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Abstract

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IMMUNOMORPHOLOGICAL AND MORPHOMETRIC CHARACTERIZATION OF CENTRAL AND PERIPHERAL ORGANS OF IMMUNOGENESIS IN MICE UNDER PUTRESCINE ADMINISTRATION TO EXPERIMENTAL ANIMALS

We studied the influence of exogenously administered putrescine on morphofunctional state of the central and peripheral organs of immunogenesis of experimental animals. White mice were exposed to single intravascular administration of putrescine (Sigma, USA) at a concentration of 10^{-9} mg/mL per 100 g animal body weight. Animals were euthanized in 2 and 8 hours after putrescine administration observing all standards set forth by the Yerevan State Medical University (YSMU) Committee on Bioethics for investigations involving laboratory animals.

Structural changes corresponding to the notion of "accidental involution" occurred in the cortical layer of thymus in experimental animals 8 hours after putrescine administration. Structural shifts manifested as a targeted activation of B-dependent zones and layers were observed in spleen and lymph nodes. The performed immunomorphological studies revealed that activation of B-dependent zones expresses a marked increase in number of B-lymphocytes containing IgG, as well as an increase of IL-1α content in these cells. It is not excluded that putrescine at rather low concentrations (similar to those determined in blood serum of intact mammals) in spleen and lymph nodes exerts targeted immunomodulating action and selectively activates B-lymphocytes populations, which, in turn, are responsible for activation of humoral immunity reactions.

Key words: putrescine, organs of immunogenesis, structural shifts, B-lymphocytes, interleukin 1 α, immunomodulation.

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Резюме

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ІМУНОМОРФОЛОГІЧНА ТА МОРФОМЕТРИЧНА ХАРАКТЕРИСТИКА ЦЕНТРАЛЬНИХ І ПЕРИФЕРИЧНИХ ОРГАНІВ ІМУНОГЕНЕЗУ МИШЕЙ ПРИ УВЕДЕННІ ПУТРЕСЦИНА ЕКСПЕРИМЕНТАЛЬНИМ ТВАРИНАМ

Ми дослідили вплив екзогенно введеного путресцина на морфофункціональний стан центральних та периферичних органів експериментальних тварин. Білим мишам було зроблено однократне внутрішньовенне введення путресцина (Сігма, США) концентрацією 10^{-9} мг/мл на 100 г маси тіла тварини. Через 2 та 8 год після введення путресцина, тваринам проводили евтаназію дотримуючись усіх положень Протоколу про дослідження з використанням експериментальних тварин, затверджених Комітетом по біоетиці Єреванського державного медичного університету.

Структурні зміни, або так звана «акцидентальна (випадкова) інволюція», спостерігалися в корковому шарі тимуса експериментальних тварин через 8 год після введення путресцину. Ми помітили структурні зміни у селезінці та лімфатичних вузлах, що проявлялися у вигляді активації В-залежних зон та шарів. Проведені імуноморфологічні дослідження показали, що активація В-залежних зон характеризується помітним збільшенням кількості В-лімфоцитів, які містять імуноглобулін G, а також збільшенням вмісту інтерлейкіну IL-1α в цих клітинах. Не виключено, що путресцин у селезінці та лімфатичних вузлах навіть у досить малих концентраціях (подібних до тих, що були визначені у сироватці крові інтактних ссавців) активує імуномодуляцію, а також вибірково активує популяцію В-лімфоцитів, які, врешті решт, відповідають за активацію реакцій гуморального імунітету.

Ключові слова: путресцин, органи імуногенезу, структурні зміни, В-лімфоцити, інтерлейкін 1 α, імуномодуляція.

Резюме

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ИММУНОМОРФОЛОГИЧЕСКАЯ И МОРФОМЕТРИЧЕСКАЯ ХАРАКТЕРИСТИКА ЦЕНТРАЛЬНЫХ И ПЕРИФЕРИЧЕСКИХ ОРГАНОВ ИММУНОГЕНЕЗА МЫШЕЙ ПРИ ВВЕДЕНИИ ПУТРЕСЦИНА ЭКСПЕРИМЕНТАЛЬНЫМ ЖИВОТНЫМ

Мы изучали влияние экзогенно введенного путресцина на морфофункциональное состояние центральных и периферических органов экспериментальных животных. Белым мышам было сделано однократное внутривенное введение путресцина (Сигма, США) концентрацией 10^{-9} мг/мл на 100 г массы тела животного. Через 2 и 8 ч после введения путресцина животным проводили эвтаназию следуя положениям Протокола про исследования с использованием экспериментальных животных, утвержденным Комитетом по биоэтике Ереванского государственного медицинского университета.

Структурные изменения, или так называемая «акцидентная (случайная) инволюция», наблюдались в корковом слое тимуса экспериментальных животных через 8 ч после введения путресцина. Мы также заметили структурные изменения в селезенке и лимфатических узлах, которые были представлены активацией В-зависимых зон и слоев. Проведенные иммуноморфологические исследования показали, что активация В-зависимых зон характеризировалась заметным увеличением количества В-лимфоцитов содержащих иммуноглобулин G и увеличением содержания интерлейкина IL-1α в этих клетках. Не исключено, что содержание путресцина в селезенке и лимфатических узлах даже в очень маленьких концентрациях (похожих на те, что были определены в сыворотке крови интактных животных) активирует иммуномодуляцию, а также выборочно активирует популяцию В-лимфоцитов, которые, в конечном счете, отвечают за активацию реакций гуморального иммунитета.

Ключевые слова: путресцин, органы иммуногенеза, структурные изменения, В-лимфоциты, интерлейкин-1α, иммуномодуляция.

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Introduction

It is generally recognized that biological effects of biogenic polyamines (putrescine, spermidine and spermine) refer exclusively to all integrative systems of the organism, the realization of which occurs at all levels of their structural organization [7, 11, 8].

However, immunomodulatory effects of polyamines in the organism of mammals [5] are very insufficiently studied, whereas immune homeostasis ensures normal performance of numerous essential functions realized, particularly affectiong nervous [10], cardiovascular [2, 6, 1] and endocrine [4, 3, 9] systems of the organism.

Current literature lacks evidence on the influence of dose-dependent putrescine concentrations towards the morphofunctional state of the central and peripheral organs of immunogenesis. Hence, to our mind, study on the immunomodulatory effects of putrescine appears quite promising, especially with approbation of its low concentrations that are similar to those determined in blood serum of mammals.

The present investigation studies the morphological, morphometric and immunomorphological shifts in thymus, spleen, and the lymph nodes of mice under conditions of putrescine administration to experimental animals.

Material and Methods

The study was performed on white male mice weighing 35–40 g. The control group involved intact animals. Animals of experimental series were divided into 3 groups. Animals of experimental groups 1 and 2 were withdrawn from the experiment appropriately 2 and 8 hours (h) after a single intravascular administration of putrescine (Sigma, USA) given as 10^{-9} mg/ml per 100 g animal body weight, under observance of all generally accepted bioethical regulations. Animals of the control group were exposed to intravascular administration of 0.1 mL saline, i.e. such an amount of saline that was used to dissolve putrescine for administration to animals of experimental groups.

Animals were euthanized under the Nembutal anesthesia.

Material under investigation (specimens of thymus, spleen and lymph nodes after fixation in Karnua liquid and further treatment with high alcohol concentrations) were embedded in paraffin Histomix plus (Russia). Sections with thickness of 3–5 μm were stained according to generally accepted morphological methods by hematoxylin eosin and according to Brachet for RNA determination.

Morphometric analysis was performed with the help of histiostereometric network containing four squares with evenly distributed 25 points in each. The studied objects (lymphocytes) were counted in case if they randomly coincided with the points. With this aim, paraffin cross-sections were prepared from spleen and the lymph nodes. Ten sections were prepared from different sites of each paraffin block (i.e. after obtaining the subsequent section, 3–4 precedent ones were discarded). Five nets were applied to each section, and then the arithmetical mean value was calculated and included in the variational series.

For immunomorphological studies frozen-tissue cryostat sections derived from the spleen and lymph nodes were obtained and used for morphometric analysis, according to the principle similar to hematoxylin eosin stained cells count.

Immunomorphological studies for determination of B-lymphocytes populations were performed through the direct reaction of fluorescence using anti-mouse IgG-FITC antibody produced in rabbit (Sigma, USA). According to protocol, the initial volume of serum, 2 mL, was diluted 60 times in 0.01 M phosphate buffer at pH 7.4 (0.01M phosphate buffer saline, pH 7.4).

Determination of IL-1α in immunocompetent cells of peripheral organs of immunogenesis was done by the indirect fluorescence reaction. For this purpose, anti-interleukin-1α antibody produced in goat (Sigma, USA) was applied to the cryostat-derived sections at the first stage. According to instructions, these antibodies selectively react only with the immunocompetent cells of mice. One milligram of lyophilized serum was dissolved in 10 mL 0.01 M salt phosphate buffer at pH 7.4. At the second stage of investigation fluorescein isothiocyanate (FITC)-labeled anti-goat IgG (Sigma, USA) was applied to the cryostat sections; at a dilution 1:200. Upon implementation of mentioned studies we strictly adhered to all points

set forth in the instructions enclosed to each reagent and those of immunofluorescence investigations protocol (Sorin, USA), including the appropriate negative control and control excluding the autoluminescence background.

Preparations stained with hematoxylin and eosin and for RNA determination according to Brachet for RNA were examined with a light microscope (Micros, Austria). Cryostat sections treated with appropriate antisera were examined using a luminescent microscope (BOECO, Germany). Microphotographs were taken using a digital photocamera (Canon A640, Japan).

All statistical analyses were performed using SPSS version 13.0 software package for Microsoft Windows (SPSS, Inc., Chicago, IL). Data are presented as mean ±SEM. Comparisons between groups were performed using Student's t test and ANOVA.

Results and Discussion

The results of morphological investigation revealed that the cytoarchitectonics of thymus in experimental animals was similar to that observed in mice of control group in 2 hours after putrescine administration. Eight hours after putrescine administration, the initial stages of "accidental involution" processes were observed in thymus of experimental animals. In particular, in the superficial layers of the cortex the compact orientation of lymphocytes was disturbed; this latter was manifested by the presence of light optical microfoci, in which single lymphocytes were encountered. The content of lymphoblasts significantly decreased in subcapsular part. In the superficial layers of the cortex, on the background of their decreased mitotic activity, signs of pycnosis and rhexis of small lymphocytes were recorded more frequently compared with the control group.

In 2 hours after putrescine administration cytoarchitectonics of the spleen and lymph nodes was preserved, i.e. it did not differ from that of rats in control group. However, already 8 hours after putrescine administration the structure was changed, signifying the predominant activation of B-dependent zones in both organs of immunogenesis. Follicles of 2 types were revealed in the splenic white pulp. In one type of follicles two-three layers of circularly oriented small lymphocytes presented the periarterial zone. In the other type of follicles, lymphocytes of marginal zone were lacking. Optically light spaces appeared in perivascular areas.

The follicle centers appeared to be slightly widened and edematous and consisted mainly of reticular cells, among which single lymphocytes and macrophages were revealed. The mantle and marginal zones also appeared widened and edematous and were presented by plasmatic cells and small lymphocytes, amongst which single lymphoblasts were identified (Fig. 1a). Single hypertrophic follicles and the fused follicles were also revealed. In the red pulp of spleen the pulpar strands were rather contoured in relief, appeared to be somehow thickened and were mainly presented by small lymphocytes and plasmatic cells (Fig. 1b).

The cytoangioarchitectonics of the lymph nodes was also preserved. In the cortical substance, there were encountered lymphoid follicles of small and medium dimensions, with widened center, the cellular content of which was mainly presented by reticulocytes, with single macrophages and lymphocytes among them. The border of paracortical zone was contoured not distinctly. This zone consisted of loosely oriented small lymphocytes, single reticulocytes, macrophages, and lymphocytes. The medullary layer of lymph nodes appeared widened as well and was presented by strands, compactly and/or loosely oriented towards each other. Medullary strands consisted of plasmatic cells, small and medium lymphocytes (Fig. 1c). Sinuses appeared to be widened; lymphocyte line cells, among which there were recorded single monocytes and neutrophilic leukocytes, were encountered in lumen of sinuses.

Thus, in B-dependent zones, upon morphological analysis, structural changes were revealed indicating the increase of small lymphocytes and plasmatic cells in the mentioned zones.

In order to identify B-lymphocytes we performed immunological studies for the B-population of lymphocytes in their representative structures and zones of the spleen and lymph nodes.

As it was shown in the results of immunofluorescent investigation the number of B-lymphocytes populations, including plasmatic cells as well, markedly increased in all studied structures and zones of peripheral organs of immunity 8 hours after the putrescine administration.

B-lymphocytes populations and plasma cells were characterized by a specific fluorescence when FITC-labelled serum anti-mouse IgG was applied to slides of spleen and lymph nodes.

Fluorescence was revealed in both cytoplasm of plasmatic cells and on the surface of small and

average lymphocytes as a circularly oriented limbus; in small lumps and grains along the entire perimeter of lymphocytes localized at the site of spleen strands; in reactive centers of the spleen and lymph nodes; in the mantle and the marginal zones of spleen follicles (Fig. 2a), and marginal parts of lymph node follicles. We also registered the specific (homogenous and small-grain) fluorescence in cytoplasm of plasmatic cells predominantly localized in the medullary strands of lymph nodes and pulpar strands of spleen.

As indicated by results of the quantitative fluorescent microscopy analysis, the content of B-lymphocytes populations in all studied layers and zones of spleen and lymph nodes did not actually differ from the controls in 2 hours after the putrescine administration (Table 1).

Eight hours after the putrescine administration, the content of B-lymphocytes populations increased in studied structures of spleen. In particular, the number of B-lymphocytes in strands of the spleen, reactive centers of follicles, mantle and marginal zones (the summary index) was higher than in controls by 2.0, 2.2 and 1.95 times, respectively.

Similar picture was observed in the lymph nodes. The number of B-lymphocytes increased 2.6 times in the reactive centers of follicles in cortical layer compared with that in animals of control group, in marginal parts of follicles the increase was 1.7-fold, likewise the pulpar strands of the medullary layer increased 1.7-fold.

The next stage of our investigation was immunomorphological study for determination of specific cell populations responsible for IL-1α synthesis in spleen and lymph nodes.

According to our research findings, in the peripheral organs of immunogenesis of animals in the control group single lymphocytes and macrophages were identified; the specific fluorescence was recorded on the surface and in cytoplasm. Such cells (mainly small lymphocytes and single macrophages) were characterized by predominant localization in the lymphoid follicles of spleen and, to a lesser extent, in the strands of spleen. Thus, these cells predominantly localized in the mantle and marginal zones of lymphoid follicles in mice of the control group.

In reactive centers of spleen follicles no presence of lymphocytar and macrophageal line cells containing IL-1α was revealed. The availability of immunocompetent cells (lymphocytes and macrophages) in lymph nodes and spleen with a specific fluorescence in their cytoplasm was recorded, and observed mainly in the marginal parts of small and average lymphoid follicles. It should be emphasized that only single lymphocytes, in cytoplasm of which a specific fluorescence was observed, were revealed in medullary strands of lymph nodes in animals of the control group. The character and topical peculiarities of the immunocompetent cell distribution in peripheral organs of immunity were similar to those determined in the spleen and lymph nodes of the controls in 2 hours after the putrescine administration to experimental animals, in an indirect immunofluorescence reaction.

Eight hours after the putrescine administration, the content of immunocompetent cells with a specific fluorescence in cytoplasm significantly increased. Thus, the presence of IL-1α was recorded in a group of lymphocytar line cells participating in formation of splenetic pulpar strands and medullar strands of lymph nodes (Fig. 2b,c).

As is obvious from Table 2, the number of IL-1α containing lymphocytes in the red pulp of spleen was increased 2.5-fold 8 hours later after the putrescine administration as compared with the control group. The number of small lymphocytes in mantle and marginal zones of spleen follicles (the summary index), in cytoplasm of which a specific fluorescence was recorded, significantly increased as well. In particular, in the mentioned zones the number of small lymphocytes containing IL-1α increased 1.9-fold, as compared with the content of similar immunocompetent cells in animals of the control group. Similar pattern was observed in the lymphoid follicles of lymph nodes as well, namely: the number of IL-1α containing lymphocytes increased 2.6-fold in marginal parts compared with the similar index in the control group animals. In the medullary strands of lymph nodes the content of similar lymphocytes, though insignificantly, increased as well. It should be particularly mentioned that we did not register immunocompetent cells (macrophages and B-lymphocytes) containing IL-1α in the reaction of indirect immunofluorescence in the reactive centers of lymphoid follicles of spleen and lymph nodes of animals in both control and experimental groups.

Table 1

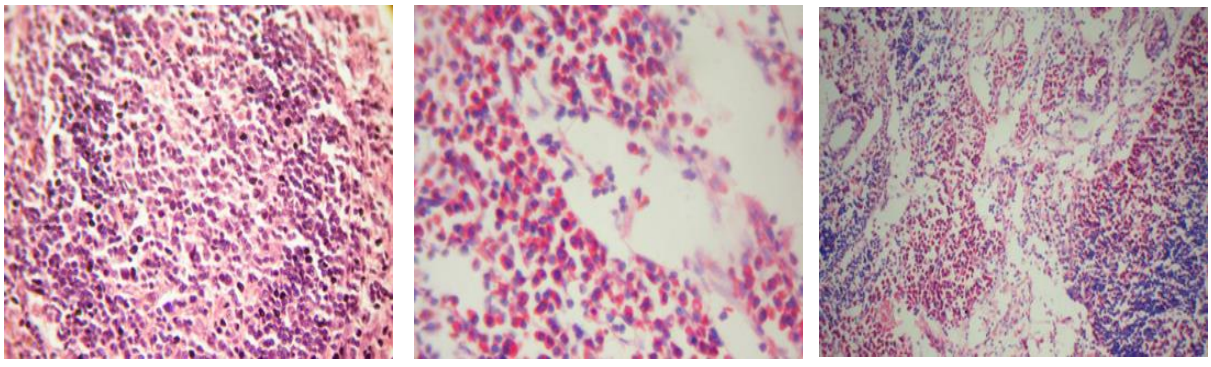
The content of B-lymphocytes populations in the spleen and lymph nodes of experimental animals in 2 and 8 hours after putrescine administration

Study groups, n = 16	Content of B-lymphocytes populations					
	Spleen			Lymph nodes		
	Red pulp strands of spleen	Reactive center of follicles	Mantle and marginal zones of follicles	Cellular strands of medullary layer	Reactive center of follicles	Marginal parts of follicles
Control	24.6 ± 6.7	9.1 ± 2.3	28.7 ± 5.1	35.8 ± 6.1	7.4 ± 2.3	23.8 ± 4.1
Experimental group 1 (in 2 hours)	29.3 ± 5.4 0.4 > P > 0.25	11.3 ± 3.8 0.4 > P > 0.25	24.2 ± 3.9 0.25 > P> 0.1	40.2 ± 6.9 0.4 > P > 0.25	8.8 ± 2.1 0.4 > P > 0.25	26.1 ± 3.3 0.4 > P > 0.25
Experimental group 2 (in 8 hours)	50.1 ± 8.2 0.025 > P > 0.01	19.7 ± 3.4 0.025 > P > 0.01	56.2 ± 7.8 0.01 > P > 0.005	61.4 ± 8.0 0.005 > P > 0.0005	19.6 ± 3.5 0.01 > P > 0.005	39.7 ± 5.4 0.01 > P > 0.005

Table 2

The number of lymphocytes in which the presence of IL-1α was recorded in structural components of spleen and lymph nodes of experimental animals under the conditions of putrescine administration

Study groups n = 16	Number of IL-1α-containing lymphocytes			
	Spleen		Lymph nodes	
	Splenetic strands of red pulp	Mantle and marginal zones of follicles	Marginal parts of follicles	Medullary strands
Control	6.4 ± 1.9	3.7 ± 1.2	4.3 ± 1.6	3.8 ± 1.1
Experimental group 1 (in 2 hours)	8.2 ± 2.7 0.4 > P > 0.25	3.2 ± 0.7 0.4 > P > 0.25	3.0 ± 1.8 0.4 > P > 0.25	5.2 ± 0.8 0.25 > P > 0.1
Experiment group 2 (in 8 hours)	16.1 ± 3.5 0.025 > P > 0.01	9.5 ± 2.1 0.025 > P > 0.01	8.7 ± 1.6 0.05 > P > 0.025	7.4 ± 1.6 0.05 > P > 0.025



a **b** **c**

Fig 1. Structural shifts in the peripheral organs of immunogenesis in experimental mice 8 hours after the putrescine administration. **a** – Mantle zone of spleen follicle appears unevenly widened and is presented by cells of lymphocytar line. Hematoxylin and eosin. Obj. 20; oc. 7; **b** – Pulpar strands of spleen are widened and characterized by compact localization in reticular stroma of lymphocytes and plasmatic cells. Brachet reaction for RNA. Obj. 20; oc. 10; **c** – Medullar layer of the lymph node is presented by compactly and loosely oriented "hypertrophied" medullar" strands mainly presented by lymphocytes and plasma cells. Brachet reaction for RNA. Obj. 10; oc. 7

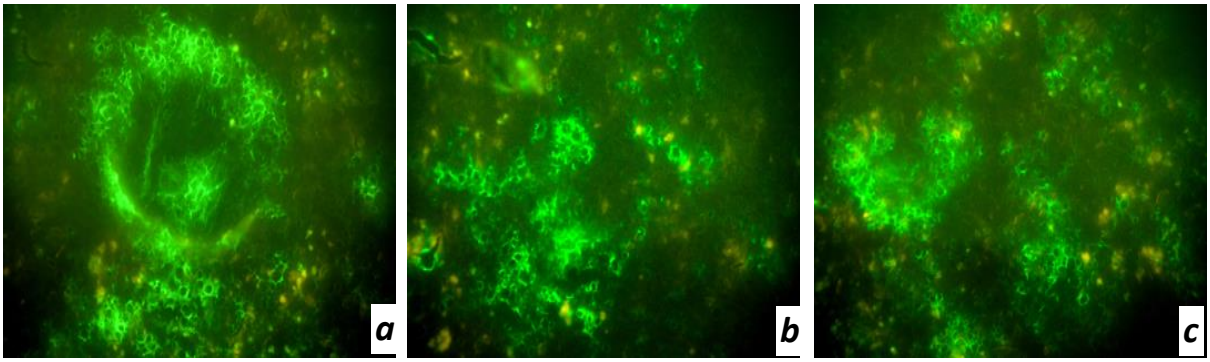


Fig. 2. Immunomorphological shifts in the peripheral organs of immunogenesis of experimental mice 8 hours after the putrescine administration. **a** – Presence of B-lymphocytes populations in the reactive center and marginal zone of follicle of spleen. Direct fluorescence reaction for revealing IgG in cells of lymphocytar and plasmacytar line. Obj. 40; oc. 7; **b** – Presence of IL-1α in lymphocytes of pulpar strands of spleen. Indirect immunofluorescence reaction for revealing IL-1α in cells of the lymphocytes and macraphageal line. Obj. 40; oc. 7; **c** – Presence of IL-1α in the lymphocytes of medullary strands of a lymph node. Indirect immunofluorescence reaction for revealing IL-1α in lymphocytes and macrophages. Obj. 40; oc. 7

Conclusion

Single intravascular administration of putrescine to experimental animals was followed by differently directed shifts in the central and peripheral organs of immunogenesis. Eight hours after the putrescine administration, the initial stages of "accidental involution" process of the cortical layer were recorded in the thymus of mice.

Eight hours after the putrescine administration a structural rearrangement of lymphoid tissue occurred in the spleen and lymph nodes of experimental animals; this latter was manifested by the signs of B-dependent zones activation with the subsequent increase of B-lymphocytes populations, which served as a source of IL-1α enhanced synthesis.

On the basis of performed morphological, morphometric and immunomorphological studies a supposition might be made: a rather low (similar to those in blood serum of mammals) concentrations of putrescine administered experimentally produce a direct stimulating influence on the intercellular cooperation formation processes, which are engaged in activation of humoral immunity in immunocompetent cells of the spleen and lymph nodes.

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