EVALUATION OF THE EFFECT OF INFUSION OF COMPOSITE HYPEROSMOLAR SOLUTIONS ON THE STRUCTURE OF NEUROIMMUNOENDOCRINE SYSTEM ORGANS IN BURN DISEASE

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ABSTRACT
The paper presents data relating to structural changes in rat adenohypophysis, thymus and adrenal cortex in experimental burn disease and its treatment with composite hyperosmolar solutions. The findings showed that intravenous hyperosmolar solutions acted as cytoprotectors.

Keywords: burn disease, cytoprotection, electron microscopy.

INTRODUCTION
Admittedly, burn disease is the specific symptom complex developing after thermal injury (if the superficial burn surface exceeds 10-12%, and deep burn surface exceeds 5-6%) and accompanied by the damage of all auto regulated systems [5, 8]. First of all, it concerns the neuroimmunoendocrine system [2, 3], the major organs of which are the hypophysis, adrenal glands and thymus [1]. Since intoxication, among other processes, plays an important role in starting mechanism of pathological reactions in burn disease [2, 3, 8], the developments of the novel methods of detoxification and comparative analysis of different infusion medications are crucial [4, 6].
PURPOSE

The paper was aimed at the evaluation of the efficacy of infusion of composite hyperosmolar solutions of disintoxicative, rheological and antishock effect on the adenohypophysis, adrenal cortex and thymus structure in burn disease.

MATERIALS AND METHODS

The experimental study of morphological changes in adenohypophysis, thymus and adrenal cortex in burn disease (in 1, 3, 7, 14, 21, 30 days after burn injury) and in condition of the effect of HAES-LX-5% hyperosmolar infusion medications of disintoxicative, rheological, energy and antishock effect, and lactoprotein with sorbitol has been made on 90 Wistar male rats, weighted 155-160 g.

Lactoprotein with sorbitol is a protein-salt product that contains albumin (5%), sorbitol (6%), sodium lactate (21.1%), as well as electrolytes in balanced amount. Osmoarity of medication is 1020 mOsm/l [4].

HAES-LX-5% is colloid-hyperosmolar medication which contains hydroxyethylated starch with MM 130,000, xylitol, sodium lactate, salts of sodium, potassium, calcium and magnesium as a colloid base. Osmolarity of the medication is 890 mOsm/l, which is three times higher than the osmolarity of sodium chloride isotonic solution and osmolality of blood plasma.

Animal housing and experiments on them have been carried out in compliance with the “General Ethic Rules for Conducting Experiments on Animals”, approved by the First National Bioethics Congress (Kyiv, 2001), and the requirements of international principles of the “European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes” (Strasbourg, 1985), as well as the principles of “Good laboratory practice for safety tests on chemicals (GLP)”.

The animals were assigned to 7 groups: Group I – intact animals; Group II, III, IV – rats without thermal injury, administered with 10 ml/kg of 0,9% NaCl solution, HAES-LX-5% and lactoprotein with sorbitol, respectively; Group V, VI, VII – animals with the burn, administered with the investigated agents according to regimen and dose, similar to Group II, III, IV animals.

The burn (after proper premedication) was induced by placing of four copperplates, pre-soaked in water at 100 °C for 6 min, to the lateral surfaces of the animals’ body (two plates per each side). The total area of burn in rats accounted for 21-23% in 10 sec exposure that was sufficient for the formation of the second-degree burn, i.e., dermal superficial burn and the development of moderate state of shock.

The investigated solutions were administered intravenously during 5-6 min in a dose of 10 ml/kg body weight. The infusion was conducted in the lower hollow vein with its catheterization in aseptic conditions through the femoral vein. Catheter, installed in femoral vein, was sewed subcutaneously. Its lumen was filled with heparin titrating solution (0,1 ml heparin per 10 ml 0,9% NaCl solution) along the entire length after each administration of agents. The first administration of solutions was made after 1 hour after modeling of pathological state, and the follow-up infusions were performed daily during 7 days.

The control group with clear dermal burn without treatment (when the animals’ lethality is definitely maximal) was not used during the experiment since the study considered the structural mechanisms of cytoprotection in burn disease. To control the therapeutic effect of hyperosmolar solutions we used the group of burn-induced animals, administered with 0,9% NaCl solution.

In the group of animals with skin burn injury, administered with 0,9% NaCl solution, a progressing increase of the lethality rate from 5% after day 1 to 11% in the interval from day 4 to day 7 with the follow-up gradual reduction of this rate to 3% in the interval from day 22 to day 30 after the dermal burn was detected. The overall rate of lethality in the group of male rats, administered with 0,9% NaCl solution after skin burn accounted for 43.5%. The specific therapeutic course treatment of rats with skin burn injury with
HAES-LX-5%, similar to lactoprotein with sorbitol, significantly prevented the deaths of animals throughout the observation.

Material sampling was made under anesthesia. After animals’ decapitation the autopsy of head, abdominal and thoracic cavities was made. Small pieces of investigated organs were cut using a razor. Material for morphological analysis was prepared in compliance with the conventional technique.

Ultrathin slices were prepared in the “LKB” ultramicrotom, analyzed and photographed on PEM-125K electron microscope. Semithin slices were stained with toluidine and methylene blue, analyzed and photographed, using Olympus BX51 microscope.

The experiment was carried out on the basis of the Research Center at N.I. Pirogov Vinnitsa National Medical University. Electron microscope analysis was made on the basis of the Department of Electron Microscopy (supervisor, Professor K.A. Stechenko) at the Institute of Pathology Problems at Bogomolets National Medical University.

RESULTS AND DISCUSSION

After day 1, 3, 7 and 14 of the experiment (time periods when the increase and stabilization of the value of lethality rate was recorded) in adenohypophysis, thymus and adrenal cortex of rats with skin burn injury, administered with 0.9% NaCl solution, the most specific common manifestation of pathomorphological changes was the alteration of functionally different cells of organs and hemomicrocirculatory stream’s vascular walls, occurrence of splinter hemorrhages, and development of pronounced intercellular and paravasal edemas. The reason for hemorrhages and edemas is the thinning of the endothelial lining, dilatation of interendothelial gaps, as well as detachment of endotheliocytes from the basal membrane, caused by the focal lysis of the basal membrane and strength reduction of endotheliocytes’ integrity in the monolayer, accompanying by transformation of endotheliocytes’ shapes and configuration of its pericontact zones.

Structural manifestations, mentioned above, could be the reflection of correlation of several factors: 1) impairment of plasmolemma flexibility, modified by the oxidative stress [7]; 2) disproportions of cytoplasmic matrix hydration in cell’s water-electrolytic balance (evident by the detected impairments of cytoplasmic matrix’s electron density); 3) excessive organization and distortion of cytoskeleton kinetic potency (associated with multifocal damages of its fibrillar structures and impairments of its contacts with plasmolemma).

The wide variation in the degree of interendothelial joints dilatation in the wall of the blood capillaries and venules of the investigated organs, identified in burn disease, leads to a number of unfavorable consequences. Dilatation results in conversion of interendothelial joints from the macromolecular substances transport pathways, controlled by the cell, on hematointerstitial pressure gradient into open-ended ducts with unlimited passing ability, far exceeding the transport capacity by means of micropinocytotic vesicles. Consequently, almost free path is opened for any coarse plasma components, including endotoxins (initiating and supporting the development of multiple organ dysfunction syndrome in burn disease) to be entered into an organ. In the areas where the regions of endothelial lining thinning and dilatation of interendothelial joints matches with the basal membrane lysis loci the open-ended transmural defects are formed (Fig. 1; Fig. 2). The described transmural defects coupled with the adjacent and dilated (as a result of the edema development) intercellular spaces are the places of plasma and blood cells leakage and intraorganic penetration, leading to edema and haemorrhages progressing.

In rats with burn injury (Group VI and VII), administered which hyperosmolar solutions according to the regimen of the experiment, no significant damage to vascular walls and haemorrhage was detected in adenohypophysis, thymus and adrenal cortex, as well as no structural signs of paravasal and intercellular
edema were recorded. This indicates about the angioprotective properties of the applied composite hyperosmolar solutions, providing with rather specific membranoflexible effect if used in combination with lactoprotein with sorbitol.

After 3 days the amorphous electron-dense contents, which is typically spread along the luminal boundary of endotheliocytes (forming the electron-dense border relevant to its configuration) was detected in the lumen of some blood capillaries and venules of the investigated organs of animals with burn injury, administered with lactoprotein with sorbitol (experimental Group VII), which penetrated beyond the lumen boundaries into intercellular interstitial matrix through "leakage" and "penetration". It should be noted that the total electron density of this intravascular contents is typically significantly less than the density of the cytoplasmic matrix of red blood cells in the vascular lumen (Fig. 3; Fig. 4). The synergy of organs’ endotheliocytes and paravasal cells resulted in the strengthening of the vascular wall and the formation of specific intraorganic membrane-like structures in animals from experimental Group VII only and exclusively.

It has been found that some areas of organs’ parenchyma contain membrane-like structures, and the others have (Fig. 5) a quirky geometric shape or are represented by the specific fragments (Fig. 6). Nevertheless, we hypothesize that the described membrane-like structures form a continuous complex, where prevalence, shape and size of its fragments are defined by the relevant parameters of the dilated intercellular gaps (emerging as a result of the development and spread of intercellular edema). Therefore, a formed (unified and integral?) membrane-like complex becomes an important (changing the cytoarchitecture), permanent (but simulated) barrier structure of the organs, which, however, is characterized by the locality (mosaicism) of allocation of its areas in the parenchyma.

In pathogenetic mechanism of initiation of structural changes in adenohypophysis, adrenal cortex and thymus the microvascular walls damage and impairment (and follow-up recovery in infusion therapy) of its barrier function is the most important but not the only factor that is inseparably connected with the complex of responses of endocrine and immune cells of the analyzed organs according to the principle of mutual potentiation of the effects. A number of vaguely differentiated phases-components of the pathological process can be defined with a certain formality in the development of structural changes in the analyzed organs of neuroimmunoendocrine system. Once occurred, they are also manifested at all subsequent stages of pathological situations with varying intensity. Destructive changes are mainly superpositioned onto clearly pronounced signs of the cell preceding adaptive adjustment: hypertrophy and hyperplasia of the organelles, populating the dilated perinuclear zone and forming more or less considerable clusters in the peripheral portions of cell. In this case, polymorphic, unusually large mitochondria and small hypoplastic organelles are located nearby, and their single short cristas, embedded into matrix’s electrooptical density are exposed to fragmentation and homogenization.

Severe irreversible changes in cell lead to its death by necrosis (typical for cells of analyzed organs of burned animals, infused with 0.9% NaCl solution) or apoptosis (typical for burned animals, administered with HAES-LX-5% or lactoprotein with sorbitol). In cell necrosis (Fig. 7) we observed membrane integrity damaging, modification of nucleus (pyknosis, rhexis, lysis) and cytoplasm (edema), cell destruction. In cell apoptosis (Fig. 8; Fig. 9) we observed condensation of chromatin and cytoplasm, reduction of cell volume (shrinkage), plasmolemmotic vesiculation, cell fragmentation, generation of apoptotic corpuscles.

The resulted data show that the applied composite hyperosmolar solutions prevent cell necrosis, and sporadic irreversibly damaged cells are eliminated by apoptosis. At the same time, the remnants of their apoptotic corpuscles sometimes are densely packed in the intercellular matrix of newly generated granules, fibrils and membranes. The degree of apoptotic corpuscles degradation in the intercellular spaces, filled with electron-dense substance is different. Apparently, part of the degraded material of the destroyed apoptotic
corpuscles integrates into preexisting membrane-like structures, generating (Fig. 10; Fig. 11) their dilated areas (the material of which looks heteromorphous and heterogeneous on the electron diffraction patterns).

Balanced combination of barrier and communicative functions of membrane-like complex in the investigated organs of neuroimmunoendocrine system of burned rats in condition of infusion of lactoprotein with sorbitol does not exclude lateral diffusion of macromolecules, incorporated in membranes (leading to membranes’ reconfiguration and "aging"). Hypothetically, the described membranes perform not only shape-forming function, but also engaged actively in regulatory metabolic processes, placing the functions of the endocrine and immunocompetent cells into varying degree of dependence on the physical-chemical characteristics of significantly modified stroma.

The resulted data show that no full recovery (full regeneration) of cellular and noncellular structures of neuroimmunoendocrine system organs in animals with burn injury after infusion of HAES-LX-5% and lactoprotein with sorbitol occurs (it rather would be expected in complex therapy of burn disease). At the same time, the effect of infusion with investigated hyperosmolar solutions is different because of the fact that the effect of the most important pathogenic factor (represented by endotoxins) is leveled due to enhancement of the barrier function of hematointerstitial barriers [2, 3]. It allows concluding about the originality of structural mechanisms of cytoprotective effect of HAES-LX-5% and lactoprotein with sorbitol.

Fig. 1. Stasis of red blood cells and their aggregation in the dilated lumen of rat adenohypophysis blood capillary after 3 days of burn disease development on condition of administration of 0.9% NaCl solution. Transmural defect (“leakage”) in blood capillary wall is marked by arrow. 1 – red blood cells in the blood capillary wall. Original magnification × 10000.
**Fig. 2.** Thinning of the endothelial lining, dilatation of interendothelial joints and formation of transmural defects (marked by arrow) in rat adrenal cortex blood capillary wall after 3 days of burn disease development on condition of administration of 0.9% NaCl solution. 1 – cellular detritus in blood capillary lumen. Original magnification × 10000.

**Fig. 3.** Electron-dense contents (1) in the venule lumen, spreading through the “leakage” (marked with single arrow) and “penetration” (marked with double arrows) in rat adenohypophysis after 3 days of burn disease development on condition of administration of lactoprotein with sorbitol. 2 – red blood cell in the venule lumen Original magnification × 20000.
Fig. 4. Red blood cells (1) in electron-dense contents of blood capillaries lumen, “decorating” the dilated intercellular gaps of vascular wall as though “disseminating” around rat thymus microvessels after 7 days of burn disease development on condition of administration of lactoprotein with sorbitol. Original magnification × 6000.

Fig. 5. Specific membrane-like complex (marked with arrows) in rat adenohypophysis after 7 days of burn disease development on condition of administration of lactoprotein with sorbitol. Original magnification × 8600.
Fig. 6. Fragments (marked with arrows) of membrane-like complex in rat adenohypophysis after 7 days of burn disease development on condition of administration of lactoprotein with sorbitol. Original magnification × 16000.

Fig. 7. Necrosis of endocrine cell in rat adrenal cortex after 14 days of burn disease development on condition of administration of 0.9% NaCl solution. 1 – nucleus of endocrine cell with typical necrotic lesions. Original magnification × 20000.
Fig. 8. Apoptosis (1) of somatotropocyte in rat adenohypophysis after 21 days of burn disease development on condition of administration of HAES-LX5%. Original magnification ×16000.

Fig. 9. Apoptosis of endocrine cell in rat adrenal cortex after 14 days of burn disease development on condition of administration of HAES-LX5%. 1 – nucleus of endocrine cell with typical apoptotic lesions. Original magnification ×20000.

Fig. 10. Apoptotic corpuscles in the area (marked with arrows) of electron-dense intercellular matrix in rat adenohypophysis after 21 days of burn disease development on condition of administration of lactoprotein with sorbitol. Original magnification × 14000.
CONCLUSIONS

1. Common manifestation of lesions in the organs of neuroimmunoendocrine system in skin thermal injury and developed burn disease is alteration of their histohematogenous barriers.

2. Structural equivalent of alteration of histohematogenous barriers in the organs of neuroimmunoendocrine system in burn disease is the development of edema and hemorrhages, as well as formation of the open-ended transmural defects ("leakage") in the wall of the blood capillaries and some venules and the relevant intraorganic intercellular dilatations ("penetrations").

3. In burn disease lactoprotein with sorbitol and HAES-LX-5% has cyto- and angioprotective effect, inhibits the development of edema, prevents the occurrence of hemorrhages and alteration of cells, contributes to reparation of organs.

4. Application of lactoprotein with sorbitol promoted clear visualization of «leakage" and "penetration", typical in burn disease, into the organs of neuroimmunoendocrine system and evaluated all those structural modifications, associated with the formation of “membrane-like complex” in the analyzed organs, as the compensatory ones.

The perspectives of the subsequent research will encompass the study of the individual effect of each component of lactoprotein with sorbitol and HAES-LX-5% on structural mechanisms of cytoprotection in the inner organs in experimental burn injury of animals.
REFERENCES


