METHODS

Experimental Model of Peritoneal Adhesion Formation

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Experimental model of peritoneal adhesion formation developing in rats and rabbits as a result of dosed autohemoperitoneum is described. The role of peritoneal macrophages, their microenvironment cells, and cytokines in the development of peritoneal adhesions is demonstrated.

Key Words: *autohemoperitoneum; fibroblasts; peritoneal adhesions*

The majority of interventions on abdominal organs are associated with the formations of postoperative connective tissue adhesions (PCA) [3,6,7]. These adhesions are morphological substratum of ileus, infertility, cholestasis, disorders in the pancreatic juice passage, *etc.*, which can result in the development of fatal pathological processes and conditions [3]. The hypotheses on the causes and mechanisms of PCA formation are based on the phenomenological approach. The models of adhesive process in the abdominal cavity are models of experimental peritonitis of bacterial etiology. The improvements in surgical technologies and introduction of not easily available endoscopic interventions in planned and urgent surgery necessitate the development of models of aseptic inflammation of the peritoneum. An adequate universal model of intraperitoneal adhesion formation can be based on the idea of the key role of the peritoneal fluid (PF) fibrin and fibroblasts, present in the tissues or migrating into foci of lesions, as the biochemical and morphological factors of adhesive process [5].

Hence, the search for new methods for prevention of postoperative PCA necessitates creation of a model of aseptic adhesive process, sufficiently manifest, simple, not provoking early death of experimental animals, inexpensive, and requiring no highly sophisticated equipment.

We developed an experimental model of an aseptic peritoneal adhesive process.

MATERIALS AND METHODS

Experiment was carried out on adult male Chinchilla rabbits (n=22; 2500-3200 g) and outbred male albino rats (n=50; 180-200 g) with consideration for the predominance of the proliferative phase of inflammation in response to alteration in these animal species. All animals were kept in a vivarium of Omsk State Medical Academy before and during the experiment under standard conditions in accordance with standard recommendations. Autoblood (1% body weight) was collected from the marginal ear vein in rabbits and from the dorsal caudal vein in rat under ether narcosis. Venous blood was injected into the abdominal cavity through a puncture in the white line no later than 20 sec after collection. Heparin treatment of venous blood or use of other anticoagulants for prevention of blood clotting in the syringe was absolutely ruled out. In order to prevent injuries to the intestinal walls and other hollow abdominal organs during the manipulation, the animal was held with its head down and the injection was made into the caudal third of the white line. The pathological process in the ab-

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dominal cavity developing as a result of autoblood injection eventuated in the formation of fibrinous depositions and then connective tissue adhesions between the peritoneal leaflets.

The efficiency of the studied method for prevention of adhesions was evaluated 7 days after intraperitoneal injection of autoblood in rabbits and 7-10 days postinjection in rats. The adhesions between the peritoneal leaflets and PF were examined.

All animals received balanced diet throughout the entire observation period. After the end of observation, median laparotomy and thorough revision of the abdominal cavity were carried out under ether narcosis under aseptic conditions. Special attention was paid to the presence, location, number, and physical characteristics (solidity, transparency, elasticity, vascularization) of adhesions between the peritoneal leaflets. The PF was collected by a sterile syringe into flasks, PCA were resected and after morphological examination fixed in 10% formalin. After manipulations, narcotized animals were sacrificed (rabbits by pulmonary artery embolism induced by injection of 20 ml atmospheric air into the marginal ear vein, rats were decapitated).

The sections of PCA were stained with hematoxylin and eosin (for microscopy with inspection of the general picture of tissue structure) and with picrofuchsin after van Gieson (for detection of collagen fibers). Collected PF specimens were fractionated by centrifugation. Macrophagic transformation of mononuclear cells was studied in the cell precipitate [2].

Measurements of the supernatant concentrations of TNF-α (by ELISA with horseradish peroxidase), hydroxyproline (obligatory component of collagen, the main connective tissue protein), and glycosaminoglycans [1] were carried out.

The normality of distribution was verified using Shapiro—Wilk test. The distribution was close to normal in the majority of cases. The relationships between the data in various groups were analyzed by calculating Pearson coefficients of correlations and linear regression coefficients.

RESULTS

Simulation of autohemoperitoneum led to the formation multiple PCA in the abdominal cavity of experimental animals (8.17±1.84 per rat and 8.59±2.32 per rabbit).

PCA were predominantly located in the inferior (caudal) part of the abdominal cavity (64.7%) and were viscero-visceral (75.6%) or viscero-parietal (24.4%). Macroscopically, adhesions were classified as cord-like (17%), planar (7.9%), film-like (44.1%), arachnoid (4.6%), and mixed (26.4%).

By day 7, the adhesions were presented by young fibrous connective tissue with well-discernible intercellular substance, orderly location of collagen fibers, and cell elements: fibrocytes, fibroblasts, solitary plasma cells, mast cells, and multinuclear cells (resembling foreign body giant cells). Mature cicatricial connective tissue with ordered collagen fibers formed on day 10; it contained homogeneous sites and fibrous structures. The number of fibroblast cells decreased significantly.

The content of hydroxyproline and hyaluronic acid (main biochemical markers of intensive connective tissue formation) was measured in rabbit PF [1,8]. A direct and close correlation between the content of the studied biochemical substrates in PF and number of adhesions was detected, with 0.93 correlation coefficient for hyaluronic acid and 0.95 for hydroxyproline. Hence, in our experiment the levels of hyaluronic acid and hydroxyproline in rabbit PF characterized anabolic activity of fibroblasts.

Fibroblasts in the connective tissue are involved in intricate cascades of interactions with microenvironmental cells [4]. In our experiment, the formation of pronounced and extensive adhesions in the abdominal cavity in autohemoperitoneum was associated with the appearance of numerous mononuclears in the peritoneal exudation. On day 10, the count of nuclear cells in PF was (5.4±3.5) $\times 10^9$ cell/liter and was represented mainly by lymphocytes and monocytes (28% lymphocytes, 40% monocytes, and 1% neutrophils). The PF monocytes were characterized by high capacity to transformation into macrophages: 62.3±8.2% of them differentiated into macrophages within 24 h. A direct correlation (r=0.91) between transformed macrophages and number of adhesions in rat abdominal cavity was detected.

High functional activity of mononuclear cells manifested in additional production of cytokines [5]. The levels of TNF-α and IL-1 in rat PF on day 10 after intraperitoneal injection of autoblood were 236.1±76.3 and 9.7±1.0 pg/ml, respectively. Direct correlations between the number of adhesions and concentrations of TNF-α (r=0.42) and IL-1 (r=0.87) were detected.

Hence, the proposed model of adhesive process in the abdominal cavity is universal for various species of laboratory animals (albino rats and rabbits) and by the initiating factor is maximally close to real surgical situations. Immunological, morphological, and cytological characteristics of

the process were studied; the model is proposed for studies of the etiology and pathogenesis of PCA, evaluation of the efficiency and safety of possible methods for its prevention and correction.

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