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Comparative Assessment of Inflammatory Reaction in Experimental Animals after Pleurodesis with Solutions of Hydrogen Peroxide and Talc

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Abstract

The aim of our research was to compare the nature and severity of the inflammatory process in the lungs, in the leaves of the visceral and parietal pleura, and in the adjacent subpleural tissues of the chest wall in experimental animals after pleurodesis with solutions of 3% and 6% hydrogen peroxide, and talc.

Methods and Results: The experiment was carried out on 200 Wistar rats, weighing 160-180 grams, 10 specimens in a subgroup, depending on the time of the experiment, i.e. 50 specimens in each study group, including the control group. The main criterion by which we determined the comparative characteristics of the effectiveness of talc and 3% and 6% solutions of hydrogen peroxide as preparations used for chemical pleurodesis in the rats was a morphological characteristic of inflammation. This criterion was confirmed by counting free cell populations in lung tissue (lymphocytes, macrophages, neutrophils, histiocytes). All comparison groups were characterized by a gradual increase in the number of lymphocytes, macrophages and histiocytes, ranging from minimum to maximum values, and by a gradual decrease in the number of neutrophils, starting with max and ending with minimum values. The number of lymphocytes, macrophages and histiocytes were increasing faster. But at the same time, for the most part, their number was lower after pleurodesis with 6% hydrogen peroxide. The minimum number of neutrophils and the fastest possible reduction in all cases was observed in pleurodesis with 6% hydrogen peroxide.

Conclusion: Pleurodesis with a 6% solution of hydrogen peroxide as a chemical agent significantly affects the quality of the inflammatory response, reducing its duration and severity in the organs and tissues of the rats' chests, compared with a solution of 3% hydrogen peroxide and talc. (**International Journal of Biomedicine. 2021;11(3):291-295.**)

Key Words: pneumothorax • pleurodesis • talc • hydrogen peroxide • free cell populations

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Introduction

According to the latest data, spontaneous pneumothorax (SP) has been identified in 6.2%–7.1% of patients with nonspecific lung diseases. This pathology tends to increase steadily, and currently there are about 15 patients per 100,000 inhabitants per year. It is important to note that the prevalence of SP is 7.4-18 cases per 100,000 men and 1.2-6 cases per 100,000 women per year. According to the results of several studies,[1] SP accounts for 11.2% of all acute pathology faced by thoracic surgeons. Note that this pathology is often found among people suffering from chronic obstructive pulmonary disease (COPD) - 26 cases per 100,000 population per year.[2] According to modern conceptions, the cause of nonspecific SP in 94.5% of cases is the destruction of emphysematous altered bulls, thereby once again confirming that SP is a complication of pulmonary emphysema and COPD.[1,3-5]

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The question of preventing the recurrence of SP remains relevant. A significant number of authors consider it necessary to use chemical pleurodesis in cases of pneumothorax occurrence, and even more in cases of its recurrence. Currently, various chemical agents are used for chemical pleurodesis: talc, olive oil, 40% glucose solution, hypertonic sodium chloride solution, acromycin, 96% alcohol solution and many others.[1,6-8]

An important and common drawback of the chemical agents used is the development of an excessive inflammatory response with severe pain and the development of various complications in the future.

Questions persist: What is the optimal chemical agent to perform pleurodesis? Taking the above data into consideration, we can claim that this scientific study is relevant.

The aim of our research was to compare the nature and severity of the inflammatory process in the lungs, in the leaves of the visceral and parietal pleura, and in the adjacent subpleural tissues of the chest wall in experimental animals after pleurodesis with solutions of 3% and 6% hydrogen peroxide, and talc.

Materials and Methods

The experiment was carried out on 200 Wistar rats, weighing 160-180 grams, 10 specimens in a subgroup, depending on the time of the experiment, i.e. 50 specimens in each study group, including the control group.

SP was simulated in rats under ether anesthesia by injection of air in the volume of 2 ml through a Velish needle.

After 1 hour under ether anesthesia, one of the chemical agents with a volume of 1.0 ml (hydrogen peroxide solution at concentrations of 6% or 3%, talc) was sprayed with a Velish needle and the air from the pleural cavity was removed. Then the animals were observed and killed in groups on Days 3, 5, 7, and 30 of the experiment. *In vivo* experiments were carried out in accordance with the legislation of the Russian Federation, in strict compliance with the European Convention for the protection of animals used for experimental and other purposes (Strasbourg, France, 1986), the provisions of Directive 210/63/EU of the European Parliament and the Council of the European Union of 22 September 2010 on the protection of animals used for scientific purposes (Article 27).

At opening of pleural cavities of the experimental animals, organs and tissues of the thorax were sampled for histological research. Pieces of lungs with adjacent parts of the chest wall were fixed in 10% neutral formalin and stained using standard histological techniques. Paraffin sections 6-7 microns thick after de-embedding (removing of paraffin) were stained with H&E for review.

In histological examination, we performed a comparative analysis of the severity of inflammatory changes in the interstitium of the lungs and commissure formation, depending on the agent used in pleurodesis.

The main criterion by which we determined the comparative characteristics of the effectiveness of talc and 3% and 6% solutions of hydrogen peroxide as preparations used for chemical pleurodesis in the rats was a morphological characteristic of inflammation. This criterion was confirmed by counting free cell populations in lung tissue (lymphocytes, macrophages, neutrophils, histiocytes).

Statistical analysis was performed using Microsoft Excel software package. For descriptive analysis, results are presented as median (Me), first quartile (25th percentile) and third quartile (75th percentile). A non-parametric Kruskal-Wallis test was used for comparisons of median values among groups.

Results

The results of descriptive statistics for free cell elements in tissues after chemical pleurodesis are presented in Table 1. The distribution of the compared values in most samples was abnormal.

The data in Table 2 indicate that the method of pleurodesis significantly affects the number of free cell elements. The differences between all pairs were estimated by the Kruskal-Walliss criterion as significant, as the significance levels were <0.05.

On Day 3 after pleurodesis with 6% hydrogen peroxide, the number of lymphocytes was less than after 3% hydrogen peroxide by 13.33% and after talc by 33.33%, but more than in the control group by 13.33%. The number of macrophages was greater than after 3% hydrogen peroxide by 8.33%, less than after talc by 25%, and more than in the control group by 25%. The number of neutrophils was less than after 3% hydrogen peroxide by 21.15% and after talc by 25%, but more than in the control group by 48%. The number of histiocytes on Day 3 after pleurodesis with 6% hydrogen peroxide was less than after 3% hydrogen peroxide by 8.33% and after talc by 16.66%, but more than in the control group by 8.33%.

On Day 5 after pleurodesis with 6% hydrogen peroxide, the number of lymphocytes was less than after 3% hydrogen peroxide by 8.33% and after talc by 20.83%, but more than in the control group by 45.83%. The number of macrophages was greater than after 3% hydrogen peroxide by 20.0%, less than after talc by 6.66%, and more than in the control group by 46.66%. The number of neutrophils was less than after 3% hydrogen peroxide by 27.5% and after talc by 37.5%, but more than in the control group by 25%. The number of histiocytes on Day 5 after pleurodesis with 6% hydrogen peroxide was less than after 3% hydrogen peroxide by 18.18% and after talc by 27.77%, but more than in the control group by 36.36%.

On Day 7 after pleurodesis with 6% hydrogen peroxide, the number of lymphocytes was more than after 3% hydrogen peroxide by 11.53% and after talc by 3.85% and more than in the control group by 73.5%. The number of macrophages was greater than after 3% hydrogen peroxide by 6.25%, less than after talc by 12.5%, and more than in the control group by 43.75%. The number of neutrophils was less than after 3% hydrogen peroxide by 33.3% and after talc by 42.2%, but more than in the control group by 15.5%. The number of histiocytes on Day 7 after pleurodesis with 6% hydrogen peroxide was less than after 3% hydrogen peroxide by 18.18% and after talc by 36.36%, but more than in the control group by 63.63%.

On Day 10 after pleurodesis with 6% hydrogen peroxide, the number of lymphocytes was more than after 3% hydrogen peroxide by 8.95%, less than after talc by 8.95% and more than in the control group by 68..65%. The number of macrophages was greater than after 3% hydrogen peroxide by 10.52%, less than after talc by 15.78%, and more than in the control group by 52.63%. The number of neutrophils was less than after 3% hydrogen peroxide by 19.23% and after talc by 30.76%, but more than in the control group by 11.53%. The number of histiocytes on Day 10 after pleurodesis with 6% hydrogen peroxide was less than after 3% hydrogen peroxide by 13.33% and after talc by 26.66%, but more than in the control group by 46.66%.

On Day 30 after pleurodesis with 6% hydrogen peroxide, the number of lymphocytes was less than after 3% hydrogen peroxide by 6%, after talc by 28%, and by 58% more than in the control group. The number of macrophages was greater than after 3% hydrogen peroxide by 9.09%, less than after talc by 13.63%, and more than in the control group by 59.09%. The number of neutrophils was less than after 3% hydrogen peroxide by 16.66% and after talc by 29.16%, but more than in the control group by 8.33%. The number of histiocytes on Day 10 after pleurodesis with 6% hydrogen peroxide was less than after 3% hydrogen peroxide, on average, by 11.11% and after talc by 22.22%, but by 61.11% more than in the control group.

Table 1.

Descriptive statistics for free cell elements in tissues after chemical pleurodesis

Days	Parameter	Talc			3% hydrogen peroxide			6% hydrogen peroxide			Control group		
		Me	25	75	Me	25	75	Me	25	75	Me	25	75
3	lymphocytes	15	14	15	12	12	12	10	9	10	8	7	8
	macrophages	12	11	13	8	8	9	9	9	10	6	6	7
	neutrophils	52	52	53	50	49	50	39	39	40	14	14	14
	histiocytes	10	9	10	8	7	8	6	7	8	4	3	4
5	lymphocytes	24	23	24	21	20	21	19	18	19	8	8	9
	macrophages	15	14	15	11	10	11	14	13	14	6,5	6	7
	neutrophils	40	40	40	37.5	37	38	25	25	26	15	15	15
	histiocytes	11	11	13	10	9	10	8	7	8	4	4	4
7	lymphocytes	25	25	26	23	22	23	26	26	27	7	7	7
	macrophages	16	16	17	13	13	13	14	14	14	7	7	7
	neutrophils	33	33	34	30	30	30	19	18	19	14	13	14
	histiocytes	14	14	14	12	11	12	10	10	10	3	3	3
10	lymphocytes	33.5	33	34	28	27	28	31	31	31	8	7	8
	macrophages	19	19	19	13.5	13	14	16	16	16	6	6	6
	neutrophils	26	33	34	22.5	22	23	18	18	18	15	15	15
	histiocytes	15	15	16	13	13	14	11	11	12	4	4	5
30	lymphocytes	50	50	51	39	38	39	36	36	37	7	7	8
	macrophages	22	21	22	17	16	17	19	18	19	6	6	7
	neutrophils	24	24	24	21	21	21	17	16	17	15	15	15
	histiocytes	18	17	18	16	16	16	14	13	14	3	2	3

Table 2.

The results of data processing by Kruskal-Walliss criterion (P-level)

Comparison groups	Lymphocytes					Macrophages					Neutrophils					Histiocytes				
	Day 3	Day 5	Day 7	Day 10	Day 30	Day 3	Day 5	Day 7	Day 10	Day 30	Day 3	Day 5	Day 7	Day 10	Day 30	Day 3	Day 5	Day 7	Day 10	Day 30
All	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Talc – 3% hydrogen peroxide	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Talc– 6% hydrogen peroxide	0.001	0.001	0.009	0.002	0.001	0.001	0.005	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Talc – control	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
3% hydrogen peroxide - 6% hydrogen peroxide	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
3% hydrogen peroxide –control	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
6% hydrogen peroxide –control	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

All comparison groups were characterized by a gradual increase in the number of lymphocytes, macrophages and histiocytes, ranging from minimum to maximum values, and by a gradual decrease in the number of neutrophils, starting with max and ending with minimum values. The predominance of neutrophilic leukocytes over other cell populations indicates an acute inflammatory reaction to the introduction of the drug; a further increase in the level of lymphocytes, macrophages, histiocytes and, accordingly, a decrease in the level of neutrophils indicates the transition of acute inflammation to chronic. By comparing the dynamics of the number of analyzed free cell elements, we found that the number of lymphocytes, macrophages and histiocytes were increasing faster. But at the same time, for the most part, their number was lower after pleurodesis with 6% hydrogen peroxide. The minimum number of neutrophils and the fastest possible reduction in all cases was observed in pleurodesis with 6% hydrogen peroxide. In the comparison group, only fluctuations in the number of the initial level of free cell elements were observed.

Conclusion

- The dynamics of the number of free cell elements in all comparison groups was estimated as stereotypical.
- The differences in all the investigated pairs were evaluated by Kruskal-Walliss criterion as significant.
- Kruskal-Walliss analysis suggests that the method of pleurodesis significantly affects the number of free cell elements involved in the inflammatory response.
- Pleurodesis with a 6% solution of hydrogen peroxide as a chemical agent significantly affects the quality of the inflammatory response, reducing its duration and severity in the organs and tissues of the rats' chests, compared with a solution of 3% hydrogen peroxide and talc.

Competing Interests

The authors declare that they have no competing interests.

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