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Research Article



# Effectiveness of various regimens of systemic anti-inflammatory therapy with glucocorticoids in the development of acute LPS-induced lung damage in the experiment

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**BACKGROUND:** When studying new and effective methods of treating acute respiratory distress syndrome, an immunogenic model of lung injury occupies a special place. To date, the search for the optimal strategy and regimen for the use of glucocorticoids in the development of acute respiratory distress syndrome is relevant.

**AIM:** The article evaluates the effectiveness of various schemes of systemic anti-inflammatory therapy with glucocorticoids in an experimental model of acute LPS-induced lung injury.

**MATERIALS AND METHODS:** The study was conducted on 100 outbred male rats. Acute lung injury was modeled using an experimental model of direct acute lung injury by a single intratracheal injection of lipopolysaccharide (LPS) from the cell wall of the bacterium *Salmonella enterica* (Sigma-Aldrich) at a dose of LD50 (20 mg/kg). All animals were divided into groups (20 each): 1 — intact rats; 2 — control group (LPS + saline); 3 — LPS + dexamethasone 0.52 mg/kg (small doses); 4 — LPS + dexamethasone 1.71 mg/kg (average doses); 5 — LPS + dexamethasone 8 mg/kg (high doses). The drugs were administered intraperitoneally once a day for 3 days. Dexamethasone doses were calculated using the interspecies dose transfer method using a factor that takes into account differences in body surface area.

**RESULTS:** It has been established that an experimental model based on the endotracheal administration of *S. enterica* leads to the development of mortality from pulmonary causes. According to a preclinical study, the systemic use of low doses of dexamethasone (0.52 mg/kg) was found to be better than higher doses (1.71 mg/kg, 8 mg/kg) in the treatment of acute LPS-induced lung injury.

**Keywords:** acute lung injury; acute respiratory distress syndrome; dexamethasone; glucocorticoids; immunogenic model of lung injury; lipopolysaccharide; preclinical study.

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Научная статья

# Эффективность применения различных схем системной противовоспалительной терапии глюкокортикоидами при развитии острого ЛПС-индуцированного повреждения легких в эксперименте

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**Актуальность.** При изучении новых и эффективных методов лечения острого респираторного дистресс-синдрома особое место занимает иммуногенная модель повреждения легких. На сегодняшний день актуальным является поиск оптимальной стратегии и режима применения глюкокортикоидов при развитии острого респираторного дистресс-синдрома.

**Цель исследования** — оценка эффективности применения различных схем системной противовоспалительной терапии глюкокортикоидами на экспериментальной модели острого липополисахарид-индуцированного повреждения легких.

**Материалы и методы.** Исследование проведено на 100 беспородных крысах-самцах. Острое повреждение легких моделировали посредством экспериментальной модели прямого острого повреждения легких методом однократного интратрахеального введения липополисахарида клеточной стенки бактерии *Salmonella enterica* («Sigma-Aldrich») в дозе ЛД50 (20 мг/кг). Все животные были разделены на группы (по 20 каждая): 1 — интактные крысы; 2 — контрольная группа (липополисахарид + физиологический раствор); 3 — липополисахарид + дексаметазон 0,52 мг/кг (малые дозы); 4 — липополисахарид + дексаметазон 1,71 мг/кг (средние дозы); 5 — липополисахарид + дексаметазон 8 мг/кг (высокие дозы). Препараты вводили внутривентриально один раз в день в течение 3 сут. Дозы дексаметазона рассчитывались по методу межвидового переноса доз с применением коэффициента, учитывающего разницу в площади поверхности тела.

**Результаты исследования.** Установлено, что экспериментальная модель на основе эндотрахеального введения *S. enterica* приводит к развитию летальности от легочных причин. По данным проведенного доклинического исследования установлена лучшая эффективность системного применения малых доз дексаметазона (0,52 мг/кг) по сравнению с более высокими дозами (1,71 мг/кг, 8 мг/кг) при лечении острого липополисахарид-индуцированного повреждения легких.

**Ключевые слова:** глюкокортикоиды; дексаметазон; доклиническое исследование; иммуногенная модель повреждения легких; липополисахарид; острое повреждение легких; острый респираторный дистресс-синдром.

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## BACKGROUND

In the search for new and effective methods of treating acute respiratory distress syndrome (ARDS), an immunogenic model of lung injury is significant [1, 2]. The most studied models of immunogenic ARDS are those with endotracheal administration of lipopolysaccharide (LPS). Endotoxin (LPS of the cell wall of gram-negative bacteria), which has a high immuno- and pyrogenicity, administration can cause both local (massive infiltration of neutrophils into the lungs, microthrombosis, interstitial and alveolar edema, death of alveolar epithelial cells, and macrophage activation) and systemic (excessive production cytokines and chemokines, endothelial dysfunction, and impaired microcirculation) pathological processes [3–5]. To date, the systemic anti-inflammatory and membrane-stabilizing effects of glucocorticoids have been proven in various immunoinflammatory diseases, including ARDS; however, the optimal dose and mode of their use remain unresolved [1, 6, 7].

The study aimed to evaluate the efficiency of various schemes of systemic anti-inflammatory therapy with glucocorticoids in an experimental model of acute LPS-induced lung injury.

## MATERIALS AND METHODS

A preclinical study was conducted on 100 outbred male rats. Acute lung injury was modeled using an experimental model of direct acute lung injury by single intratracheal administration of LPS from the cell wall of the bacterium *Salmonella enterica* (Sigma-Aldrich, MA, USA) at a dose of LD<sub>50</sub> (20 mg/kg) [4]. All animals were divided into groups ( $n = 20$  each), where group 1 included intact rats, group 2 was the control (LPS + saline), group 3 received LPS + dexamethasone 0.52 mg/kg (small doses), group 4 received LPS + dexamethasone 1.71 mg/kg (average doses), and group 5 received LPS + dexamethasone 8 mg/kg (high doses). The drugs were administered intraperitoneally once a day for 3 days. Dexamethasone doses were calculated by interspecies dose transfer using a coefficient that considers the difference in the body

surface area [8]. During the experiment, the survival rate, laboratory and clinical parameters (physical inactivity, cyanosis of the extremities, tachypnea, and dyspnea), temperature, and bodyweight of the animals were assessed. The mass coefficient of the lungs (ratio of the mass of the lung complex to the mass of the animal) was also calculated [4].

**Statistical analysis.** To test the hypotheses, an electronic database obtained as a result of the experiment was created using Microsoft Office 365 Excel, followed by statistical processing of the results in GraphPad Prism 8.0. The study results were presented as median and upper and lower quartiles  $Me$  [Q1; Q3]. When comparing the median and relative frequency of indicators of groups with a normal distribution, the Kruskal–Wallis test was used, followed by a posteriori pairwise comparison using the Dunn test, taking into account the Bonferroni correction. The significance level was set  $p < 0.05$ . The relationship between qualitative indicators, at two levels each, was assessed by constructing four-field contingency tables and calculating Pearson's  $\chi^2$  criterion based on them, and when the number of cases per cell of the four-field table was  $< 5$ , Fisher's exact test was used.

## RESULTS

In group 2 (control group), a statistically significant increase in lung mass coefficient was noted after intratracheal administration of LPS at a dose of 20 mg/kg compared with group 1 (intact animals) ( $p < 0.0001$ ). In the intraperitoneal administration of dexamethasone at doses of 0.52 and 8 mg/kg in groups 3 and 5, respectively, significantly lower median lung mass coefficients were obtained when the values of this indicator were compared with those in the control group ( $p = 0.0016$  and  $p = 0.0003$ , respectively) (Table 1).

Compared with the control group, group 5 (8 mg/kg intraperitoneal dexamethasone) showed a statistically significant decrease in sodium concentration in the venous blood ( $p = 0.001$ ). In groups 4 and 5, significantly high median potassium (K) concentrations in the venous blood were recorded when compared with the concentration in the control group ( $p = 0.002$  and  $p = 0.008$ ).

**Table 1.** Indicators of the mass coefficient of the lungs 72 h after treatment for 3 days and their comparison with the control group\*

Group No.	Group description	Lung mass factor	$p$ (Dunn post-hoc test**)
1	Intact animals	7.48* [5.7; 8.0]	<0.0001
2	Control, LPS 20 mg/kg i/t	12.53 [12.02; 14.02]	–
3	LPS 20 mg/kg i/t + dexamethasone 0.52 mg/kg i/p	8.59* [8.25; 10.56]	0.0016
4	LPS 20 mg/kg i/t + dexamethasone 1.71 mg/kg i/p	11.84 [9.64; 12.87]	0.5
5	LPS 20 mg/kg i/t + dexamethasone 8 mg/kg i/p	8.39* [7.51; 9.72]	0.0003

\* — Differences are statistically significant relative to the values in the control group ( $p < 0.05$ , Kruskal–Wallis test);

\*\* — hereinafter, a test of aposterior intergroup comparison of variables.

The serum concentration of ionized calcium (iCa) was significantly higher in group 4 (1.71 mg/kg intraperitoneal dexamethasone) than in the control group. The median concentration of venous blood glucose was significantly higher in groups 3–5 than in the control group (Table 2).

During intraperitoneal administration of dexamethasone, significant intergroup differences were noted in the absolute count and percentage of lymphocytes, monocytes, and granulocytes in groups 3–5 (Table 3).

In these groups (dexamethasone), a statistically significant increase was found in soluble fibrin–monomer complexes (SFMC) after intratracheal administration of LPS at a dose of 20 mg/kg compared with group 1 (intact animals) ( $p < 0.001$ ), which probably indicated thrombogenesis activation. Moreover, an increase in the dose of dexamethasone was associated with higher SFMC rates (Table 4).

Statistically significant differences were found in the incidence of clinical parameters such as physical inactivity, cyanosis of the extremities, tachypnea, and dyspnea between groups 1 and 3–5 and the control group (2) ( $p < 0.001$ , Fisher's exact test).

In the intragroup comparison, bodyweight changes were statistically significant in all test groups compared with baseline values at the start of the experiment ( $p < 0.05$ , pairwise Wilcoxon test). In addition, no significant differences in the weight changes were found between the groups.

In the analysis of survival rates in the groups, the use of intraperitoneal dexamethasone at low doses (0.52 and 1.71 mg/kg) once a day for 3 days positively affected the survival rate in acute LPS-induced lung injury, as on day 4 in the corresponding groups, only one lethal outcome (5%) was detected. In rats treated with dexamethasone at a dose of 8 mg/kg, the lethality rate was 25% ( $n = 5$ ), whereas in the control group, it was 45% ( $n = 9$ ).

**Table 2.** Blood sodium, potassium, and glucose levels of laboratory animals, 72 h after treatment for 3 days and their comparison with the control group

Group No.	Group description	Indicators			
		Na, mmol/L	K, mmol/L	iCa, mmol/L	Glucose, mmol/L
2	Control, LPS 20 mg/kg i/t	142 [139; 144]	4.3 [3.9; 4.6]	1.45 [1.24; 1.49]	8.55 [7.77; 8.77]
3	LPS 20 mg/kg i/t + dexamethasone 0.52 mg/kg ip	140.5 [139.2; 141]	4.6 [4.48; 5.98]	1.44 [1.36; 1.49]	11.17* [9.99; 11.63]
4	LPS 20 mg/kg i/t + dexamethasone 1.71 mg/kg i/p	140 [139; 141]	4.9* [4.8; 5.6]	1.52* [1.47; 1.53]	12.22* [9.97; 13.72]
5	LPS 20 mg/kg i/t + dexamethasone 8 mg/kg i/p	137* [136.5; 138.5]	5.0* [4.75; 5.35]	1.43 [1.34; 1.56]	11.44* [10.68; 14.81]
$p$ (Kruskall–Wallis test)		<0.001	0.0013	0.02	<0.001

\* —  $p < 0.05$ , Dunn post-hoc test.

**Table 3.** Indicators of the clinical analysis of blood of laboratory animals

Group No.	Group description	Leuko-cytes, $10^9/L$	Lympho-cytes, $10^9/L$	Mono-cytes, $10^9/L$	Granulo-cytes, $10^9/L$	Lympho-cytes, %	Mono-cytes, %	Erythro-cytes, $10^{12}/L$	Hemo-globin, g/L	Platelets, $10^9/L$
2	Control, LPS 20 mg/kg i/t	7.7 [7.2; 10.2]	5.4 [4.6; 5.8]	0.4 [0.3; 0.5]	1.5 [1.3; 1.7]	74 [73; 75]	5 [5; 6]	7.5 [7.4; 7.7]	15.2 [15; 16]	519 [515; 592]
3	LPS 20 mg/kg i/t + dexamethasone 0.52 mg/kg i/p	6.1 [5.8; 6.8]	1.8* [1.7; 2.1]	0.8 [0.7; 0.9]	3.2* [3.1; 3.8]	32* [29; 36]	13* [11; 14]	7 [7; 7]	15 [15; 16]	548 [504; 599]
4	LPS 20 mg/kg i/t + dexamethasone 1.71 mg/kg i/p	8 [7; 11]	2* [1; 3]	1* [0.5; 1.5]	5* [4; 6]	22* [20; 30]	12* [11; 15]	7 [7; 8]	15 [15; 17]	511 [444; 568]
5	LPS 20 mg/kg i/t + dexamethasone 8 mg/kg i/p	8.5 [7.2; 10.1]	2.4* [1.6; 3]	1.3* [1; 1.4]	4.5* [4.1; 6]	30.5* [29; 34]	15.3* [13.3; 15.7]	7.7 [7.3; 7.9]	16.3 [15.5; 16.4]	410* [335; 449]
$p$ (Kruskall–Wallis test)		<0.001	0.012	0.009	<0.001	<0.001	0.001	0.28	0.19	0.021

\* —  $p < 0.05$ , Dunn post-hoc test.

**Table 4.** Content of SFMC in the blood of laboratory animals

Group No.	Group description	SFMC, g/L × 10 <sup>2</sup>
1	Intact animals	6.75 [5.8; 9]
2	Control, LPS 20 mg/kg i/t	9 [8.9; 10]
3	LPS 20 mg/kg i/t + dexamethasone 0.52 mg/kg i/p	13* [12; 17]
4	LPS 20 mg/kg i/t + dexamethasone 1.71 mg/kg i/p	12.5* [10.75; 17.5]
5	LPS 20 mg/kg i/t + dexamethasone 8 mg/kg i/p	14.5* [12.5; 18.75]
<i>p</i> (Kruskall–Wallis test)		<0.001

\* —  $p < 0,001$ , Dunn post-hoc test.

Thus, the induction of acute lung injury in laboratory animals by endotracheal administration of *S. enterica* LPS leads to early mortality (45%, 9/20) and deterioration of clinical, laboratory, and morphological (lung mass coefficient) parameters. By the end of day 3, a statistically significant decrease in the mass coefficient of the lungs was noted in the group treated with dexamethasone at doses of 0.52 and 8 mg/kg, compared with the control group and the group treated with 1.71 mg/kg. The use of intraperitoneal dexamethasone at low doses (0.52 and 1.71 mg/kg) once a day for 3 days exerted a positive effect on the survival rate in acute LPS-induced lung injury. Animals receiving dexamethasone had higher levels of glucose, potassium, ionized calcium, and SFMC, which was probably due to the side effects of glucocorticoid therapy. An increase in the dose of dexamethasone was associated with the activation of thrombogenesis. No significant differences were found in the dynamics of clinical parameters between groups receiving dexamethasone.

## CONCLUSIONS

1. The induction of acute lung injury on a model of small laboratory animals through endotracheal administration of *S. enterica* LPS leads to early mortality (45%, 9/20) and deterioration of clinical, laboratory, and morphological (lung mass coefficient) parameters.

2. By the end of day 3, a statistically significant decrease was found in the mass coefficient of the lungs in

animals treated with dexamethasone at doses of 0.52 and 8 mg/kg, compared with the control animals and animals receiving 1.71 mg/kg of dexamethasone.

3. The use of intraperitoneal dexamethasone at low doses (0.52 and 1.71 mg/kg) once a day for 3 days positively affected the survival rate in LPS-induced lung injury, as the mortality rate by the end of day 3 was 5% (1/20).

4. Dexamethasone-treated animals had higher levels of glucose, potassium, ionized calcium, and SFMC, which is probably due to the side effects of glucocorticoid therapy. Notably, an increase in the dose of dexamethasone was associated with the activation of thrombogenesis. No significant differences were found in the changes in clinical parameters over time between the groups receiving dexamethasone.

## ADDITIONAL INFORMATION

**Funding.** The study had no external funding.

**Conflict of interest.** The authors declare no conflict of interest.

**Ethical considerations.** The study was approved by the local ethics committee of the S.M. Kirov Military Medical Academy, Ministry of Defense of the Russian Federation (Protocol No. 258 dated 12/21/2021).

**Author contributions.** All authors made a significant contribution to the study and preparation of the article, read and approved the final version before its publication.

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