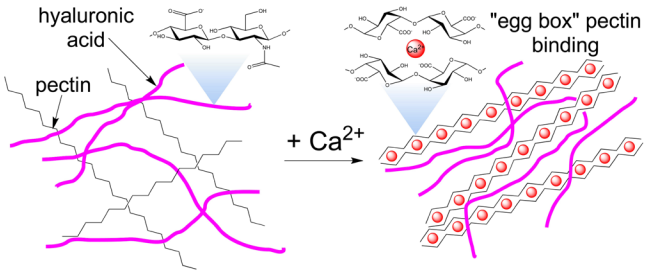

Fig. 2. *Illustration of Ca^{2+}-induced gel formation of pectic molecules in the presence of HA.*

Previously, we studied the gelation of HA with various pectins in the presence of calcium chloride at different concentrations and tested the gelation of pectin concentrations from 0.1% to 5.0%, HA from 0.01% to 2.0%, and $CaCl_2$ from 0.1 to 2.0%. All pectins and HA used in the work had a molecular weight above 300 kD. It was found that the most effective gelation occurred at the pectin concentrations of 3% or 5%, HA concentration of 0.05%, and $CaCl_2$ concentration of 2.0%.

The formation and morphology of gel particles can be influenced by various parameters. Earlier it was shown that the concentration of HA 1g/l is minimal for obtaining hyaluronic gel particles, and the concentration of HA 6 g/l leads to solutions that are too viscous. Between these boundaries, the concentration of HA does not have a significant effect on the morphology of gel particles. They have a spherical shape with a smooth surface. Gel particles obtained from pectic polysaccharides have a spherical shape. We have established that all tested hyaluronan-pectic complexes also form spherical gel particles.

The morphological characteristics of HPGPs obtained by us are presented in Table 1. The diameters of dry HPGPs obtained on the basis of pectins AP, LMC, SVC and TVC were 1.35±0.11 mm, 0.98±0.03 mm, 0.93±0.04 mm and 0.83±0.04 mm, respectively. Thus, the largest gel particles were formed in the variant with apple pectin. Similar patterns were also observed with respect to surface area and volume of HPGPs. Other researchers have obtained hyaluronic gel particles with a diameter from 8.8 μm to 28.1 μm.[22] S. Lim and colleagues[21] obtained gel particles from HA, from chitosan and from a complex of HA with chitosan with average dimensions of 19.91±1.57 μm, 29.47±3.58 μm and 28.60±1.34 μm, respectively ($P<0.05$). That is, HPGPs we have obtained are larger than the particles from HA, from chitosan or from the complex of HA with chitosan.

Table 1.

Morphological characteristics of dry HPGPs

Gel particles	Diameter, mm	Area surface, mm^2	Volume, mm^3	Density, mg/mm^3
HA + AP (1)	1.35±0.11	5.80±0.91	1.32±0.31	0.48±0.13
HA + LMC (2)	0.98±0.03	3.04±0.16	0.50±0.04	0.92±0.07
HA + SVC (3)	0.93±0.04	2.67±0.21	0.42±0.05	0.83±0.09
HA + TVC (4)	0.83±0.04	2.18±0.21	0.30±0.04	1.66±0.28
Statistics	F=1273.0453 P=0.0000 P_{1-2}=0.0000 P_{1-3}=0.0000 P_{1-4}=0.0000 P_{2-3}=0.0000 P_{2-4}=0.0000 P_{3-4}=0.0000	F=1119.5633 P=0.0000 P_{1-2}=0.0000 P_{1-3}=0.0000 P_{1-4}=0.0000 P_{2-3}=0.0000 P_{2-4}=0.0000 P_{3-4}=0.0000	F=845.9725 P=0.0000 P_{1-2}=0.0000 P_{1-3}=0.0000 P_{1-4}=0.0000 P_{2-3}=0.0024 P_{2-4}=0.0000 P_{3-4}=0.0000	F=908.9258 P=0.0000 P_{1-2}=0.0000 P_{1-3}=0.0000 P_{1-4}=0.0000 P_{2-3}=0.0007 P_{2-4}=0.0000 P_{3-4}=0.0000

With respect to the density of HPGPs, an opposite pattern was observed. The highest density was found in HPGPs prepared on the basis of the tansy pectin TVC, and the lowest density was found for the gel particles obtained on the basis of apple pectin AP .

In sum, the largest HPGPs were formed from HA and apple pectin AP, and the densest gel particles were from HA and pectin of callus culture of tansy TVC.The swelling and degradation of pectic particles and HPGPs under conditions of an artificial environment of GIT have been studied. Gel particles derived from pectins (control) and pectins in combination with HA (experiment) were characterized by the degree of swelling of the gel (SD). The gel particles based on apple pectin were completely degraded in the SIF medium (control and experiment). Particles from the duckweed pectin gel were degraded in the SIF+P incubation medium in the control, whereas in the experiment they were degraded in combination with HA only in the SIF+P medium after 30 minutes. The particles of hyaluronan-pectin gels, obtained on the basis of pectins of callus cultures of campion and duckweed, did not differ significantly in the degree of swelling. It has been found that only particles of hyaluronan-pectic gels formed on the basis of the pectin of the tansy callus culture are less prone to biodegradation and have the greatest resistance in the artificial GIT as compared to other tested pectins; they do not completely break down even after 24 hours of incubation in the SIF+P medium (Fig. 3).

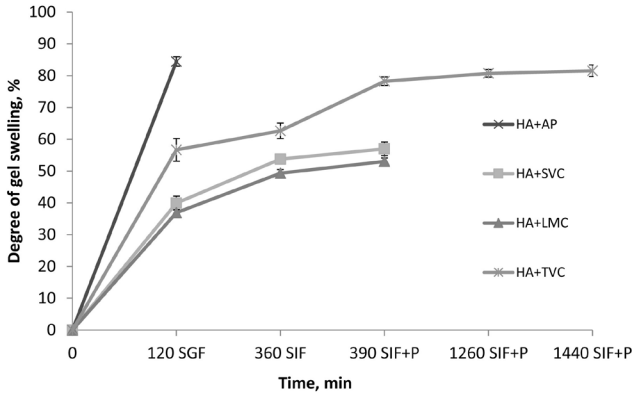

Fig. 3. *Swelling and degradation of HPGPs under conditions of the artificial environment of GIT.*

M. Fatnassi and colleagues showed that the hyaluronic gel particles they studied during incubation in the Tris buffer (pH 7.4) were completely degraded after 22 hours of incubation.

HPGPs formed on the basis of tanacetan—tansy callus culture pectin—have fairly high stability under conditions of the artificial environment of GIT. It can be assumed that the differences in the stability of HPGPs formed from different pectins are associated with differences in molecular sizes and in the fine structure of pectic macromolecules.[1,2]

The obtained data may indicate the prospect of further studies of the properties of hyaluronan-pectic gels, the microparticles of which can be tested as directed drug delivery systems in the small and large intestine.

Competing interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 7(4) (2017) 315-318
http://dx.doi.org/10.21103/Article7(4)_OA9

ORIGINAL ARTICLE

Epidemiology/Population

INTERNATIONAL JOURNAL OF BIOMEDICINE

Methodical Approaches to the Comprehensive Assessment of Environmental Risks on the Health of the Population of the Megacity

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Abstract

This article considers methodological approaches to a comprehensive assessment of the impact of environmental factors on the health of a megacity population for the purpose of making targeted management decisions. We propose a methodology for constructing a Risk Assessment Map (RAM) in tabular and graphical (using GIS technologies) variants and consider using data from the ecological and hygienic monitoring. The stage of risk mapping consists of systematization and generalization of data on emissions of leading industrial enterprises, power engineering and motor transport objects, taking into account the main meteorological conditions and objective characteristics of the impact of pollution of environmental objects on the health status of the population in various areas of the megacity. By the method of expert assessments, we suggest assigning ranks for each criterion of the risk factor being evaluated, followed by the derivation of the general rank of the risk probability. **(International Journal of Biomedicine. 2017;7(4):315-318.)**

Key Words: risk • environmental factors • public health • risk mapping • risk assessment • risk probability • risk impact

Introduction

One of the most characteristic features of the modern development of civilization is urbanization. The concentration of a large number of industries, vehicles, buildings and people in the limited territory of a modern city creates an urbanized residential environment that is significantly different in quality from the natural human habitat. [1,2]

The formation of anthropogenic load in urban areas is characterized by considerable variability in exposure to the multisided and total effects of chemical elements. In the hierarchy of factors in the urbanized environment that create environmental risks for public health, the leading place belongs to air pollution by harmful chemicals from various sources. In addition to harmful emissions from stationary sources, it is necessary to take into account the significant contribution of motor vehicles to the contamination of the surface layer of the atmosphere. More than 200 particularly toxic substances (sulfur dioxide (SO_2), nitrogen dioxide (NO_2), carbon monoxide (CO), aldehydes, ketones, alcohols, ethers, hydrocarbons, heavy metals) have been detected in the exhaust gases from automotive vehicles. In summer, the toxicity of air increases due to photochemical reactions, with a sharp increase in the concentrations of NO_2, ozone, aldehydes and organic peroxides.[3,4] In addition to automobile exhaust, chemical, fine dust, and aerosol contaminants contribute to increasing pollution due to deterioration of the road surface, tires, and brake pads, and the leakage of motor oils and process fluids.[5]

The rapid growth of the car fleet in megacities has led to an increase of more than 50% in the amount of harmful impurities in the air and an increase in noise levels on urban highways by 5-10 dB.[6] One of the negative characteristics of transport noise is its spread over vast areas and almost constant impact throughout the day. The problem of the harmful effects of environmental factors on the health of the population is becoming increasingly important every year. Risk is the probability and severity of an adverse effect/event occurring to people or the environment following exposure, under defined conditions, to a risk source(s). [The probability of adverse effects caused under specified circumstances by an agent in an organism, a population or an ecological system].[7] Identifying cause-effect relationships between habitat risks and pathological changes in the health status of a population or an individual is one of the tasks of hygienic diagnosis. A key element in the methodology

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of hygienic diagnosis is the analysis and assessment of the risk of adverse environmental factors for public health.[8]

In the methodology of risk analysis, there are two main, interrelated, but fundamentally different components:

1) Risk assessment for human health as a medical, biological and hygienic task. The four basic steps in the risk assessment process as defined by the NAS are:

Hazard identification — characterization of innate adverse toxic effects of agents.

Dose-response assessment — characterization of the relation between doses and incidences of adverse effects in exposed populations.

Exposure assessment — measurement or estimation of the intensity, frequency, and duration of human exposures to agents.

Risk characterization — estimation of the incidence of health effects under the various conditions of human exposure. [9]

2) Risk management as a complex social, economic and political task.

All the data obtained after the risk analysis are transferred to the organizations responsible for risk management to develop targeted management solutions aimed at preventing or minimizing the impact of the risk on the health of the exposed population.

During the implementation of the first stage, the goals and objectives of the planned studies should be clearly formulated; then it is necessary to identify the risks existing in the study area. One of the effective and visual tools of such work is the development of Risk Assessment Map (RAM).

Stage of risk mapping

In order to prioritize activities aimed at minimizing environmental risks in large urban agglomerations, it is necessary to identify all major sources of environmental pollution, including sources in the adjacent areas, due to the possibility of spatial distribution of the pollution, and to determine the number of exposed populations.

The following is the structure of environmental risks for the health of the megacity population:

- Chemical factors (the pollution of atmospheric air, drinking water, soil, food products by xenobiotics)
- Physical factors (noise, vibration, electromagnetic radiation, radiation)
- Biological factors (bacteria, viruses, fungi, rickettsia)
- Social factors (working and living conditions)

In the course of ongoing research and expert work at the stage of hazard identification, the composition of all potentially hazardous environmental factors that could have a negative impact on the health of the population is determined. Then a list of priority (most dangerous) factors is formed. Identification of factors involves identifying the most significant qualitative and quantitative characteristics:

- Place of risk factor occurrence
- Source of risk factor occurrence
- Emission (emission volume, parameters necessary for calculating the maximum one-time and average annual concentrations)
- Points of influence (primarily polluted environment, transport media, accumulating or transforming chemicals)
- Level of interrelations between factors (synergism, antagonism)
- Critical organs and systems of the human body that are affected by the risk factor
- Probability of risk factor occurrence (acceptable, unacceptable risk)
- Effect of exposure to a risk factor (carcinogenic risk, non-carcinogenic risk)

The leading criteria for the selection of priority (indicator) pollutants are: (1) their toxic properties and the amount of a substance entering the environment; (2) high persistence of a substance in an environmental object; (3) ability for bioaccumulation and inter-environment migration, which causes simultaneous contamination of several environmental objects and spatial distribution of the pollution; and (4) the ability to cause harmful effects in the human body (irreversible, remote, having high medical and social significance). At this stage, the potentially affected population size is determined and characterized.

Once the list of priority factors is formed, it is advisable to develop a conceptual model of the study territory to set the goal and tasks of assessing the risk of environmental factors for the health of the exposed population. It can be a graphic or descriptive representation of the possible interrelationships between the exposed population groups, sources of environmental pollution, and routes of exposure to pollutants (initially polluted environments; the environments transporting, storing or transforming chemicals; the pathways of possible entry of potentially hazardous chemicals into the human body from contaminated media).

It is necessary to pay attention to the temporal focus of research. The temporal focus of research on risk assessment can be retrospective, current and prospective. If the research is aimed at assessing the health risk of a population caused by a particular object, for example an industrial enterprise, the most important source is information about the qualitative and quantitative composition of the emissions of this object, their spatial and temporal characteristics. In addition to stationary sources of emissions, the influence of vehicles on the contamination of the surface layer of the atmosphere of the study area is also taken into account. In this case, retrospective studies will include an assessment of the risk caused by the previous impacts of chemicals contained in emissions from industrial facilities polluting the environment in the region. The current risk assessment will be related to the chemical contamination of environmental objects at the time of the study. A prospective risk assessment will determine the levels of risk over a certain time period under a specific scenario of chemical contamination of environmental objects.

The following methodology is proposed for constructing RAM of the impact of the megacity environment on the health of the population. Based on the data obtained, an RAM is formed. An RAM can be constructed both in tabular and in graphical form. To build a tabular map, all the risk factors selected for further investigation are summarized in a single table (Table 1). Risk factors are placed on the

lines, and their quantitative and qualitative characteristics are indicated in the columns (i.e. a source of occurrence, possible effect of exposure, probability of manifestation of the effect, degree of danger, etc.), and medical-demographic indicators (morbidity, mortality) among exposed population. The number of characteristics given is not constant; in each case there should be an individual approach depending on the features of the study area, the nature of the environmental risk factors (chemical, physical, biological, social), their sources, emissions, the number of the potentially exposed population, and the causal relationship between the exposure level of the risk factor and the number of cases or the severity of adverse effects that have occurred in the studied population.

For systematization and generalization of data on emissions of leading industrial enterprises, power engineering, and motor transport objects, we use the data of ecological and hygienic monitoring, taking into account the main meteorological conditions and objective characteristics of the effect of environmental object pollutions on the health of the population in different areas of the megacity.

The number of qualitative and quantitative variables and indicators is determined by a group of researchers and experts. Then, by the method of expert assessments, we suggest assigning ranks for each criterion of the risk factor being evaluated, followed by the derivation of the general rank of the risk probability. Based on the obtained ranks, RAM of the impact of the megacity environment on the health of the population is formed (Table 2).

The map is filled out by putting a value of "general rank" in the cells corresponding to a certain level of the risk probability. We recommend the construction or correction the RAM at least once a year.

Based on the RAM, management decisions are made:

1. Risks that are in the first columns of probability and danger (unlikely and acceptable) are controlled. In accordance with these risks, it is necessary to develop a plan of measures to prevent the transition into the category of permissible or dangerous.

2. Risks located in the second columns (likely and permissible) require systematic monitoring and development of the annual action plan.

3. For the risks in the third columns (very likely and dangerous), a plan of immediate (priority) measures should be developed to minimize their impact on the environment and the health of the exposed population.

RAM of the impact of the megacity environment on the health of the population can be represented in graphical form. To do this, when evaluating analytical data, it is recommended to use GIS technologies to visually display the location of potential pollution sources and sampling points relative to residential areas. This will allow to determine the degree of representativeness of the potential exposure of the studied risk factors to the population and to justify the extrapolation of the obtained data to the entire study area.

The methodology for constructing RAM of the impact of the megacity environment on the health of the population using GIS technologies consists of the following stages:

- Selection of the licensed GIS software for building RAM;
- Putting the results of calculating risks on the GIS platform and building RAM.

Conceptually, RAM can be presented in the form of an "Atlas of ecological and sanitary-epidemiological risks on the health of the population of the megalopolis."

Table 1.

Risk factors selected for constructing RAM of the impact of the megacity environment on the health of the population

Risk factor (RF)	Route of impact					Exposed population (people)	Critical organs and systems	Health effects corresponding to various hazard categories	Carcinogenic hazard index (for carcinogens)	Non-carcinogenic hazard index (for non-carcinogens)*	Reference levels for acute and chronic effects **
	Source of RF	Level of RF emission	Perceiving environment (the object of the environment)	Impact point (the place of potential human contact with RF)	Ways of RF entering into the human body						
1	2	3	4	5	6	7	8	9	10	11	12

* risk of development of non-carcinogenic effects is carried out on the basis of hazard ratio calculation;
** in contrast to the MPC, are recommendatory criteria and are used exclusively for the purpose of assessing the possible impact of chemicals on public health

Table 2.

RAM of the impact of the megacity environment on the health of the population

Risk factor RF	Probability of occurrence of RF			Hazard of RF		
	unlikely	likely	very likely	acceptable	permissible	dangerous

Thus, the development and application of RAM of the impact of the megacity environment on the health of the population helps to better evaluate the medical and environmental situation in the megacity, determine the mutual influence of environmental factors, and assess the population health risks.

Forecasting the hygienic and epidemiological situation with the assessment of population health risks during the development of town planning decisions contributes to the selection of optimal management solutions aimed at optimizing the environment and minimizing the impact of the risk on the health of the exposed population.

Competing interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 7(4) (2017) 319-323
http://dx.doi.org/10.21103/Article7(4)_OA10

ORIGINAL ARTICLE

Epidemiology/Population

INTERNATIONAL JOURNAL OF BIOMEDICINE

Prevalence of Overweight, Obesity and Abdominal Obesity among the Adult Population of Yakutia

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Abstract

This study describes data obtained as a result of a one-stage epidemiological study for levels of CVD risk factors among indigenous and non-indigenous populations of the Republic of Sakha (Yakutia) (RS(Y)).

A total of 1,856 indigenous residents (Yakuts) and non-indigenous residents (Russians) were examined: 512 men (mean age, 47.5±15.1) and 1,344 women (mean age, 48.1±14.2). Among the surveyed adult population of Yakutia, the average values of BMI, regardless of ethnicity, were high with a regular increase in this indicator with age, especially in women. Overweight is more common for indigenous men (40%) compared with indigenous women (34.5%). Among the non-indigenous residents, there were no gender differences. In the sample of the indigenous population, obesity was more common in women (24.3%) than in men (18.7%). Among non-indigenous residents, similar differences were not obtained, except for a higher incidence of obesity in older women. The average waist circumference in men and women of both ethnic groups is not high, but the indicators are higher for men than for women; the gender differences are leveled in the older age group in both ethnic groups. Prevalence of abdominal obesity (AO) is extremely high in indigenous residents (61.6%); in both ethnic groups, the prevalence of AO is higher among women than men. The incidence of overweight, obesity and AO was significantly higher in Yakut people.(**International Journal of Biomedicine. 2017;7(4):319-323.)**

Key Words: overweight • obesity • abdominal obesity • metabolic syndrome

Abbreviations

AO, abdominal obesity; **BW**, body weight; **BMI**, body mass index; **CVD**, cardiovascular diseases; **HC**, hip circumference; **MS**, metabolic syndrome; **WC**, waist circumference.

Introduction

Throughout the world, especially in developed countries, obesity, having a non-infectious pandemic character, has become a major health problem. According to the WHO 39% of adults aged 18 years and over were overweight in 2016, and 13% were obese.[1] The proportion of obese men and women in Europe ranges from 10% to 20% and from 15% to 25%, respectively. In Russia, more than 60% of the adult population suffer from being overweight and about 26% from obesity. Life expectancy in morbid obesity is reduced by 9 years for women and by 12 years for men.[1,2]

An increase in BMI leads to an increase in the risk of developing concomitant diseases. The risk of complications, especially cardiovascular and metabolic, depends not only on the degree of obesity, but also on its type (localization of fatty deposits). The most unfavorable to health and most common for men is AO, when fat is deposited between internal organs in the waist region.[3]

The role of overweight and obesity in the development of CVD is not clearly established at this time. In studies, different criteria for obesity are often used, and therefore reports of CVD and obesity are contradictory.[3] At the same time, obesity increases the development and progression of such conditions as hypertension, diabetes mellitus, dyslipidemia, and MS. In

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addition, obesity is an easily determined marker of risk.

For all the variety of reasons leading to the development of the main manifestations of MS, of primary importance in its pathogenesis is irrational nutrition. Nutrition is one of the main factors determining the health of the population; on the one hand, nutrition can act as a disease prevention tool, and on the other, as an etiological factor or risk factor for many common chronic, noncommunicable diseases.[4,5]

In the past two decades, interest in the study of the physical status of humans in the North has increased.[6-10] Numerous data confirm the presence of morphofunctional features in the indigenous people of the North that can be considered as a product of a long evolution and adaptation of humans to unfavorable conditions of existence.[8,9,11]

The aim of our study was to estimate the prevalence of overweight and obesity, including AO, among the indigenous and non-indigenous residents of Yakutia.

Materials and Methods

The Republic of Sakha (Yakutia) (RS(Y)) is the largest subject of the Russian Federation. About 40% of the territory of Yakutia lies above the Arctic Circle. Limiting factors for the human body in the extreme conditions of Yakutia are both climatic and man-made.[12] The study was carried out within the framework of the project "Multifactor study of the health status of the indigenous and non-indigenous populations of RS(Y) aimed to optimize the regional programs for improving the quality of life of the inhabitants of the republic, taking into account the territorial and ethnic characteristics in the context of modern socioeconomic development" (State Project No. 6512 of September 6, 2017). The study protocol was reviewed and approved by the Ethics Committee of North-Eastern Federal University named after MK Ammosov. All participants provided the written informed consent.

A total of 1,856 indigenous residents (Yakuts) and non-indigenous residents (Russians) were examined: 512 men (mean age, 47.5±15.1) and 1,344 women (mean age, 48.1±14.2). The surveyed respondents were divided into 2 age groups: Group 1, between 18 and 59 years, and Group 2, over 60 years.

The survey program included a comprehensive exam with a developed map questionnaire. The questionnaire consisted of several sections: socio-demographic, anamnestic data, heredity, physical activity, bad habits, and anthropometry.

The methods of basic screening and facultative studies were standardized and carried out in accordance with the recommendations adopted for epidemiological studies. The length of the body was measured with a digital anthropometry device, Tanita, and a digital anthropometry device, Martin, during the expedition surveys, with an accuracy of 0.1cm. BW was measured without clothing using medical scales with an accuracy of 50 g. WC and HC were measured with a centimeter tape with an accuracy of 0.1 cm. BMI is calculated using Quetelet's formula:

$BMI = \text{body weight(kg)}/\text{height(cm)}^2$

BMI value between 25 kg/m^2 and 30 kg/m^2 was assessed as overweight, BMI value above 30 kg/m^2 was assessed as obesity.

WC was measured in the smallest circle, below the rib cage and above the navel; HC was measured at the buttock level, where there is the greatest circumference. According to the NCEP ATP III definition (2001), AO is defined as WC > 102 cm for men and >88 cm for women.

*S*tatistical analysis was performed using statistical software package SPSS v. 19.0. Baseline characteristics were summarized as frequencies and percentages for categorical variables and as means and SDs for continuous variables. Analysis of the distribution of values obtained was performed using the Kolmogorov-Smirnov test. For data with normal distribution, inter-group comparisons were performed using Student's t-test. The Mann-Whitney U Test was used to compare the differences between the two independent groups (for nonparametric data). Categorical variables were analyzed using the Chi-square test. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

The obtained data from anthropometric studies are presented in Table 1. The average BMI among the indigenous residents of Yakutia was high and amounted to 26.7±5.13 kg/m^2. With age, this indicator increased in Group 2 for women. In men, such dynamics were not observed.

The average WC in indigenous residents of Yakutia was 88.4±13.6 cm. This indicator was higher in men (91.8±12 82 cm) than in women (87.2±13.65 cm) with statistical significance in Group 1 (Table 1).

Table 1.

Anthropometric parameters of indigenous residents of Yakutia

Group	Total	Men	Women	P
BMI (kg/m^2)				
Total	26.7±5.13	26.5±4.61	26.8±5.31	0.502
Group 1	26.6±5.17	26.5±4.71	26.6±5.32	0.967
Group 2	27.2±4.98	26.6±4.32	27.4±5.21	0.08
P_{1-2}	<0.01	0.545	<0.01	
WC (cm)				
Total	88.4±13.58	91.8±12.82	87.2±13.65	<0.001
Group 1	87.5±13.40	91.6±13.33	86.1±13.12	<0.001
Group 2	91.5±13.72	92.5±11.34	90.9±14.55	0.214
P_{1-2}	<0.001	0.194	<0.001	

In indigenous residents, 36% of cases were found to be overweight and 22.9% were obese (Table 2). We identified the gender differences in the prevalence of overweight and obesity. In males, the frequency of overweight was higher than in women (40% vs. 34.5%, respectively), but obesity, on the contrary, was more common for women (24.3%) than for men (18.7%). These differences were more pronounced in Group 2. With age, an increase in the frequency of overweight was found only for men; obesity did not differ significantly

in men and women (Table 2). Similar data were obtained by T.Klimova and colleagues[6] for the indigenous rural population of Yakutia. According to the BMI categories, they found low BW in 2% of men and women; normal BW in 44% of men and 38% of women; pre-obesity in 37% and 34%, respectively; and obesity in 18% and 26%, respectively.

Table 2.

Prevalence of overweight and obesity (by BMI) and AO among the indigenous population of Yakutia

Group	Total	Men	Women	P
Overweight				
Total	36.0	40.0	34.5	<0.05
Group 1	34.5	37.0	33.6	0.246
Group 2	41.2	49.6	38.0	<0.05
P_{1-2}	<0.05	<0.05	0.164	
Obesity				
Total	22.9	18.7	24.3	<0.05
Group 1	22.5	20.2	23.4	0.211
Group 2	24.0	14.2	27.8	<0.01
P_{1-2}	0.533	0.154	0.123	
AO				
Total	61.6	42.0	68.5	<0.001
Group 1	58.8	39.9	65.5	<0.001
Group 2	70.8	48.5	78.8	<0.001
P_{1-2}	<0.001	0.130	<0.001	

The prevalence of AO among indigenous residents of Yakutia was extremely high (61.6%): 42% for men and 68.5% for women.(Table 2) A significant excess of AO in women compared with men persisted in the analysis by age group. In women, the increase in the frequency of AO with age was more pronounced than in men (79% among women of Group 2), which influenced the high incidence of AO in the general population. According to T.Klimova et al., the prevalence of AO (by IDF criteria) among the rural indigenous population of Yakutia was also high: 34% for men and 62% for women.[6]

The results of studies in the North that evaluate the fat component of the body, provide the distinctive anthropometric features in indigenous inhabitants: 1) Distribution of fat is almost the same for all age groups with the greatest manifestation in the abdomen, chest, back and shoulder, regardless of age; 2) The average number of skin fat folds of the shoulder (the front and back surfaces), forearm, abdomen, hip, and lower leg was significantly higher in middle-aged men than in older men; 3) The age-related changes in BW of women are associated with the development of the predominantly fat component in the abdomen, with pronounced age differences.[6-9] The results of the research show that the indigenous northern populations, in the process of adaptation to extreme climatic conditions, developed the specific features of their constitution (high body density, stocky physique with well-developed musculoskeletal mass), which were aimed to slow heat transfer. The rural population of Yakutia is characterized by typical features inherent in the northern adaptive type—small body length with relatively large mass, WC and HC, and a favorable lipid spectrum.[6]

It has been established that the indigenous population of the Far North is characterized by a protein-lipid type of nutrition, which contributes to the formation of the "polar metabolic type." [13-15] In recent years, the change in the lifestyle of the indigenous people has led to a change in the traditional diet. A few studies on the features of the actual diet of the Yakut population revealed that the diet of the elderly is unbalanced in fat and carbohydrate components: a high proportion of total fat intake, including saturated fat. A high content of refined sugars was noted against a background of a low intake of complex carbohydrates, dietary fiber, both in the Yakut and Caucasian ethnic groups.[16] Thus, an analysis of the current trends in nutrition of people 60 years of age and over in Yakutia, with an assessment of the impact of these dietary habits in the formation of overweight, obesity, and AO indicates a high prevalence of these factors in the population.

In the non-indigenous population of Yakutia, mean BMI was not significantly different between males and females of Group 1 (Table 3).

Table 3.

Anthropometric parameters of non-indigenous residents of Yakutia

Group	Total	Men	Women	P
BMI (kg/m^2)				
Total	25.4±5.1	25.47±4.1	25.46±5.5	0.963
Group 1	25.1±4.8	25.38±4.2	24.93±5.2	0.236
Group 2	29.7±5.6	26.72±2.9	30.61±6.0	<0.01
P_{1-2}	<0.001	0.182	<0.001	
WC (cm)				
Total	81.6±13.7	86.18±12.7	79.28±13.6	<0.001
Group 1	80.4±13.0	85.74±12.7	77.69±12.4	<0.001
Group 2	94.3±14.3	92.67±11.2	94.83±15.3	0.583
P_{1-2}	<0.001	<0.05	<0.001	

In Group 2, the mean BMI was significantly higher in females than in males, as in the indigenous population of Yakutia; however, these differences were leveled in the older age group (Table 3). With age, both in the group of men and women, the mean values of WC increased.

In the non-indigenous population, 31.1% of cases were found to be overweight and 16.2% were obese (Table 4). We found gender- and age-related differences in the prevalence of obesity. In Group 2, obesity was found in 47.2% of non-indigenous women and in 16.7% non-indigenous men ($P<0.05$). The incidence of AO was significantly higher in women than in men, regardless of age.

A comparative analysis of the BMI values revealed a high prevalence of overweight and obesity among the

indigenous residents of Yakutia. The prevalence of AO among these residents was also about 2 times higher than in non-indigenous residents (Table 5).

Table 4.

Prevalence of overweight and obesity (by BMI) and AO among the non- indigenous population of Yakutia

Group	Total	Men	Women	P
Overweight				
Total	31.1	35.1	29.1	0.076
Group 1	30.1	33.7	28.2	0.115
Group 2	42.3	55.6	37.7	0.186
P_{1-2}	<0.05	0.060	0.147	
Obesity				
Total	16.2	13.8	17.4	0.187
Group 1	14.1	13.6	14.3	0.797
Group 2	39.4	16.7	47.2	<0.05
P_{1-2}	<0.001	0.719	<0.001	
AO				
Total	36.7	24.1	43.0	<0.001
Group 1	32.9	22.7	38.1	<0.001
Group 2	78.9	44.4	90.6	<0.001
P_{1-2}	<0.001	<0.05	<0.001	

Table 5.

Prevalence of overweight and obesity (by BMI) and AO among the indigenous and non- indigenous populations of Yakutia

Variable	Total	Indigenous	Non-indigenous	P
Overweight	34.6	36.0	31.7	<0.05
Obesity	20.7	22.9	16.4	<0.001
AO	53.0	61.6	37.4	<0.001

Thus, the high level of metabolic risk factors (high prevalence of overweight, obesity and abdominal obesity) in Yakuts suggests the cause lies in the dietary imbalance [16] and allows predicting negative trends in the development of metabolic disorders with an increase in the prevalence of MS.

Conclusion

1. Among the surveyed adult population of Yakutia, the average values of BMI, regardless of ethnicity, were high with a regular increase in this indicator with age, especially in women.

2. Overweight is more common for indigenous men (40%) compared with indigenous women (34.5%). Among the non-indigenous residents, there were no gender differences. In the sample of the indigenous population, obesity was more common in women (24.3%) than in men (18.7%). Among non-indigenous residents, similar differences were not obtained, except for a higher incidence of obesity in older women.

3. The average WC in men and women of both ethnic groups is not high, but the indicators are higher for men than for women; the gender differences are leveled in the older age group in both ethnic groups.

4. Prevalence of AO is extremely high in indigenous residents (61.6%); in both ethnic groups, the prevalence of AO is higher among women than men.

5. The incidence of overweight, obesity and AO was significantly higher in Yakut people.

Competing interests

The authors declare that they have no competing interests.

Sources of Funding

The study was carried out within the framework of the project "Multifactor study of the health status of the indigenous and non-indigenous populations of RS(Y) aimed to optimize the regional programs for improving the quality of life of the inhabitants of the republic, taking into account the territorial and ethnic characteristics in the context of modern socioeconomic development" (State Project No. 6512 of September 6, 2017).

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International Journal of Biomedicine 7(4) (2017) 324-326
http://dx.doi.org/10.21103/Article7(4)_CR1

CASE REPORT

INTERNATIONAL JOURNAL OF BIOMEDICINE

A Rare Case of Charcot-Mari-Tooth Disease Type 2S in a 20-year-old Man

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Abstract

Charcot-Marie-Tooth disease type 2 (CMT2S) is rare form of Charcot-Marie-Tooth disease (CMT) that is characterized by a mutation in the *IGHMBP2* gene. This gene encodes a helicase superfamily member that binds a specific DNA sequence from the region of the immunoglobulin mu chain switch. Mutation of this gene leads to spinal muscle atrophy with respiratory distress type 1 and CMT2S. This case report presents a 20-year-old male with genetically confirmed CMT2S having clinical respiratory involvement and symmetrically involved lower extremities. DNA sequencing revealed a previously unknown heterozygous mutation in the exone 2 of the *IGHMBP2* gene leading to the replacement of the amino acid in the 46 position of the protein (chr11q13.3: 68673587 G>C). These atypical features widen the clinical spectrum of CMT2S. This is the first described case of a previously unknown mutation in the Russian population with confirmation of its genetic study. In describing this clinical case, we also improve diagnostic management and try to increase the alertness of various doctors towards neuromuscular diseases, including CMT. **(International Journal of Biomedicine. 2017;7(4):324-326.)**

Key Words: Charcot-Marie-Tooth disease • hereditary neuropathy • chromosome 11q13.3 • heterozygous mutation

Abbreviations

CMT, Charcot-Marie-Tooth disease; **CMT2,** CMT type 2; **CMT2S**, CMT type 2S; **DNA**, deoxyribonucleic acid; **EMG**, electromyography; **HSMN2S**, hereditary sensory and motor neuropathy type 2S; **NCV**, nerve conduction velocity; **IGHMBP2**, immunoglobulin mu binding protein 2.

Introduction

Hereditary sensory and motor neuropathy (HSMN) is a group of common neuromuscular disorders with heterogeneous clinical presentations and genetic causes. Detailed neuromuscular evaluations, including nerve conduction studies, laboratory testing, and histopathologic examination, can assist in identification of the inherited component beyond family history. Neurophysiologic studies, including needle EMG, are very useful for distinguishing acquired from inherited mechanisms. Chronic motor unit potential changes on a needle EMG, characterized by amplitude >2 mV and duration >15–20 msec, can help establish the chronic nature of the inherited neuropathy.[1] However, genetic testing increasingly enables definitive diagnosis of a rare form of HSMN.

CMT is genetic heterogeneous form of HSMN. CMT2 is an axonal (non-demyelinnating) HSMN characterized by distal muscle weakness and atrophy. Nerve conduction velocities are usually within the normal range. However, occasionally they fall in the low-normal or mildly abnormal range (35-48 m/sec), where peripheral nerves are not enlarged or hypertrophic. CMT2 shows extensive clinical overlap with CMT1. However, in general, individuals with CMT2 tend to be less disabled and have less sensory loss than individuals with CMT1. A threshold of 38 m/sec for *n. medianus* conduction is often used clinically to distinguish CMT1 from CMT2.[2]

Molecular genetic testing is possible for pathogenic variants in numerous genes associated with CMT2 phenotypes.

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An alternative genetic testing strategy is the use of a multi-gene panel that includes genes associated with CMT and other genes of interest.[3] Panels exist for dominantly and recessively inherited CMT as well as demyelination and axonal forms. Larger (all-inclusive) panels may also be available. Most individuals diagnosed as having autosomal dominant CMT have an affected parent, although occasionally the family history is negative. However, CMT2S is extremely rare, so the prevalence among the population is unknown.[4]

Case Report

A 20-year-old male has been observed at the University Clinic since he was 18. He first applied in June 2016 because of snoring. There was no complaint of any major illness in the past. At the first visit, on examination a large number of subcutaneous hematomas on the lower extremities attracted attention. It turned out that he works as a loader and when lifting the load with his hands bent at the elbows, the cargo often falls out of his hands. This problem is not present if he lifts the cargo with outstretched hands. He was examined by a neurologist.

Detailed evaluations

When communicating, the patient was apathetic, the mood was low, and he carried out some commands very slowly. Results of psychological tests were that he has a depressive syndrome.

We found the reduced sensation with stocking-glove distribution in the distal limbs; his feet and lower third of the shins were pale by the type of high socks, with local hypothermia 2 degrees Celsius below the temperature of the proximal parts of the limbs and trunk. There were a distal hyperhidrosis of the hands and feet, dystrophic changes in the skin and nails of both feet with a local anthrite, thinning of the skin, and minor skin lesions covered with hemorrhagic crusts, mainly on the back of the feet and the front surface of the lower third of the tibia at compression sites when wearing shoes.

Cranial nerves: the adjusting horizontal nystagmus with gaze to the sides, the weakness of convergence on the left and the dysarthria of the 1st degree.

The motor sphere: the hypotrophy and hypotonia of the muscles of both hands, especially *m. hypotenari*. A decrease in their strength to 3-4 points. Bicepital reflexes were moderate on both sides, carporadial reflexes were low. There was moderate hypotension and hypotrophy of the muscles of the feet and the lower third of the shins, and clew toes of both feet. Knee reflexes were moderate, Achilles reflexes low (less on the right). Standing on the toes was possible, but without vision control there was a risk of falls. Standing on heels was difficult with the risk of falling and with the formation of postures to compensate balance. Execution of the heel-knee test was difficult due to a sensitive ataxia; it was performed by the patient slowly with mild bilateral dysmetry. In the Romberg position he was stable; without vision control, there was a disturbance of balance. Violations of pain sensitivity were not found.

Hereditary anamnesis

There were no manifestations of this disease in the first- or second-degree relatives.

Laboratory testing

Biochemical analysis: the level of creatine kinase and lactate dehydrogenase was normal.

EMG/NCV tests: low speed of motor conduction on *n. medianus* 29.8 m/sec and *n. peroneus* 43.8 m/sec and decrease of M-response *n. medianus* 2.22 (-75.3%), *n. peroneus* 2.3 (-34.4%).

Esthesiometry: From the distal sections of the lower limbs (ankles), there was a significant asymmetric (more to the left) decrease in vibration sensitivity at frequencies 8, 16, 32, 63, 125, 250, and 500 Hz, with a tendency to fall out at high frequencies.

Cardiorespiratory monitoring: there are no data for sleep apnea syndrome. Podography: high arch, claw toes.

DNA sequencing data

The search for pathogenic mutations associated with hereditary neuromuscular diseases was carried out in the laboratory of molecular pathology, the GENOMED laboratory (Moscow, Russia). The search revealed a previously unknown heterozygous mutation in the exone 2 of the gene *IGHMBP2* leading to the replacement of the amino acid in the 46 position of the protein (chr11q13.3: 68673587 G>C).

Discussion

The term “CMT” includes a clinically and genetically heterogeneous group of disorders, which are the most common inherited neuromuscular disorders with an estimated prevalence of one in 2,500 individuals.[5] Not only does CMT present with a significant genetic heterogeneity but it may also segregate with different Mendelian patterns: autosomal-dominant (AD), autosomal-recessive (AR) or X-linked.[6]

Previously it was believed that the described variant of the genetic mutation in the *IGHMBP2* gene leads to the emergence of only spinal muscular atrophy with diaphragm paralysis (OMIM: 604320).[7] However, in 2014, cases of patients from England, America, Serbia, Poland, Italy, Korea, and Vietnam were first described with a mutation in the same gene leading to the clinical presentation of HSMN2S (OMIM: 616155).[8]

The young man turned to the doctor with complaints of a breathing disorder, which is typical for the clinical picture of spinal muscular atrophy with diaphragm paralysis. However, the conducted examinations showed no significant deviations from the norm. To clarify the diagnosis, DNA sequencing was recommended.

Exome sequencing techniques have non-standardized, highly variable coverage; of particular note are regions of the exome refractory to accurate sequencing by this method (including genes with pseudogene, highly repetitive coding regions, and large deletions and duplications). It is for this reason we use this method.

DNA sequencing revealed a previously unknown heterozygous mutation in the exone 2 of the gene *IGHMBP2* leading to the replacement of the amino acid in the 46 position of the protein (chr11q13.3: 68673587 G>C). Homozygous and compound heterozygous mutations in this gene are described in patients with CMT axonal form 2S type disease. In spite of

the fact that in this clinical case the mutation is heterozygous, the clinical picture corresponds to CMT2S. This type has been described quite recently; the clinical picture is not yet fully formed. Presenting this case, we are replenishing the world database. The boy was admitted to the University Clinic at the age of 18 with an extensive clinical picture. Earlier he had undergone routine medical examinations in educational institutions and in various private clinics, but none of the doctors suspected the disease. He never went for additional examinations or consultations with outside doctors. In describing this clinical case, we also improve diagnostic management and try to increase the alertness of various doctors towards neuromuscular diseases, including CMT.

Conclusion

The data from clinical and EMG/NCV tests of the patient, as well as the presence of a mutation in the heterozygous state in the *IGHMBP2* gene, revealed by the targeted sequencing, has allowed us to establish the final diagnosis: CMT2S (OMIM: 616155), heterozygous carrier of the mutation 68673587 G>C in the exon 2 of the *IGNMBP2* gene, a sporadic case first identified; distal peripheral upper paraparesis with predominant involvement of ulnar nerves and the ulnar group of muscles of both hands of 1 degree of severity, with moderately pronounced atrophy and hypotension of the hypotenor muscles (forming a "flat" hand), the initial signs of angiotrophoneuritic syndrome at the level of the hands; distal peripheral lower paraparesis in the predominant lesion of the tibial nerves and the tibial muscle group, with deformity of both feet as hollow, sensory dinamostatic ataxia of 1 degree of severity and angiotrophoneurotic syndrome at the level of the feet and lower third of the tibia with trophic changes in the skin and its derivatives, cornflowers on the rear of the foot.

While visiting a neurogeneticist, the patient received explanations about the disease, prognosis of life, ability to work, about the methods of habilitation and way of life. With the help of psycho-correction techniques, the depressive syndrome regressed. At the time of the last admission, the young man was going to college to become an electrician. He plans to enter the university, marry and take care of his health. This is the first described case of a previously unknown mutation in the Russian population with confirmation of its genetic study.

Competing interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 7(4) (2017) 327-329
http://dx.doi.org/10.21103/Article7(4)_CR2

CASE REPORT

INTERNATIONAL JOURNAL OF BIOMEDICINE

Bactrim Induced Hemolysis and Thrombocytopenia in a Patient with Pernicious Anemia

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Abstract

This case report presents a 87-year-old female who had a history of pernicious anemia and was given Bactrim, which suppressed folic acid and caused a more profound anemia, thrombocytopenia and hemolysis. **(International Journal of Biomedicine. 2017;7(4):327-329.)**

Key Words: pernicious anemia • thrombocytopenia • hemolysis • Bactrim • cyanocobalamin • folic acid

Abbreviations

AML, acute myelogenous leukemia; **CBC**, complete blood count; **DHFR**, dihydrofolate reductase; **Hb**, hemoglobin; **Hct**, hematocrit; **IF**, intrinsic factor; **INR**, international normalized ratio; **LDH**, lactate dehydrogenase; **LFTs**, liver function tests; **MCV**, mean corpuscular volume; **MDS**, myelodysplastic syndrome; **PMH**, past medical history; **Plt**, platelets; **PABA**, para-aminobenzoic acid; **PT**, prothrombin time; **PTT**, partial thromboplastin time; **RBC**, red blood cells; **TIBC**, total iron-binding capacity; **TMP-SMX**, trimethoprim–sulfamethoxazole; **TSH**, thyroid stimulating hormone; **UTI**, urinary tract infection; **WBC**, white blood cells.

Introduction

Vitamin B_{12} deficiency anemia is common in the US with a prevalence of 5%-10% among subjects older than 60 years of age.[1] Pernicious anemia is a common cause of B_{12} deficiency with a prevalence of 0.1% in the general population and 1.9% among patients over 60 years old.[1] Causes of pernicious anemia can be diet, inadequate absorption (chronic atrophic gastritis in 90% of cases and autoimmune production of autoantibodies against IF), gastrectomy, gastritis, infection, intestinal disorder, medication toxic effect, and sometimes heredity.[2] Clinical presentation can vary and may require extensive diagnostic workup Sulfonamides can interfere with the folic acid-tetrahydrofolate synthesis pathway, which is important for purine, DNA and amino acid synthesis.[3] Drug interactions should also be considered.[3] Elderly patients are at higher risk of B_{12} deficiency anemia and treatment with sulfonamides should be given cautiously, and followed up with routine lab work including CBC and monitoring of liver and kidney function tests.[3]

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Case Report

Our patient is an 87-year-old female with PMH of untreated pernicious anemia for more than one year, cholelithiasis, dementia, and UTI (treated with Bactrim). She was admitted to the hospital due to an episode of syncope. According to the patient's family, she was mostly bedridden, had nausea for several months, poor *per os* intake, weight loss, generalized weakness and deteriorating functional status for the past several months. Family members reported that she had no overt bleeding. On admission, the patient's vitals were within the normal range with the exception of tachycardia. The physical exam illustrated a cachectic, malnourished appearance, loss of orientation, with inappropriate responses to questions and minimal communication to her family members. Laboratory results showed Hb–4.3g/dL, Hct–12.7%, RBC–1.35×10^{12}/L,

MCV–94 (80.4–95.9), and Plt of 142×10^9/L. Subsequently, the patient developed thrombocytopenia after transfusion of 3 units of pRBC. Her platelet level dropped to 8×10^9/L and WBC dropped to 3.7×10^9/L. After one unit of donor platelet transfusion, the patient had an anemia and hemolysis workup. Results supported the diagnosis for pernicious anemia with hemolysis: low level of B_{12}–88 pg/mL and folate–3.8 ng/mL, and IF antibodies. There was no evidence of iron deficiency as she had a high level of ferritin–1861 ng/mL, iron–192 ug/dL, with low levels of TIBC, haptoglobin–<20 mg/dL, while her direct anti-globulin profile was negative. TSH was in normal limits, fibrinogen level–176 mg/dL, and INR–1.29. PT was slightly elevated and PTT was in normal limits. Renal and hepatic tests were normal, except for elevated total bilirubin–2.3 mg/dL, direct bilirubin–1.4 mg/dL, and LDH–272 U/L, which revealed hemolysis. A peripheral smear did not show platelet clumping, nor schistocytes, which ruled out thrombotic thrombocytopenic purpura or hemolytic uremic syndrome. Patient had good response to platelet transfusion ×1 and to intramuscular cyanocobalamin 1000 mcg supplementation daily for 7 days. At discharge, patient's Hgb improved to 8.8 g/dL, Hct to 25.3%, WBC to 4.3×10^9/L, and Plt to 136×10^9/L. It was recommended to continue to inject cyanocobalamin intramuscularly once a week for 4 weeks, then once a month for the rest of her life and continue folate 5 mg daily by mouth. One month later on a clinic follow-up, the patient's blood profile showed: Hb–10.2 g/dL, RBC–3.39×10^{12}/L, WBC–15.4×10^9/L, and Plt–215×10^9/L.

Discussion

The risk of vitamin B_{12} deficiency and pernicious anemia increases with age while the prevalence of vitamin B_{12} deficiency is 5%-10% after 60 years of age.[1,4] Clinical presentation can vary and may require extensive diagnostic intervention. Both deficiencies of vitamin B_{12} and folate can lead to megaloblastic anemia; however, vitamin B_{12} deficiency will additionally lead to neurological damage,[2] and it may take years for symptoms of vitamin B_{12} deficiency to develop due to large body storage, while symptoms of folate deficiency will develop in 4-5 months if dietary intake of folate is diminished. Hematological presentation of Vitamin B_{12} deficiency presents with macroovalocytic anemia with elevated level of iron, indirect bilirubin, LDH, low level of haptoglobin due to ineffective erythropoiesis and failed maturation, which leads to an increase in the destruction of red blood cells in bone marrow and periphery.[5,6] Macrocytosis is not specific for vitamin B_{12} or folate deficiency. In severe cases of deficiency, pancytopenia can develop.[5] Patients with vitamin B_{12} deficiency have elevated both methylmalonic acid and homocysteine whereas patients with folic acid deficiency have only homocysteine elevated.[7]

Our patient had a 1-year history of untreated pernicious anemia. Due to a prior UTI, the patient received Bactrim treatment, which aggravated her condition due to additional folate suppression as its side effect. Bactrim interferes with the tetrahydrofolate synthesis pathway as it is a structural analog of PABA.[3,8,9,2] Sulfonamides compete with PABA to bind to dihydropteroate synthase and inhibit conversion of PABA and dihydropteroate diphosphate to dihydrofolic acid, or dihydrofolate.[1,2,3,8,9,7] Inhibiting the production of dihydrofolate intermediates interferes with the normal bacterial synthesis of folic acid.[1,2,3,8,9,7] Trimethoprim serves as a competitive DHFR inhibitor; it also inhibits the de novo synthesis of tetrahydrofolate, the biologically active form of folate. Folic acid is an essential product for DNA and amino acid synthesis in bacterial growth.[6] Trimethoprim is a weak DHFR inhibitor, and in high doses, it has been implicated in megaloblastic pancytopenia.[2,8,9] Co-administration of folinic acid can prevent or reduce the antifolate activity of TMP-SMX without affecting its antimicrobial activity.[3,10] Bactrim is metabolized by the liver (10-20%) and the rest is excreted by the kidneys. Dosing adjustments should be made for patients with any kidney impairment.[3,10] A main contraindication of trimethoprim use is megaloblastic anemia due to folate deficiency; because our patient had a history of pernicious anemia, this fact should have been taken into consideration.

S. Yeruva et al. reported that hemolysis was observed in 1.5% of vitamin B_{12} deficiency cases.[11] Hemolysis with thrombocytopenia can develop secondary to ineffective erythropoiesis from B_{12} deficiency anemia plus a recent use of Bactrim, which in turn inhibits erythropoiesis. Ineffective erythropoiesis can lead to pancytopenia, but the morphological bone marrow picture may mimic that of MDS.[12] For this reason, bone marrow aspiration/biopsy before a therapeutic trial with vitamin B_{12} is not indicated.[12] Severe vitamin B_{12} deficiency, if accompanied by folic acid deficiency, can present with transient chromosomal abnormalities.[13]

M. Wollan et al. described a pediatric patient who, due to combined deficiency of the folic acid and vitamin B_{12}, developed nonrandom del(7q), a clonal abnormality usually associated with MDS or secondary AML.[14] Treatment with both folic acid and vitamin B_{12} corrected the clinical as well as the marrow morphologic and cytogenetic.[14] Dr. Kim in his study presented 12 patients with pancytopenia that were misdiagnosed as MDS and were successfully treated with vitamin B_{12}.[15] Our patient recovered after the blood transfusion and vitamin B_{12} IV replacement. If her anemia did not resolve, the patient would require a bone marrow biopsy and malignancy workup.

Conclusion

Elderly patients are at a higher risk of B_{12} deficiency anemia, and treatment with sulfonamides should be given with accuracy and careful planning. Furthermore, each patient should be followed up with routine lab tests, such as CBC, LFTs, and kidney function monitoring. Our patient had a history of pernicious anemia and was given Bactrim, which suppressed folic acid and caused a more profound anemia, thrombocytopenia and hemolysis.

Competing interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 7(4) (2017) 330-334
http://dx.doi.org/10.21103/Article7(4)_PV1

POINT OF VIEW

INTERNATIONAL JOURNAL OF BIOMEDICINE

Mental Activity as an Attractor of Evolutionary Development of Homo Sapiens

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Abstract

We view the psyche of HS as an active distributed system, in which the emergence of new subsystem, creativity, about 50,000 years ago created conditions for a sharp jump-over to a new quality level of the system as a whole, into a new class of systems. As a result of the separation of the image of the goal (IG) from the "reactive behavior," the creation and projection of the creative product (CC) into the external environment, and the subsequent perception of CC as an objectively existing fragment of the world (with control functions relative to the subject), a new hominid need arose: to achieve parametric equilibrium with a virtual construction—a symbol. Satisfaction of this need created a primary frustration—the desire to achieve a symbolic goal that is beyond reach. We believe that the main factor in the evolutionary development (ED) of HS is the mechanism for satisfying this need by resolving frustration, accompanied by the development of technological support for the purposeful forms of HS behavior. The period of formation of the HS psyche, as a system at a new level, coincides in time and meaningful content with the 11th phase transition of the planetary evolution (Panov-Snooks), being the initial segment of the ascending part of the hyperbolic trajectory of ED. We believe that the psyche of this new representative of HS, which creates frustration constructs (virtual motivators), has become an attractor for ED. By an attractor, we mean the finite region of the inevitable convergence of phase trajectories of a complex system, the attraction of which draws into itself the set of possible trajectories of systems determined by different initial conditions. The actions of the attractor create conditions under which the future state of the system, represented by the the final state of the system, has a determinative influence on the present system. **(International Journal of Biomedicine. 2017;7(4):330-334.)**

Key Words: creativity • symbol • frustration • evolutionary development • mental activity

Abbreviations

CC, creative construct; **ED**, evolutionary development; **HS**, Homo sapiens; **IG**, image of the goal.

Basic Part

The purpose of this research was to determine the role and mechanisms of mental activity of HS in ED.

Creativity, as a specific species feature of the HS psyche, is the ability to produce prognostic hypotheses that cannot be derived directly from the initial conditions. This ability appeared in HS as a result of a genetic mutation,[1] or other (exogenous or interfering) causes,[2] about 50,000 years ago, and led to the possibility of separating the IG from targeted forms of behavior. The creative product (CC), originally the IG, was transformed into a fragment of the environment [2-4] with the functions of the control object. The emergence and complication of projective ways of depicting the CC triggered the emergence of pictorial techniques and primary cultural skills. The combination of the "image and meaning" in the CC has formed the more complex forms of abstract, symbolic thinking, inevitably accompanied by interpretations at the initial stages of the development of evaluation criteria and decisions, before the formation of the final CC, which becomes a symbol that is not subjected to significant additions and changes. Being an upper, finite segment of the pyramid consisting of primary representations, mythologems, myths, logical systems, etc., in its complete form the symbol has a finite number of characters

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(possibly coinciding with the Mueller number), correlated with a volume of random attention, with the possibility of being perceived as an integral (indivisible) object, an appeal to which actualizes the entire pyramid of evidentiary reasoning.

Empowering the symbol with controlling properties initiated the creation of attributes of the control object (symbol) consisting of an arsenal of capabilities that were inaccessible to HS (the mechanism for the appearance of mythologies, myths, cults, etc.).

We view the psyche of HS as an active distributed system consisting of functional subsystems, in which the emergence of a new subsystem, creativity, created conditions for a sharp jump-over to a new quality level of the system as a whole, with a change in functional properties and a transition into a new class of systems.

The result of the projection of CC into the external environment, the subsequent perception of it as an objectively existing fragment of the surrounding world, with managerial (in relation to the subject) functions, was the emergence of a new need for hominids: equilibrium in the parametric relationships with the virtual construct (CC) that created frustration, at the resolution of which, the isometric coincidence of the parameters with the ultimate goal (symbol) is unattainable. At the same time, homomorphic degrees of similarity, approximation to the symbol, have no quantitative restrictions. The intensity of frustration, which is maximal at the starting position, periodically decreases with the achievement of the step-like homomorphic similarities of the desired result (inverse positive connection), the achievement of temporal parametric equilibrium with the step-by-step goal determining the vector leading to a decrease in the intensity of frustration.

The complication of the information landscape as a result of including a mental product (CC) as a significant fragment of the environment has formed and developed new operational systems of the psyche: creativity, imagination, symbolic thinking, etc.

Integration into social groups (regardless of scale), formation of hierarchies, creation of boundaries around what is possible and permissible (ethics), complication of mythologems, and creation of mythological systems that determine ethics. Negentropic processes of socialization have several main goals, of which we note the following:

a) optimization of cooperative behavior to ensure vital needs (permissible and acceptable, the alimentary, defensive, sexual and other behaviors);

b) reduction of the frustration tension as a result of adaptive purposeful behavior, with vectors determined by IGs (symbols) formed by common mythologies (motives) of various degrees of complexity common to a social group.

The principal difference between these forms of behavior is that, in the behavioral algorithm of type “a”, positive feedback emerges only when the final goal (meeting the need) is achieved, eliminating the integral units of behavior (i.e., neuronal structures for ensuring purposeful behavior); in behavior “b”, positive feedback, temporarily reducing frustration tension, emerges at the stage of awareness (“insight”) and decision-making, (i.e., at the completion of the first purposeful behavior and in the following sequence leading to the final result).

With the behavior “a”, the need (and the providing systems: neural, metabolic and other) is eliminated by positive feedback as a result of vital behavior—achieving the ultimate goal; with behavior “b”, there is a temporary decrease in the frustration tension, which does not lead to the elimination of the need, to demotivation, due to the impossibility of achieving the ultimate goal—the symbol. In other words, to eliminate integral units of purposeful behavior of type “a” (the first signaling system), it is necessary to lack an afferent information flow from the receptor fields of the organism (an elimination of negative feedback); for behavior of type “b” (the second signaling system), it is sufficient to achieve a virtual intermediate (graded) goal (a reverse feedback, which does not have afferent receptor equivalents before the insight stage), temporarily reducing the frustration tension. We agree with L. White [5] that since symbolic behavior is the most important sign of culture, one of HS’s main abilities is to give symbols a major role in any culture. It is asserted [5-7] that in the interconnected elements of culture, the dominant driving factors are “techno-economics,” technological systems that are the basis of sociocultural transformations with a main tendency to increase information for impact on technologies that, according to the dominant point of view, are the main factors of evolution.

We believe that the main factor of evolution is the new need for hominids: the achievement of the current parametric equilibrium with CC, the symbol, and the resolution of frustration, where the technologies, despite rapid, exponential growth, have an auxiliary meaning, namely, the provision of technical means to aid targeted behavior. Unlike the ultimate goal (symbol) that is beyond the achievable, homomorphic (in relation to the symbol) forms of behavior have a set of specific, step-by-step goals that stimulate the development of applied technologies in accordance with realistic goals. These technologies provide both vital needs and mechanisms for reducing the frustration voltage (i.e., technologies, often having a double purpose, or more, are not significant in themselves but as a means of achieving the goal).

The theory of the evolution of the biosphere and society is formulated as a synergetic model of history,[8-10] where the crises (resource, techno-humanitarian, ecological) and environmental degradation can be productive (productive phase transitions). One of the decisive factors in the periods of phase crises is the excessive internal diversity of the system. In other words, at the onset of the evolutionary crisis, certain species forms that did not previously have evolutionary advantages find a deterministic equivalent response, an adaptive response to the crisis, which makes them a new factor in the formation of systems.[9] We believe that the emergence of creativity, symbolic thinking, the production of various forms of adaptive behavior that initiated technological development, and increasing socialization have modified one of the subspecies of hominids (HS), transforming it into a significant and then leading evolutionary factor with the potential for technological transformation of the environment and aggressive species (also intraspecific) behavior, as an

effective way of achieving the goal. The mechanism described above for the emergence of new functions of the psyche created the conditions for the transition of the HS psyche to a new systemic level, one of the manifestations of which was the phenomenon of frustration, as a derivative of a new need for hominids, initiating adaptive forms of purposeful behavior with a vector of achieving a goal that is beyond reach. The period of the supposed formation of the psyche, as a system at a new level, dates from the final stage of the Upper Paleolithic (about 50,000 years ago) and coincides with the 11th position of the planetary revolutions.[9,11] It was at the end of the Upper Paleolithic that the development of hunting technologies, accompanied by improvement in the conditions and life span of HS, led to the destruction of populations (and species) of animals, to the reduction in the resource base, and to the intensification of intraspecific competition. The way out of the crisis was the transition from the appropriating (hunting, gathering) to the producing economy (farming, cattle breeding).

In the phase transition diagrams (Fig.1 and Fig.2) separating the qualitatively different phases of the evolution of the planetary system, there is a transformation of the rising plateau from the 7th phase transition (Olduvai, Paleolithic revolution) to the 11th phase transition—the exponentially rising segment of the evolutionary chart: the period of the Upper Paleolithic Period ending (more than 2 million years).

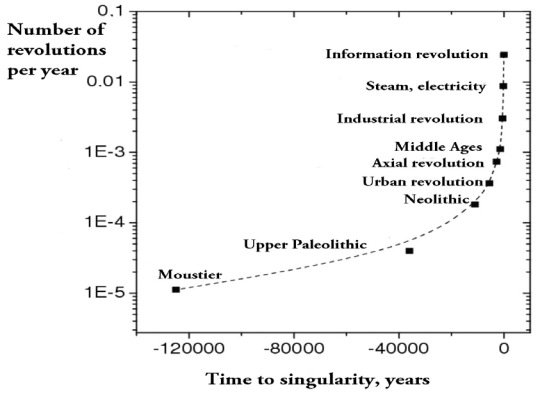

Fig. 1. Singularity of evolution and exacerbation regimes. *The points corresponding to a few last phase transitions (cited by A. Panov, 2005).*

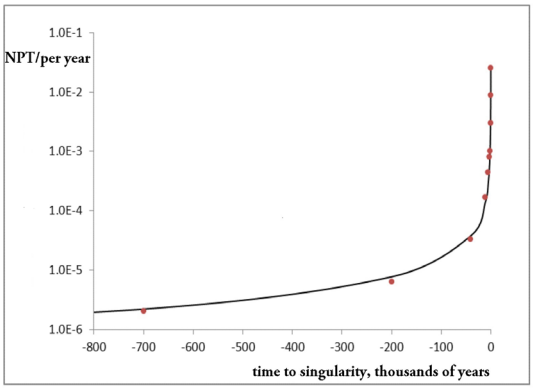

Fig. 2. The Snooks-Panov curve. *The points of phase transitions 9-18. NPT- number of phase transitions (cited by La Rose R & Bates B, 1990).*

The reasons for the phase transitions, according to A. Panov [11] are "oxygen crisis" - phase 1, "Cambrian explosion" - phase 2, etc. We believe that it is possible to state that the causes of the 11th phase transition (the Paleolithic cultural revolution) are those described above and earlier [2-4] qualitative changes in the HS psyche, which formed the psyche of a new class: a creative psyche producing frustration constructs—motivating, purposeful forms of behavior that require cooperation—and developing forms of communication, where the symbol becomes the dominant factor in ED of HS. In other words, the psyche of a new class that creates motivating frustration constructs (virtual motivators) becomes an attractor of the evolutionary development of HS. In our understanding, the attractor is the final region of the inevitable convergence of phase trajectories of a complex system, the attraction of which draws into itself a multitude of possible trajectories of systems determined by different initial conditions. The actions of the attractor realize the determinative function of the future state of the system. [12] In other words, the final state has not yet been reached, but as a goal chosen by the system it already affects that system.

Beginning in the Upper Paleolithic, HS is becoming an increasingly important participant in ED with the accelerating and increasing role of the HS community in the scale of the planetary biosphere. The advantages of the psyche at a new level allowed HS to accelerate the development of technologies in all types of activities, both appropriating and subsequently extractive (from cultural, agrarian transformations to information globalization). We do not list the achievements of technological growth, elaborated in detail by many authors and partly by Panov.[9,11] While the pace of technological development advances, it should be noted that the development of the ethical coordinates of society (cultural regulators), which could compensate for the destructive aspects of technology, lag behind.[8] The disparity between the advanced dynamics of technological development and the lagging development of "cultural regulators" creates the risks of applying technological advances to realize the species aggressive potential as an effective way of implementing purposeful behavior,[4] which can influence the vectors of ED. That is, the dynamics of technological development can have vectors that do not coincide with the vectors of sociocultural evolution.

A quantitative study of the sequence of points of phase evolutionary transitions showed that each subsequent phase of the evolution of the planetary system is, on average, 2.67±0.15 times shorter than the previous one.[11] We consider that the following events are related: 1) the initial phase of the ascending segment of the evolutionary exponent; and 2) the emergence and sharp growth of a new component of planetary (not only biological) evolution—the technological results of the mental activity of HS. In the absence of known factors of the all-planet (geological, climatic, etc.) scale, with the potential to influence the vector of ED, we believe it is possible to assert that the results of the mental activity of HS, the attractor of ED from the end of the Upper Paleolithic, were the accelerator for the development of this vector, which sets the parameters and the speed of development. A number of authors affirm [11,13] that genetic changes (as well as technological ones) are processes in

which the exit of one paradigm is the entrance of the other, which gives the "acceleration effect." The dynamics of accelerating and contracting phase transitions of the evolution of HS is defined [11,14-16] as a sequence having a limit ("singularity" of evolution). Since the "singularity point" is a mathematical abstraction, meaning an inevitable change in the parameters of global evolution, it is proposed to consider it not a "point" but a "singularity period," similar in meaning to a demographic transition.[17]

We consider it advisable to consider the computational (and other) capabilities of computer technology as a functional complement to the HS psyche, at this stage of development, connected with the operating systems of the HS brain by the main communication channel through the eyes (sensory organ taken out of the brain, but related anatomically and functionally). We consider the analogy of constructive interrelations in systems to be admissible: 1) "psyche of HS - computer" with 2) "psyche of HS - creative product," with the mechanism of projection into the external environment and perception of projection as an independent object (symbol). The principal difference between this system and other types of systems is the absence of an intermediary, the absence of liability and other restrictions of an ethical nature, the possibility of direct dialogue with an immediate unambiguous response (i.e. a constant cycle of positive encouragement, known as a way of regulating behavior).[18] In other words, communications are a two-way channel between the "recipient" (person) and the information "donor" (computer), where the "donor" influences the psyche by means of imposing and changing adaptive behavioral responses and ethical attitudes, rendering the recipient's responses and communications archaic, changing the influence of cultural regulators, and modifying the human behavior. The addition of written messages with graphic symbols greatly expands the information capacity due to the emotional component sewn in the iconography. The use of graphic symbols and acronyms (symbolic abbreviations of whole phrases, for example, IMHO and many others) brings together the IRC-writing with the primitive writing of the initial cultures. The behavior of HS in a nonlinear information environment reveals, in addition to the archetypal information environment, also fundamentally new phenomena for the psyche, namely, virtual "reality," "drift of goals," virtual associations of individuals with unusual properties of the psyche, elimination of the age threshold, and many others. Thus, the interaction and mutual influence in the system HS-computer, combining the archetypal and constructively new qualities of both the recipient and the donor (with a periodic change of roles), has a vector of accelerating convergence, expansion, and interference of the spheres of interaction (i.e., mutual adaptation). The emergence of a new (external, technological) object communicating with the psyche has already led to the emergence of new operational capabilities. With the accumulation of quantitative changes (unknown to us), conditions can be created for adaptive transformation, modification of the psyche HS with the vector of maximum communication contact with the "donor" (computer) and transition to a new qualitative level of the system with a change in configuration, functional properties and access to a new systemic class (possibly with the migrating topography of the "decision center" and the expansion of the boundaries of personalization).

It is not the task of the present paper to consider the prognostic vectors of evolutionary development when the singularity is achieved. We believe that the projected problems and tasks of the post-singular evolutionary phase transition are a subject to resolution with a possible change in the development vector by the already active evolutionary attractor, the modifying psyche of HS, which is in the stage of developing a new configuration, the transformation of which (amplification, change of properties, qualities, etc.) occurs at present, with the accelerating development (exceeding the limits established by Moore's law) of the external, technological element of the attractor of ED.

Conclusion

We believe that the basic initiating factor of the exponential growth of ED was the spasmodic qualitative change in the psyche of HS, which appeared at the end of the Upper Paleolithic, with a change in functional properties and a transition to a new class of systems. The result of this phenomenon was a new need for hominids, namely, the achievement of the current parametric equilibrium with the symbol, projected into the external environment by the creative construct of the psyche, endowed with control functions. Satisfaction of this need has activated the development of the phenomenon of frustration, which has initiated adaptive forms of purposeful behavior with the vector of achieving a symbolic goal that is beyond reach. Beginning with the era of the Upper Paleolithic, the initial segment of the exponential growth of the evolutionary chart, which sets the parameters for the growth of ED, we presume the accelerator of ED has been the mental activity of HS with the qualities and properties of the attractor of planetary evolution.

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International Journal of Biomedicine 7(4) (2017) 335-340
http://dx.doi.org/10.21103/Article7(4)_IA1

IMAGE ARTICLE

INTERNATIONAL JOURNAL OF BIOMEDICINE

Vibrational Decihertz (dHz), Centihertz (cHz), Millihertz (mHz), Microhertz (μHz), Nanohertz (nHz), Picohertz (pHz), Femtohertz (fHz), Attohertz (aHz), Zeptohertz (zHz) and Yoctohertz (yHz) Imaging and Spectroscopy Comparative Study on Malignant and Benign Human Cancer Cells and Tissues under Synchrotron Radiation

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Abstract

In the current study, we have experimentally and computationally presented vibrational decihertz (dHz), centihertz (cHz), millihertz (mHz), microhertz (μHz), nanohertz (nHz), picohertz (pHz), femtohertz (fHz), attohertz (aHz), zeptohertz (zHz) and yoctohertz (yHz) imaging and spectroscopy comparative study on malignant and benign human cancer cells and tissues under synchrotron radiation. It can be concluded that malignant human cancer cells and tissues have gradually transformed to benign human cancer cells and tissues under synchrotron radiation with the passing of time. **(International Journal of Biomedicine. 2017;7(4):335-340.)**

Key Words: spectroscopy • synchrotron radiation • cancer cells • malignant cells • benign cells

Introduction

In the current study, we have experimentally and computationally presented vibrational decihertz (dHz), centihertz (cHz), millihertz (mHz), microhertz (μHz), nanohertz (nHz), picohertz (pHz), femtohertz (fHz), attohertz (aHz), zeptohertz (zHz) and yoctohertz (yHz) imaging and spectroscopy comparative study on malignant and benign human cancer cells and tissues under synchrotron radiation [1–100]. In this regard, we have experimentally investigated and compared malignant human cancer cells and tissues before and after irradiating of synchrotron radiation using vibrational decihertz (dHz), centihertz (cHz), millihertz (mHz), microhertz (μHz), nanohertz (nHz), picohertz (pHz), femtohertz (fHz), attohertz (aHz), zeptohertz (zHz) and yoctohertz (yHz) imaging and spectroscopy. It is clear that malignant human cancer cells and tissues have gradually transformed to benign human cancer cells and tissues under synchrotron radiation with the passing of time (Figure 1).[1–100]

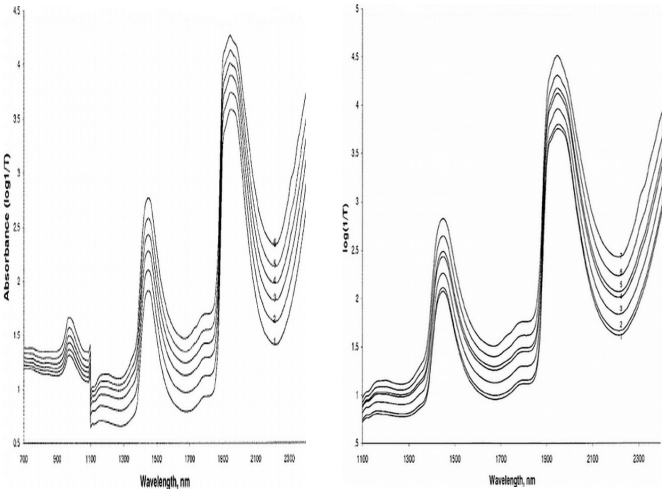

Fig. 1. *Vibrational decihertz (dHz), centihertz (cHz), millihertz (mHz), microhertz (μHz), nanohertz (nHz), picohertz (pHz), femtohertz (fHz), attohertz (aHz), zeptohertz (zHz) and yoctohertz (yHz) spectra of malignant human cancer cells and tissues (a) before and (b) after irradiating of synchrotron radiation.*[1–100]

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Furthermore, we have computationally simulated this transformation process according to the passing of time (Figure 2) and also different distributions of human cancer cells and tissues (Figure 3) using MATLAB, respectively.[1–100]

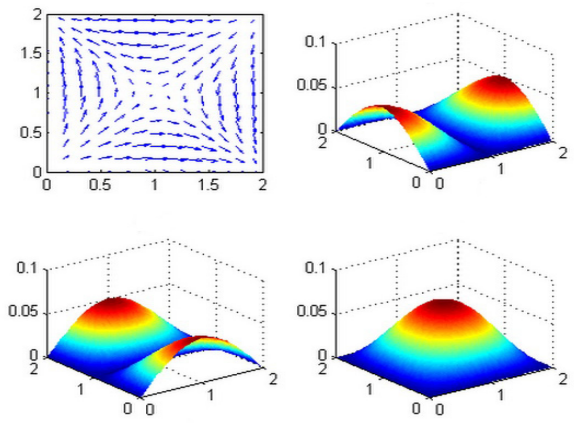

Fig. 2. *Simulation of transformation process of malignant human cancer cells and tissues to benign human cancer cells and tissues under synchrotron radiation with the passing of time using MATLAB.*[1–100]

Fig. 3. *Different simulations of transformation process of malignant human cancer cells and tissues to benign human cancer cells and tissues under synchrotron radiation accroding to the different distributions of human cancer cells and tissues using MATLAB.*[1–100]

It should be noted that different simulations of transformation process of malignant human cancer cells and tissues to benign human cancer cells and tissues under synchrotron radiation according to the different distributions of human cancer cells and tissues using MATLAB (a) before irradiating of synchrotron radiation (top left), after (b) 10 days (top right), (c) 20 days (left bottom) and (d) 30 dyas (right bottom) irradiating of synchrotron radiation was investigated (Figure 3).[1–100] It can be concluded that malignant human cancer cells and tissues have gradually transformed to benign human cancer cells and tissues under synchrotron radiation with the passing of time (Figures 1–3).[1–100]

Conflicts of interest

There are no commercial interests or conflicts of interest to declar.

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Acknowledgments

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Book: Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. *Medical Microbiology*. 4th ed. St. Louis: Mosby; 2002.

Chapter in Edited Book: Meltzer PS, Kallioniemi A, Trent JM. Chromosome alterations in human solid tumors. In: Vogelstein B, Kinzler KW, editors. *The Genetic Basis of Human Cancer*. New York: McGraw-Hill; 2002:93–113.

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