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Biochemical Changes Induced By Emphysema in Children

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Abstract

In the lung, the pathogenesis of emphysema (Em) is complicated and involves several endogenous factors: protease-antiprotease imbalance, α1-antitrypsin deficiency, oxidative stress (OS), and ion imbalance. It is our contention that an increase in the free Fe level in combination with a significant decrease of SOD activity that we detected is disadvantageous for patients with lung pathology, particularly in cases with complicated Em. GP and SOD activity decrease in patients with Em. The Zn/Cu ratio also decreases during Em, whereas the Zn in/Cu ratio is increased, when compared with patients without Em. We discovered the activation of HNE (not distinctively expressed, but obviously, prolonged over time), and have assumed an increase in cathepsin G activity (according to antiG activation). A moderate rise in the MMP-7 level can indicate an inactivation of the remodeling processes in these patients, and a part of the compensatory mechanisms. This assumption is confirmed by the MMP-7 correlation links found in cases of Em, with anti-NE and anti-G activity (r=+0.67 and r=+0.64, correspondingly) that the use of antioxidants alone has little effect, as these drugs cannot neutralize the main chain of free-radical reactions, namely Fe^{2+}. Most likely, iron chelators will need to be included in lung pathology treatment, particularly in children to prevent an intensification of OS, underlying the development of multiple organ deficiency. IJBM 2012; 2(3):222-227. © 2012 International Medical Research and Development Corporation. All rights reserved.

Key words: *emphysema, human neutrophilic elastase, matrix metalloproteinases, free ions, erythrocyte, glutathione peroxidase, superoxide dismutase.*

Introduction

According to the definition approved by the American Thoracic Society in 1962, an essential emphysema (Em) symptom is not only an expansion, but also a destruction of the respiratory system. Bullous Em is not a special form of Em. Thin-walled bullae can be formed during any type of Em, reach a significant size, and finally burst, resulting in the development of pneumothorax. Genetic causes of Em appear at a young age. Several endogenous factors are involved in the pathogenesis of Em. At the end of the nineteenth century, the predominant concept of the origin of Em was the vascular hypothesis; recently, until the end of the 1960s of the twentieth century, a "mechanical hypothesis" was accepted, which was based on the experimental reproduction of Em in dogs, on inflation of certain parts of the lungs. However, the current proteases-antiproteases hypothesis of the origin of Em is based on the examination of patients with α1-antitrypsin (α1–AT) deficiency, although only 1% of the patients with this deficiency suffer from Em. This theory confirmed that not only elastin, but other components of the alveolar cells are also involved in the development of Em [1].

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The reason for the development of Em is the disruption in the of the proteolysis-antiproteolysis system, due to the increase and/or the reduction of concentration (activity) decrease of the protease activity. In an experimental model of Em and fibrosis development, it was established that bleomycin causes an elastolitic Em that precedes fibrosis; therefore, the increase in the amount of elastase in the alveolar interstitium has a bearing on the increase in the expression of the growth factors. The breaks caused were diminished when a human neutrophilic elastase (HNE) inhibitor was used [2]. It is normally assumed that the elastic lung frame is damaged as a result of an imbalance during Em and that the elastin content in the pulmonary parenchyma decreases, while the collagen level increases [3]. There is another hypothesis stating that an excess of proteases can result from a latent virus infection. The latent adenovirus E1A was found to be secreted in all the Em patients. As a result of the disorders associated with persistent infection and a constant attraction of a significant amount of neutrophils to the pulmonary inflammation, local emphysema (more often bullous Em) is developed in patients suffering from cystic fibrosis. Protease inhibitor deficiency arises because of the genetically determined defect of synthesis or secretion (congenital deficiency of α1-Antitrypsin), or due to their excessive deactivation by the oxidative stress (OS) products. Matrix metalloproteinases (MMPs) are also involved in the development of chronic tissue damage in the lungs and bronchi, whose activation is controlled by the involvement of zinc ions [5].

Any impact by the adverse endogenous and exogenous factors from various sources can disturb the zinc-copper balance, which eventually results in the development of OS. Metabolic disturbance (or comprehensibility) of bivalent cations are assumed to be genetically determined and related to several biological effects [6]. Probably, a variation in the blood microelement composition is one of the reasons for the development of secondary immunogenicity conditions during respiratory organ diseases.

The aim of our investigation was to study the relationship between the changes in the number of bivalent cations in the blood plasma and erythrocytes, and the antioxidant activity and peroxide process activity, as well as the relationship of these parameters with the HNE content and activity, antibody activity towards HNE and cathepsin G and α1-AT and α2-macroglobulin (α2-MG) HNE inhibitors.

Materials and methods

Group characteristics.

The first group of patients with Em included children with congenital malformation of the lungs and bronchi (CML&B) between 2.3 and 16 years of age. Bullous Em was diagnosed in all the patients at the time of study. The second comparison group had 68 patients with CML&B, but without Em. The third control group involved 30 children without any bronchial or lung disease pathology. Blood was collected into tubes with heparin-lithium; whole blood 50 μl was collected for determination of glutathione peroxidase (GP) activity using Randox kits on a DU 530 spectrophotometer (Beckmann Coulter, USA). The neutrophil-enriched plasma was separated by sedimentation. Next, the blood was centrifuged, plasma was separated and its antioxidant status (AOS) was analyzed using Randox kits and its magnesium and iron ionic content was determined using an automatic Beckmann Coulter Synchron CX-5Δ analyzer (USA). The amount of zinc, copper, α1-antitrypsin, and α2-macroglobulin inhibitors present was determined spectrophotometrically using Sentinel kits (Italy). The erythrocytes were then washed thrice with cold physiological solution, following by cell sedimentation. After the last wash, 0.5 mL of the mixed erythrocyte sediment was thoroughly but carefully added to 2.0 mL of deionized water and frozen for complete cell hemolysis. Then, 0.005 mL of cells was drawn from the mixed pellet for erythrocyte counting in the probe. After hemolysis superoxide dismutase activity (SOD) in the cells was determined using the Randox standard kits and the level of free (not linked to proteins) Cu ion, Zn ion, Fe ion, Mg ion and Malone dialdehyde content was determined by a reaction with TBA. To determine the free intracellular ions after lysate defrosting, first the hemoglobin and proteins were removed using trichloroacetic acid (TCA) by slowly adding it to 5% final concentration. In the neutrophil-enriched plasma, the HNE content, anti NE and anti cathepsin G (antiG) activity, and MMP-7 content were determined using Bender Medsystems, Orgentic and Randox standard kits. The HNE activity was determined spectrophotometrically by measuring the breakdown intensity of N-metoxysuccinyl-ALA-ALA-PRO-VAL-L-nitroanilide at λ 440 nm.

Statistical data analysis was carried out using Statistical software. The differences between groups were verified statistically, significant at probability forecasting ($p<0.05$). The correlation coefficient was computed by the Pearson method.

Results

Table 1 shows that the Mg and Cu content in the blood plasma was increased, the Zn content was somewhat decreased while the Fe content did not change; the Zn/Cu ratio in blood plasma also decreased somewhat, which could be the evidence of the activation of the peroxide processes. AOS decreased in patients simultaneously, less during Em than in the second group ($p<0.05$). Some data is available showing that the increase in the copper content by 1.6-1.8 times, correlates with the increase in radical formation. Studies in the expirate showed that the Zn/Cu coefficient increased giving evidence to support AOS stability: the higher the value, the more stable the antioxidant protection was; therefore, there is a distinct negative relationship between the Zn/Cu value ratio and the intensity of radical formation (RFI) ($r=-0.993$) [7].

We have demonstrated the correlation links between AOS and Cu plasma ($r=-0.48$), AOS and Zn plasma ($r=-0.64$), and Zn/Cu ratio ($r=0.42$). The increase in the ion content of intracellular Mg was established in the affected children compared with the affected children of the second group; also, the decrease in the levels of Cu ion, Zn ion, and Fe ion during Em, compared with the affected children of the second group was noted, which was accompanied by the

Table 1
Biochemical parameters at emphysema and CML&B

Parameters	Group 1	Group 2		Group 3		
	M±m	M±m	p1/2≤	M±m	p1/3≤	p2/3≤
Blood plasma						
Mg mM/L	0.97±0.02	0.95±0.01	NS	0.9±0.016	0.05	0.05
Fe μkM/L	15.0±2.19	14.8±0.88	NS	15.0±1.3	NS	NS
Cu μkM/L	10.83±0.25	10.1±0.13	0.05	9.38±0.26	0.01	0.05
Zn μkM/L	21.9±0.31	21.2±0.12	0.05	22.5±0.3	NS	0.01
Zn/ Cu	2.04±0.06	2.14±0.35	NS	2.44±0.07	NS	NS
AOS mM/L	1.239±0.05	1.092±0.037	0.05	1.598±0.131	0.05	0.01
α1-AT mg/dL	220.0±18.5	260.2±24.9	NS	161.5±4.54	0.01	0.01
α2-MG mg/dL	210.±22.4	283.6±7.4	0.05	277.6±6.6	0.05	NS
Neutrophil-enriched plasma						
HNE UE/mL	53.9±7.6	56.6±3.6	NS	45.4±7.3	NS	NS
HNE ng/mL	157.7±25.4	126.4±9.2	NS	127.5±14.7	NS	NS
anatine UE/mL	260.9±16.4	389.5±15.6	0.01	331.4±18.0	0.01	0.05
MMP-7 ng/mL	3.52±0.21	3.16±0.07	0.05	3.05±0,16	0.05	NS
antiG UE/mL	372.±39.0	469.8±20.2	0.05	264.0±49.9	NS	0.01
Erythrocytes						
Mg ion Mm/10^{12}Er	0.386±0.02	0.387±0.013	NS	0.314±0.007	0.01	0.01
Fe ion μkM/10^{12} Er	52.5±3.13	60.1±1.62	0.05	40.7±0.95	0.01	0.01
Cu ion μkM/10^{12} Er	3.9±0.11	4.50±0.12	0.01	4.02±0.03	NS	0.01
Zn ion μkM/10^{12} Er	25.8±1.5	28.7±0.78	0.05	26.2±0.9	NS	0.05
Zn ion/Cu ion	6.7±0.15	6.33±0.1	0.05	5.02±0.42	0.01	0.01
SOD UE/10^{12} Er	124.9±24.2	146.7±12.9	NS	170.3±25.0	NS	NS
GP UE/10^{12} Er	204.7±19.7	349.1±26.2	0.01	221.7±18.5	NS	0.01
MDA μkM/10^{12} Er	26.6±3.48	30.8±2.21	NS	19.1±2.71	NS	0.05

decrease in activity of the SOD and GP antioxidant enzymes. The increase in the intracellular Zn/Cu ratio confirmed the lipid peroxidation (LP) processes revealed in our study. As mentioned above, we determined that the free ions in the cells, which were not linked to proteins, increased this coefficient which was evidence of an imbalance in ion binding with the enzyme active sites, including SOD. This ratio was maximal during Em, which corresponded to the lowest activity of SOD, a decrease in the Zn/Cu ratio accompanied by a proportional increase in the SOD activity in the second and third groups. We have demonstrated a positive correlation link between the SOD activity and Fe in (r=+0.6) at Em. The decrease in GP activity in patients with Em, and the increase in activity of this enzyme in patients with Em was revealed. This GP activity in patients with Em showed a connection with the Cu ion level (r=+0.47), and Zn ion level (r=+0.48).

The MDA content in the erythrocytes in all the patients was higher than in the control group; however, the MDA content during Em was somewhat lower than at bronchia and/or lung pathology without Em. In fact, the higher level of AOS in the first group of patients corresponded to this observation. The α1-AT level increase at Em was notably compared with the control, but its content was definitely higher than in patients without Em. This is in agreement with the data in the literature data, that α1-AT deficiency in adult patients was registered only in one case from the 104

patients studied (0.96%); also, the increase in the level of α1-AT of unclear etiology was registered in 18 patients [8]. A significant decrease in the α2-MG content in patients with Em compared with the control and to patients without Em was noted. This concurs with the author's data that showed that an increase in the elastase α2-MG complex content was observed in patients suffering with leucosis and rheumatoid arthritis. At the same time, enzyme activity did not correlate with the hardness grave (weight) and activity of the process [9]. In the group of patients with Em, the activity and the content of HNE were seen to increase; however, this increase was not statistically significant compared with the control group. The HNE activity in the second group of patients was somewhat a little higher even, than during Em, and the HNE content was at the level of the control group. The HNE activity was revealed to be correlated with the Zn/Cu ion ratio (r=+0.61) during Em. The anti-NE activity during Em was found to be lower than in the control group ($p<0.01$), and significantly lower than in the second group of patients ($p<0.01$). The anti-NE activity in the second group exceeded this control parameter ($p<0.05$). A correlation between the anti-NE activity and Mg ion level (r=+0.4) and AOS (r=0.45) during Em, was found to be absent in the second group of patients. A double increase in the anti-G activity in the second group of patients was noted, which was less expressed during Em. A correlation between anti-G activity and SOD was revealed during Em, as was the anti-G activity correlated to patient age (r=+0.52). Probably, the anti-G activity increase is compensatory in nature and reflects the increase in activity and/or cathepsin G content in these patients.

The increase in the MMP-7 level was shown during Em, which was not noted in the second group of patients, which concurred with the data related to the enhanced secretion of the MMP-7 during emphysema and transplantation, and most significantly in the lungs of the patients with MB [10]. The MMP-7 content during Em was negatively correlated with the Fe level in the blood plasma (r=-0.36), and force vital capacity (r=-0.75). A positive correlation between the MMP-7 level, Mg ion (r=+0.44), HNE content (r=+0.67), anti-G activity (r=+0.65) was revealed, and which was absent in the second group of patients.

Discussion

The primary active states (forme/forming)of oxygen are known to possess regulatory or moderate anti-microbial ability. These states include nitrogen oxide NO, exerting a vasodilatory effect, and superoxide OO^-, whose role is quite multi-dimensional. It is usually transformed into hydrogen peroxide by specialized SOD enzyme action, following by further transformation into the hypochlorite ClO^-. The macrophages utilize both these compounds to fight against bacteria. At the same time, the excess superoxide forms peroxynitrite by reacting with NO, or reduces Fe^{3+} into Fe^{2+}, which produces the hydroxyl radical OH*, or lipoxy radical LO* by reaction with H_2O_2, HClO or lipoperoxides. These radicals, as well as peroxynitrite, belong to the category of secondary radicals, possessing the ability to do irreversible damage of the membrane lipids, and also molecules of DNA, carbohydrates and proteins. During Em, such secondary radicals are probably formed, particularly as shown by us, with reduced SOD activity and an increased level of Fe ion, all the more; therefore, it is accompanied by GP activity inhibition compared with the second group of patients. Some experimental data is available which shows that Em can be spontaneously developed in middle-aged mice, and that this is connected to the oxidant/antioxidant imbalance due to the increase in the expression of the new NADPH oxidase gene and elastin degradation [11].

Currently, the gene coding α1-AT synthesis has been proved to possess an expressed polymorphism, and its 186 mutations have been fixed. Therefore, the synthesis, secretion and functional activity of α1-AT can be inhibited. Methionin oxidation at the 351 or 358 position can occur at OS, and this inactivates the anti-NE activity of α1-AT [12]. In the pathogenesis of bullous Em in the lungs, genetically determined deficiency has been shown to play no significant role, which was confirmed by our data as well as the data of the other investigators, particularly noting the specific anti protease therapy inefficiency in patients with Em [8].

MG deficiency, which we detected during Em, was believed to be an essential condition for the development of the reparative processes during aseptic inflammation. Mice devoid of the MG gene became rapidly infected; however, intentionally lethal doses of exotoxins did not exert any influence to them [13]. The α2-MG has been shown to have a "trap" section for α1-AT, which possesses a triggering mechanism, switching on only on contact with the activated elastase. Stereo structure conformation of both proteins occurs at the time of contact, which results in the transformation of the proteolysis properties of the elastase and its protection from α1-AT action. At the same time, the α2-MG in the complex with the activated elastase can migrate to the other regions of lungs, where it can trigger an alteration in the parenchyma by elastase release [8].

Generally speaking, HNE plays a dual role. On one hand, HNE can perform not only intracellular killing of the neutrophil-captured gram-positive bacteria; on the other hand, on being released extracellularly, it can play a role in the bacterial killing, by being part of the neutrophil extracellular trap (NETs). The compounds that are a part of NET, are active both against gram-positive and gram-negative bacteria, and can also destroy virulent factors that come into contact with the NETs. Besides directly killing the bacteria, HNE plays an important role in the initiation of immunity and inflammation, particularly in neutrophil recruitment and expression of the mucinic gene [4]. Cathepsin G along with Em is believes to be responsible for the development of bullous changes. At the same time, the cathepsin G expression can reflect the signaling response of the activated macrophages to an infection. The inhibition of the activity of serine proteases has been shown to enhance disease progression during tuberculosis [14]. Earlier research showed that several proteases of various groups can cooperate in tissue remodeling, in the same place, at the same time. MMPs are believed to play a specific role during acute and chronic pathogenesis conditions, connected with extracellular matrix degradation [15]. Some biochemical and morphometric investigations revealed that the collagen

in the Em lung differs from that in a normal one. There can be two reasons for this: (1) intestinal collagenases (MMP) or the other enzymes damaging the collagen fibrils, which result in a drop in the collagen content which is not restored, and/or (2) active collagen remodeling occurs; however, the newly synthesized fibrils have such a mechanical effect which cannot withstand the load. With respect to the first mechanism, it is known that various MMP's and even HNE can degrade the collagen of the first type. High oxygen concentrations (and, as a result, high concentrations of AOC) promoted the development Em and collagen degradation, without change in the elastin content [16]. Collagen antibodies were found in the serum of patients with Em. The importance of collagen in the development of Em has also been proved by the fact that Em is developed in mice with an enhanced MMP-1 expression, and no changes in the elastin content were found. This supports the fact that Em can be caused by an elastin-independent mechanism. Structural changes which initiated an enhanced reparation result in a disruption of the balance in the lungs, and eventually, in fibrosis and/or Em. In emphysematic human lungs volume breaks correlate with enlargement of wall thickness and collagen, and increased elastin content. The ultra structures of the human lung collagen during Em became thinner and disorganization of the fibrils occurred after remodeling. As not all the structures undergo inflammation and proteolytic enzyme action immediately, it is obvious that damaged and normal sections are present in the lung at the same time. An enhanced collagen and elastin turnover was observed during Em, which was expressed in an enhanced secretion of the specific degradation markers of the cross-linked elastin and collagen in the urine [15].

Conclusion

We appreciate the fact that the enhancement of the free Fe ion level along with the significant decrease in the SOD activity that we detected is disadvantageous for patients with lung pathology (LP), particularly in cases of complicated emphysema. Fe ion enhancement (compared with the control), especially expressed in the second group indicates progressive OS and involvement in a growing number of free Fe. The decrease in GP and SOD activity during Em can indicate the compensatory mechanism break-down in children with extremely severe infection. The Zn/Cu ratio is seen to decrease during Em, whereas the Zn ion/Cu ratio increases, compared with patients without Em. We identified the activation of HNE (not distinctively expressed, but obviously, prolonged over time), and have assumed an enhancement of the cathepsin G activity (according to antiG activation). A moderate enhancement of the MMP-7 level of one of a few metalloproteinases, synthesized in a healthy lung can indicate an inactivation of the remodeling processes in these patients, which are a part of the compensatory mechanisms. This assumption is confirmed by the MMP-7 correlation links found during Em, with anti-NE and anti-G activity (r=+0.67 and r=+0.64, correspondingly). Earlier studies showed that several used antibiotics and anesthetics possess pro-oxidant activity. The process of accumulation of toxic radicals is not a one-stage one, but works over a specific time frame. It is believed that using only antioxidants has very little significance, as these drugs cannot neutralize the main chain of free-radical reactions, namely Fe^{2+}. Most likely, iron chelators need to be included in the treatment of children with lung pathology, to prevent an intensification of OS, underlying the development of multiple organ deficiency [17].

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