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The state of the cytokine profile under the conditions of the immobilization stress development

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Abstract

The aim of our study is to elucidate changes in the content of pro-inflammatory interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) and anti-inflammatory cytokines interleukin-10 (IL-10) in the blood serum of guinea pigs in the dynamics of experimental immobilization stress.

The dynamics of the immobilization stress is accompanied by a pronounced progression of the proinflammatory group of cytokines - TNF-α and IL-6 against the background of declining functional activity of IL-10 at all stages of their formation (3 rd, 5 th and 15 th days) with an advantage on the 3 rd day of the experiment. The data obtained indicate an imbalance of pro- and anti-inflammatory cytokines and impaired cytokinogenesis, which is important for the pathogenesis in immobilization stress.

Key words: immobilization stress; tumor necrosis factor – α; interleukins; cytokines.

Стан цитокінового профілю за умов розвитку іммобілізаційного стресу

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Метою нашого дослідження є з'ясування змін вмісту рівня прозапальних інтерлейкіну-6 (ІЛ-6) і фактору некрозу пухлин-α (ФНП-α) та протизапальних цитокінів інтерлейкіну-10 (ІЛ-10) у сироватці крові морських свинок у динаміці розвитку іммобілізаційного стресу.

Динаміка розвитку іммобілізаційного стресу супроводжує виражену прогресію прозапальної групи цитокінів – ФНП-α та IL-6 на тлі спаду функціональної активності IL-10 на усіх етапах їх формування (3-я, 5-а та 15-а доби) з перевагою на 3-у добу експерименту. Одержані дані свідчать про розбалансування про- і протизапальних цитокінів та порушення цитокіногенезу при іммобілізаційному стресі.

Ключові слова: іммобілізаційний стрес; інтерлейкіни; фактор некрозу пухлин-α; цитокіни.

Introduction. Over the past few decades, there has been an acceleration of the pace of human life, the scientific and technical revolution and other factors determine the impact of various stresses on the human and animal body. It is appropriate to note that excessive and prolonged action of stress factors, stress reaction can become the basis for the development of diseases [1].

Stress is a complex stereotypical response to the action of pathogenic factors, which, along with specific, is characterized by non-specific reactions of the body [2]. The stress reaction is implemented with the participation of the hypothalamic-pituitary-adrenal system and the sympathoadrenal system. Activation of these systems leads to Selye's stressor triad, the first component of which is hypertrophy of the adrenal glands with increased synthesis of glucocorticoids and catecholamines [3] and includes three phases: anxiety, resistance, and exhaustion. The last phase is characterized by a violation of the body's homeostasis and the development of psychosomatic diseases. The existence of a modern person is constantly accompanied by stress reactions associated with the rapid pace of life, complex relationships

in society, difficult social factors, etc. Adaptation reactions that occur in the body against the background of excessive stress can contribute to the emergence and development of a number of pathological processes [3–5]. Strong or long-term stress suppresses the acquired, to a lesser extent, the innate immune response [6], changes the level of cytokines that affect the production of hormones of the hypothalamic-pituitary-adrenal system [2, 6].

Cytokines (CCs) are low molecular weight proteins, endogenous biologically active mediators that provide signal transmission, exchange of information between different types of cells within the same organ, communication between organs and systems, both under physiological conditions and under the influence of various pathogenic factors [7]. These are glycosylated polypeptides that regulate and determine the nature of the immune response. Depending on the nature of the pathogenic agent, the intensity and duration of antigenic stimulation, the initial state of the body's immune system, the central nervous system can act as both antagonists and synergists, complementing each other [8].

The aim of the work is to study the character of the disturbances content level of pro-inflammatory tumor necrosis factor - α (TNF-α), interleukin-6 (IL-6) and anti-inflammatory cytokine interleukin-10 (IL-10) in the serum of guinea pigs under the condition of immobilization stress (IS).

Material and methods of research. Experimental studies were performed on 40 guinea pigs (males), which were divided into four groups (10 in each): the first - intact animals - control; the second (experimental) group - animals with immobilization stress (3 rd day), the third group included guinea pigs with IS on the 5th day of the model process, to IV - animals with IS 15 th day.

Immobilization stress was reproduced by the method of PD Horizontov (1996). This model is scientifically justified [9]. Animals of the experimental groups were subjected to prolonged immobilization stress (3h) and then were removed from the experiment on the 3 rd , 5 th and 15 th day of the experiment. We selected fixed days (3 rd, 5 th and 15th) for studies that corresponded to the classic stages of acute inflammation.

All experiments on laboratory animals carried out with following the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, 1986), Council Directive 2010/63 / EU, the Law of Ukraine 3447- IV "protection animals from the cruelty," the general ethics of animal experimentation adopted by the first national Congress on bioethics in Ukraine (2001). In addition, all experiments with experimental guinea-pigs were conducted under the control of the BioEthics

Commission at Danylo Halytsky Lviv National Medical University. Laboratory animals were kept on a standard fodder in animal facility with adequate lighting and temperature conditions.

The IL-6, IL-10 and TNF-α concentration in the blood serum were defined for all groups of guinea-pigs. It was made using hard-phase immune-enzyme analysis (ELISA), by means of the test-system "Diaclone" (France). All numerical results were subjected **to** statistical processing using the arithmetic mean (M), the error of the arithmetic mean (m), and the Student's "t" test and Mann-Whitney test (U). Calculations were made using statistical and graphical analysis tools of Microsoft Excel spreadsheets of the Microsoft Office suite. In all cases of analysis, the critical level of significance was taken as 0.05.

Results and discussion. During the study of some indicators of the cytokine profile during immobilization stress, it was established that on all the investigated days of the experiment there were likely changes in the concentration of both pro-inflammatory and anti-inflammatory cytokines compared to the group of intact animals.

The dynamics of the development of immobilization stress is accompanied by a pronounced progression of the pro-inflammatory group of cytokines, namely an increase in TNF-α by 55.2% ($p \leq 0.05$) and IL-6 by 53.3% ($p \leq 0.05$) on the 3rd day against the control group. At the same time, we observe a decrease in the functional activity of IL-10 on this day of the study by 41.5% ($p \leq 0.05$) in comparison with intact animals (Fig. 1).

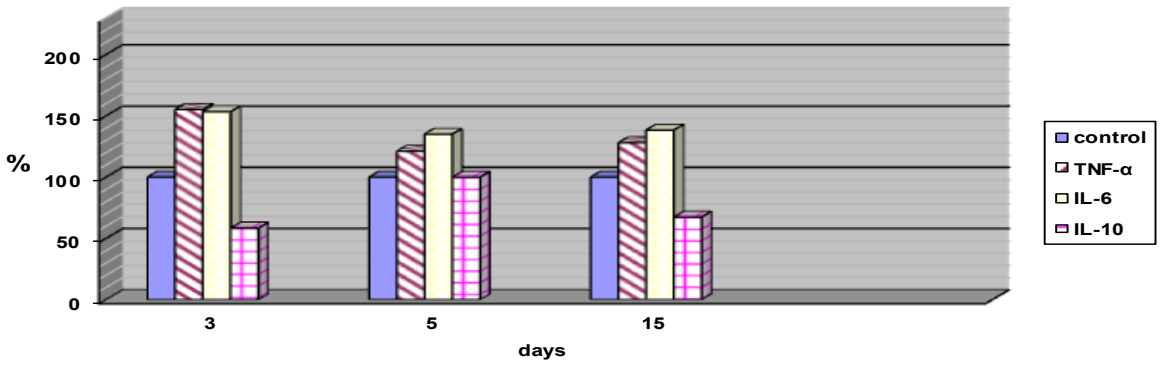

Fig.1. The level of TNF-α, IL-6 and IL-10 in the blood in the dynamics of the immobilization stress formation (% of control)

During further research, we observe changes in a similar direction, but not so significant. As for the pro-inflammatory TNF-α, a slight increase in its concentration by 21.0% ($p \leq 0.05$) on the 5 th day and by 28.0% on the 15 th day of the experiment was found in comparison with animals of the 1st group. Regarding the next indicator of IL-6, we also note its increase, but it is less compared to guinea pigs on the 3 rd day, namely, on the 5 th and 15 th days, this indicator increases by 35.0%, respectively ($p \leq 0.05$) and 38.3% ($p \leq 0.05$) relative to the control group.

Therefore, the results of studies of the cytokine profile in blood serum under IS conditions established an increase in the level of the pool of pro-inflammatory CCs, especially the most pronounced in the early period of the experiment (Fig. 1).

For a more comprehensive characterization of the cytokine status, we also studied the anti-inflammatory factor IL-10 in the blood serum of guinea pigs under immobilization stress. In the case of studying this indicator in the later periods of the experiment, we record that on the 5 th day the level of IL-10 is at the control level, and on the 15 th day it again decreases by 32.5% ($p \leq 0.05$) when compared with control group of animals.

Conclusions. So, the results obtained by us of studies of the activity of the cytokine profile indicate their significant changes in the blood depending on the observation period and reached a maximum increase in the level of pro-inflammatory CCs and, at the same time, a decrease in anti-inflammatory, on the 3 rd day of the experiment during the development of immobilization stress.

Author Contributions

The authors agree on equal distribution of partial participation.

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Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

All information is publicly available and data regarding this particular patient can be obtained upon request from corresponding senior author.

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgments

The authors declare that there are no conflicts of interest.

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