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MICROMORPHOMETRIC CHANGES IN RATS SPLEEN IN THE FIRST 7 DAYS AFTER SKIN BURNS AND UNDER APPLICATION OF INFUSION SOLUTIONS

Summary. *In the experiment, during 7 days, were studied changes in the relative volume of white and red spleen pulp of rats without burn and after burning of the skin with 0.9% solution of NaCl, lactoprotein with sorbitol or HAES-LX-5%. When introducing infusion solutions for animals without burning of skin set only 10-13% higher relative volume of white pulp and 7-11% lower values of the relative volume of red pulp when injected lactoprotein with sorbitol than with the introduction of 0.9% NaCl solution or HAES-LX-5%. On the background of skin burns set: reduction when administrating lactoprotein with sorbitol by 4.1%, and when HAES-LX-5% applied by 7.8% of the relative volume of white pulp from 1 to 7 days of the experiment; higher (14.4 and 18.0% respectively) values of the relative volume of white pulp and smaller (7.1 and 9.1% respectively) of the relative volume of red pulp in rats after 3 days after burning of the skin at the administration of HAES-LX-5% or lactoprotein with sorbitol than in animals at a given time when administering 0.9% NaCl solution; larger by 19.4% values of relative volume of white pulp and 5.8% smaller by the value of the relative volume of red pulp in rats 7 days after skin burns when HAES-LX-5% solution is injected than in animals of present the term with administration of 0.9% NaCl solution; less by 7.9% values of the relative amount of red pulp in rats 7 days after skin burns when administering lactoprotein solution with sorbitol than in animals at this time administering with 0.9% NaCl solution.*

Key words: *micromorphometry, spleen, rats, 0.9% NaCl solution, lactoprotein with sorbitol, HAES-LX-5%.*

Introduction

At present time, the spectrum of damage and compensatory changes in the immunocompetent organs (including the spleen) following a burn injury of the skin, along with macrometric rearrangements has expanded to histological, cytological and ultrastructural changes [4-6, 17]. All this leads to the treatment and prevention of complications of burn disease, guided by a preliminary assessment of the morpho-functional state of immune competent organs with subsequent adequate infusion therapy.

A promising direction of treatment of the consequences of burn intoxication is the use of domestic combined organoprotective colloidal-crystalloid drug solutions, which have several advantages over mono-preparations. In this plan, our attention was attracted by lactoprotein with sorbitol [10, 14] and HAES-LX-5% - a new blood substitute developed by the Institute of Blood Pathology and Transfusion Medicine of the Academy of Medical Sciences of Ukraine [11]. Their use requires prior careful study of morphological and morphometric changes at different levels of structural organization of the spleen, features of the cell cycle and DNA fragmentation in the correction of the effects of thermal trauma of the skin.

The *purpose* of the study was to determine, at the optical-optical level, stereological changes in the parenchyma of the spleen of rats in 1-7 days after skin burn and application of 0.9% NaCl solution, lactoprotein with sorbitol and HAES-LX-5%.

Materials and methods

Within the framework of scientific cooperation between National Pirogov Memorial Medical University, Vinnytsya and SI "Institute of blood pathology and transfusion medicine of NAMS of Ukraine" (Lviv) and National Pirogov Memorial Medical University, Vinnytsya and the National Medical University named after O.O. Bogomolets an experimental study of the effect of the control infusion drug - 0.9% solution of NaCl, reference product - solution of lactoprotein with sorbitol (serially issued by Kyiv "Biopharm" Company - certificate of state registration of the Ministry of Health of Ukraine № 464/09-300200000 dated 12.03.2009 - is a protein-saline solution that contains 5% of colloidal base and 5% sodium bicarbonate sorbitol, 6% sodium citrate, 2.1% lactate, sodium chloride 0.8%, calcium chloride 0.01%, potassium chloride - 0.0075%, sodium bicarbonate - 0.01%;

ion contain of drug has the following structure: Na^+ - 343.5 mmol/L, K^+ - 1.0 mmol/L, Ca^{++} - 0.9 mmol/L, Cl^- - 139.7 mmol/L, HCO_3^- - 1.2 mmol/L, $\text{CH}_3\text{CH}(\text{OH})\text{COO}^-$ - 187.4 mmol/L, osmolarity of the solution is 1020 mosmol/L) and the study drug HAES-LX-5% solution (developed in the in SI of "Blood Pathology and Transfusion Medicine of the National Academy of Medical Sciences of Ukraine" - as a colloidal base contains poly(0-2-hydroxyethyl) starch - 5% (the average molecular weight is 130 000 Dalton, the degree of molecular substitution 0,4), as well as polyether alcohol xylitol - 5%, ingestion of sodium lactate - 1,5%, sodium chloride - 0,8%, potassium chloride - 0,03%, calcium chloride - 0,02%, magnesium chloride - 0,01%; The ionic composition of the solution has the following structure: Na^+ - 270.7 mmol/L, K^+ - 4.0 mmol/L, Ca^{++} - 1.8 mmol/L, Mg^{++} - 1.1 mmol/L, Cl^- - 146.6 mmol/L, $\text{CH}_3\text{CH}(\text{OH})\text{COO}^-$ - 133.8 mmol/L; the theoretical osmolarity of the preparation that was studied - 890 mosmol/L, which is 3 times higher than the 0.9% NaCl solution and osmolarity of the blood plasma [11]) on the structure of the spleen of the intact rats, as well as in the early stages (1, 3 and 7 days) after a burn injury to the skin.

The research was carried out on laboratory white rats, males weighing 155-160 g, obtained from the vivarium of the Institute of Pharmacology and Toxicology of the Academy of Medical Sciences of Ukraine. During the experiment all animals were kept under vivarium of National Pirogov Memorial Medical University, Vinnytsya (indoor temperature - within 24-25 °C, humidity - within 40-60%) on a standard water and food ration, with free access to water and food. All experiments were carried out taking into account the recommendations of the European Commission on conducting medical-biological research on the use of animals and medical recommendations of the State Pharmacological Center of the Ministry of Health of Ukraine and "Rules for the clinical evaluation of safety of pharmacological agents (GLP)" [12, 16] and the rules of humane treatment of experimental animals (approved by the Committee on Bioethics of the National Pirogov Memorial Medical University, Vinnytsya - Minutes № 1 by 14.01.2010).

Laboratory research was carried out at the bases of the laboratory of functional morphology and genetics of the development of SSC National Pirogov Memorial Medical University, Vinnytsya (certified by the State Pharmacological Center Ministry of Health of Ukraine, certificate number 003/10 dated January 11, 2010) and the laboratory of the Department of Pharmacology National Pirogov Memorial Medical University, Vinnytsya (certified by the State Pharmacological Center Ministry of Health of Ukraine, certificate number 000679 dated January 11, 2008).

All solutions were injected into the lower vena cava after its catheterization in aseptic conditions through the femoral vein at a dose of 10 ml/kg body weight of the animal - the average therapeutic dose calculated by the developers HAES-LX-5% according to the existing recommendations [16]. After each administration of colloidal-hyperosmolar solutions, the lumen of the catheter under the skin was filled with titrated

heparin solution (0.1 ml of heparin per 10 ml of 0.9% NaCl solution). The first introduction of colloid-hyperosmolar solutions (within 5-6 minutes) was carried out 1 hour after the start of the experiment (depilation of the lateral surfaces of the trunk and burning of the skin), and subsequent infusions - 1 time per day during the first 7 days of the experiment.

Shaving of the lateral surfaces of the trunk of the rat, catheterization of the veins, staining of skin burns and decapitation of the animals were carried out under conditions of intravenous propofol anesthesia (at the rate of 60 mg/kg of animal weight).

180 rats were divided into 6 groups in the experiment: 1, 2 and 3 groups - rats without thermal trauma who were infused with 0.9% NaCl solution, lactoprotein with sorbitol and HAES-LX-5% in dose 10 ml per kg. In the 4th, 5th and 6th groups, 0.9% NaCl solution, lactoprotein with sorbitol and HAES-LX-5% infusion were administered to the rats at a dose of 10 ml per kg after skin burn.

Burn skin damage was caused by applying to the pre-depilated lateral surfaces of the trunk of the rats for 10 seconds by four copper plates (two plates on each side, each with a surface area of 13.86 cm²) which were preheated for 6 minutes in constant temperature water 100 C° [8, 15]. According to the formula M. O. Lee [13], the total area of skin surface damage in rats was 21-23%. This area at this exposure is sufficient for the formation of the second-third degree burns (according to the classification adopted at the 20th Congress of Surgeons of Ukraine, Ternopil, 2000) and the induction of a shock state of moderate severity [9].

The stereological study of the spleen tissues was carried out on a Laborlox S (Leitz) microscope on the demonstration screen with an increase of 40/1.25x10 using the Weibull grid [1]. The following formula was used to determine the volume density (relative volume, cm³/cm³) of the red and white pulp of the spleen: $V_{vi} = P_i / P_T$; where V_{vi} is the volume density of the corresponding parts of the spleen; P_i is the number of test points falling into the corresponding structures; P_T - aggregate number of test points.

In each of the histological preparations (6 in each group of animals) in different zones of the spleen by random selection, 5 fields of vision were selected in which the stereological parameters were determined.

The statistical processing of the obtained results was carried out in the licensed package "Statistica 6.1" with the use of nonparametric methods for evaluating the results. Evaluated the character of the distribution of signs for each of the obtained variation series, set the average values of each characteristic, studied and the values of standard quadratic deviations. The reliability of the differences between the independent quantitative values was determined using the Man-Whitney U-criterion [3].

Results. Discussion

Relative volume of white pulp in rats without burning skin injury, which was injected 0.9% NaCl solution after 1 day is 0,398±0,131 cm³/cm³, after 3 days is 0,401±0,128

cm³/cm³, after 7 days - 0,405±0,156 cm³/cm³. Relative volume of red pulp in rats without burn injuries of skin, which was injected 0.9% NaCl solution after 1 day 0,600±0,132 cm³/cm³, after 3 days is 0,599±0,128 cm³/cm³, after 7 days - 0,594±0,156 cm³/cm³.

Relative volume of white pulp in rats without burning skin injury, which was injected with lactoprotein with sorbitol solution after 1 day 0,440±0,151 cm³/cm³, after 3 days is 0,448±0,131 cm³/cm³, after 7 days - 0,460±0,165 cm³/cm³. Relative volume of red pulp in rats without burn injuries of skin, which was injected with lactobacillus solution with sorbitol after 1 day is 0,558±0,151 cm³/cm³, after 3 days, is 0,548±0,128 cm³/cm³, after 7 days - 0,534±0,161 cm³/cm³.

Relative volume of white pulp in rats without burn injury caused by HAES-LX-5% solution, after 1 day 0,403±0,124 cm³/cm³, after 3 days is 0,416±0,108 cm³/cm³, after 7 days - 0,413±0,156 cm³/cm³. Relative volume of red pulp in rats without burn injuries of skin, which was injected HAES-LX-5% solution, after 1 day 0,597±0,124 cm³/cm³, after 3 days is 0,584±0,108 cm³/cm³, after 7 days - 0,571±0,148 cm³/cm³.

An analysis of the relative volume of white and red pulp in rats without skin burn, which colloidal hyperosmolar solutions was administered during the first seven days of the experiment, did not reveal any significant changes in the values of these indices after 1, 3 and 7 days from the beginning of the experiment. Attention is drawn only to 10-13% higher relative volumes of white pulp and 7-11% lower values of the relative volume of red pulp in animals when administering lactoprotein with sorbitol than when administering 0.9% NaCl solution or HAES-LX-5%, which coincides with the peculiarities of the dynamics of structural changes in the spleen of rats at the light-optical level when infused with lactoprotein with sorbitol [2].

Relative volume of white pulp in rats 1 day after burn skin injury when administered 0.9% NaCl solution is 0,348±0,122 cm³/cm³, which is 12.6% less (p>0.05) than in rats without burn injuries of the skin which were injected with the same solution in a similar period of observation. The relative volume of red pulp is 0,647±0,123 cm³/cm³, which is 7.8% more (p>0.05) than in rats without burning skin injury, which was injected with a 0.9% NaCl solution at the same time of observation.

Relative volume of white pulp in rats 3 days after skin burn injury when administered 0.9% NaCl solution is 0,305±0,169 cm³/cm³, which is 23.9% less (p<0.01) than in rats without burn injuries of the skin which was injected 0.9% NaCl solution at the same time of observation. The relative volume of red pulp is 0,693±0,167 cm³/cm³, which is 15.7% more (p<0.05) than in non-burning skin injuries of rats, which injected a 0.9% NaCl solution at the same time of observation.

Relative volume of white pulp in rats 7 days after skin burn injury when administered 0.9% NaCl solution is 0,278±0,127 cm³/cm³, which is 31.6% less (p<0.01) than in rats without burn injuries of the skin which was injected

0.9% NaCl solution at the same time of observation. Relative volume of red pulp is 0,699±0,133 cm³/cm³, which is 17.7% more (p<0.05) than in rats without burning skin injury, which was injected with a 0.9% NaCl solution at the same time of observation.

Relative volume of white pulp in rats administered lactoprotein with sorbitol after 1 day after burn skin injury is 0,370±0,140 cm³/cm³, which is 15.9% less (p>0.05) than in non-burning skin rats injected a similar solution at the same time of observation. Relative volume of red pulp in rats administered lactoprotein with sorbitol 1 day after the burn skin injury is 0,630±0,140 cm³/cm³, which is 12.9% more (p = 0.068) than in non-burning skin rats, which were given similar solution at the same time of observation.

The relative volume of white pulp in rats by which the lactoprotein with sorbitol was injected 3 days after the skin burn injury is 0,360±0,143 cm³/cm³, which is 19.6% less (p<0.01) than that of non-burning skin rats injected a similar solution at the same time of observation. Relative volume of red pulp in rats by which the solution of lactoprotein with sorbitol was injected 3 days after the burn injury of the skin is 0,637±0,142 cm³/cm³, which is 16.2% more (p<0.01) than in non-burning skin rats injected a similar solution at the same time of observation.

Relative volume of white pulp in rats by which the lactoprotein with sorbitol was injected 7 days after the skin burn injury is 0,355±0,148 cm³/cm³, which is 22.8% less (p<0.05) than in non-burning skin rats injected a similar solution at the same time of observation. Relative volume of red pulp in rats, which was injected with a solution of lactoprotein with sorbitol 7 days after the burn injury of the skin, is 0,644±0,148 cm³/cm³, which is 20.6% more (p<0.01) than in non-burning skin rats injected a similar solution at the same time of observation.

Comparing the stereometric changes in the relative volume of the white and red pulp of the spleen in the first seven days after the burn injury of the skin and the use of a solution of lactoprotein with sorbitol, with results after burning of the skin and the use of 0.9% NaCl solution attracted the following differences: larger by 18.0% (p>0.05) value of the relative volume of white pulp and lower by 9.1% (p>0.05) the value of the relative volume of red pulp in rats 3 days after burning of the skin with the introduction of lactoprotein with sorbitol, than in animals at a given time when the 0.9% solution of NaCl was introduced I; 7.9% (p>0.05) values of the relative amount of red pulp in rats were lower in 7 days after burning of the skin with administration of lactoprotein with sorbitol than in animals at the given time when 0.9% solution of NaCl was administered.

Relative volume of white pulp in rats by which HAES-LX-5% solution was injected 1 day after the skin burn injury is 0,360±0,171 cm³/cm³, which is 10.7% less (p>0.05) than in rats without skin burn which was administered a similar solution at the same time of observation. Relative volume of red pulp in rats by which the HAES-LX-5% solution was injected 1 day after the skin burn injury is 0,640±0,171 cm³/

cm³, which is only 7.2% higher ($p > 0.05$) than in rats without burning the skin, which was injected with a similar solution at the same time of observation. This is due to the stenosis of the sinusoid, as well as the decrease of the marginal zone in the lymphoid follicles and periarterial region [7].

Relative volume of white pulp in rats by which the HAES-LX-5% solution was injected 3 days after the skin burn injury is $0,349 \pm 0,151 \text{ cm}^3/\text{cm}^3$, which is 16.1% less ($p < 0.05$) than in rats without skin burn which was administered a similar solution at the same time of observation. The relative volume of red pulp in rats, which was injected HAES-LX-5% solution, after 3 days after the skin burn injury was $0,644 \pm 0,144 \text{ cm}^3/\text{cm}^3$, which is 10.3% more ($p = 0.060$) than in non-burning skin rats which was administered a similar solution at the same time of observation.

Relative volume of white pulp in rats by which the HAES-LX-5% solution was injected 7 days after the skin burn injury is $0,332 \pm 0,108 \text{ cm}^3/\text{cm}^3$, which is 19.6% less ($p = 0.067$) than in non-burning skin rats which was administered a similar solution at the same time of observation. Relative volume of red pulp in rats by which the HAES-LX-5% solution was injected 7 days after the skin burn injury is $0,659 \pm 0,139 \text{ cm}^3/\text{cm}^3$, which is 15.4% more ($p < 0.05$) than in rats without skin burn which was administered a similar solution at the same time of observation.

Comparing the stereometric changes in the relative volume of the white and red spleen of the spleen in the first seven days after skin burn injury and the use of the HAES-LX-5% solution, with results after burning the skin and applying 0.9% NaCl solution, draw attention to the following differences: larger on 14.4% ($p > 0.05$) value of the relative volume of white pulp and lower by 7.1% ($p > 0.05$) the value of the relative volume of red pulp in rats 3 days after skin burn in the introduction of HAES-LX-5%, than in animals at the given time when the 0.9% solution of NaCl was introduced; the values of relative volume of white pulp and the smaller by 5.8% ($p > 0.05$) were higher by 19.4% ($p > 0.05$), the value of the relative volume of red pulp in rats 7 days after burning of the skin at administration of a solution of HAES-LX-5%, than in animals at a given time, with administration of 0.9% solution of NaCl.

The changes in the relative volume of the white and red spleen in the first seven days after the burn injury of

the skin and the use of infusion solutions coincide with the features of the dynamics of structural changes in the spleen of the rats at the light-optical level [2, 7].

Conclusions and perspectives of further development

1. The relative volume of white and red pulp in rats without skin burn, which during the first seven days of the experiment were administered infusion solutions practically did not change after 1, 3 and 7 days from the beginning of the experiment. Only 10-13% higher values of the relative volume of white pulp and only 7-11% lower values of the relative volume of red pulp in animals when administered with lactoprotein with sorbitol were established only when compared with administration of 0.9% NaCl solution or HAES-LX-5%.

2. The following dynamics of changes in the relative volume of white and red pulp in animals after the skin burn following the introduction of infusion solutions was established: reduction when administering of solution lactoprotein with sorbitol by 4.1%, and when administered HAES-LX-5% by 7.8% relative volume of white pulp from 1 to 7 days of the experiment; higher (14.4 and 18.0% respectively) values of the relative volume of white pulp and smaller (7.1 and 9.1% respectively) of the relative volume of red pulp in rats after 3 days after burning of the skin at the administration of HAES-LX-5% or lactoprotein with sorbitol than in animals at a given time when administering 0.9% NaCl solution; larger by 19.4% values of relative volume of white pulp and 5.8% smaller by the value of the relative volume of red pulp in rats 7 days after skin burns when HAES-LX-5% solution is injected than in animals of present the term with administration of 0.9% NaCl solution; less by 7.9% values of the relative amount of red pulp in rats 7 days after skin burns when administering lactoprotein solution with sorbitol than in animals at this time administering with 0.9% NaCl solution.

Prospects for further development: the established stereometric changes in the red and white pulp of the spleen of rats in the experimental burn disease indicate the expediency of the use of infusion solutions of lactoprotein with sorbitol and HAES-LX-5% in order to prevent the negative changes in the spleen under conditions of burn shock.

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- ploscheyu 21-23% poverhni shkiri ta yih korektsiya infuziynim rozchinom HAES-LX-5%. *Biomedical and Biosocial Anthropology*, 21, 93-97. (in Ukraine)
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МІКРОМОРФОМЕТРИЧНІ ЗМІНИ В СЕЛЕЗІНЦІ ЩУРІВ У ПЕРШІ 7 ДІБ ПІСЛЯ ОПІКУ ШКИРИ ТА ПРИ ВВЕДЕННІ ІНФУЗІЙНИХ РОЗЧИНІВ

Резюме. В експерименті на протязі 7 діб вивчені зміни відносного об'єму білої і червоної пульпи селезінки щурів без опіку і після опіку шкіри при введенні 0,9% розчину NaCl, лактопротеїну з сорбітолом або HAES-LX-5%. При введенні інфузійних розчинів тваринам без опіку шкіри встановлено лише на 10-13% більші значення відносного об'єму білої пульпи та на 7-11% менші значення відносного об'єму червоної пульпи при введенні лактопротеїну з сорбітолом, ніж при введенні 0,9% розчину NaCl або HAES-LX-5%. На фоні опіку шкіри встановлено: зменшення при введенні лактопротеїну з сорбітолом на 4,1%, а при введенні HAES-LX-5% на 7,8% відносного об'єму білої пульпи від 1 до 7 доби експерименту; більші (відповідно на 14,4 і 18,0%) значення відносного об'єму білої пульпи та менші (відповідно на 7,1 і 9,1%) значення відносного об'єму червоної пульпи у щурів через 3 доби після опіку шкіри при введенні HAES-LX-5% або лактопротеїну з сорбітолом, ніж у тварин в даний термін при введенні 0,9% розчину NaCl; більші на 19,4% значення відносного об'єму білої пульпи та менші на 5,8% значення відносного об'єму червоної пульпи у щурів через 7 діб після опіку шкіри при введенні розчину HAES-LX-5%, ніж у тварин в даний термін при введенні 0,9% розчину NaCl; менші на 7,9% значення відносного об'єму червоної пульпи у щурів через 7 діб після опіку шкіри при введенні розчину лактопротеїну із сорбітолом, ніж у тварин в даний термін при введенні 0,9% розчину NaCl.

Ключові слова: мікроморфометрія, селезінка, щури, 0,9% розчин NaCl, лактопротеїн з сорбітолом, HAES-LX-5%.

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МІКРОМОРФОМЕТРИЧЕСКИЕ ИЗМЕНЕНИЯ В СЕЛЕЗЕНКЕ КРЫС В ПЕРВЫЕ 7 ДНЕЙ ПОСЛЕ ОЖОГА КОЖИ И ПРИ ВВЕДЕНИИ ИНФУЗИОННЫХ РАСТВОРОВ

Резюме. В эксперименте в течении 7 дней изучены изменения относительного объема белой и красной пульпы селезенки крыс без ожога и после ожога кожи при введении 0,9% раствора NaCl, лактопротеина с сорбитолом или HAES-LX-5%. При введении инфузионных растворов животным без ожога кожи установлено лишь на 10-13% большие значения относительного объема белой пульпы и на 7-11% меньшие значения относительного объема красной пульпы при введении лактопротеина с сорбитолом, нежели при введении 0,9% раствора NaCl или HAES-LX-5%. На фоне ожога кожи установлено: уменьшение при введении лактопротеина с сорбитолом на 4,1%, а при введении HAES-LX-5% на 7,8% относительного объема белой пульпы от 1 до 7 суток эксперимента; большие (соответственно на 14,4 и 18,0%) значения относительного объема белой пульпы и меньшие (соответственно на 7,1 и 9,1%) значения относительного объема красной пульпы у крыс через 3 суток после ожога кожи при введении HAES-LX-5% или лактопротеина с сорбитолом, нежели у животных в данный термин при введении 0,9% раствора NaCl; большие на 19,4% значения относительного объема белой пульпы и меньшие на 5,8% значения относительного объема красной пульпы у крыс через 7 дней после ожога кожи при введении раствора HAES-LX-5%, нежели у животных в данный термин при введении 0,9% раствора NaCl; меньшие на 7,9% значения относительного объема красной пульпы у крыс через 7 дней после ожога кожи при введении раствора лактопротеина с сорбитолом, нежели у животных в данный термин при введении 0,9% раствора NaCl.

Ключевые слова: микроморфометрия, селезенка, крысы, 0,9% раствор NaCl, лактопротеин с сорбитолом, HAES-LX-5%.

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