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Morphology of Catgut Implant Destruction in the Peritoneal Cavity of Male White Rats

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ABSTRACT

Introduction: aseptic peritonitis it is reaction of the local immune system aimed at rejection of a foreign body, which, having antigenic properties, does not (unlike a pathogen) counteract the immune system. The suture materials, namely catgut thread, used in intracavitary surgical operations possess the xenogenic properties and can use for antigenic stimulation of the immune system of the peritoneal cavity. The purpose is to study the destructive changes in the catgut implant in the peritoneal cavity of albino male rats in experimental modeling of aseptic inflammation of the peritoneum.

Methods: the study involved 15 white mature male rats, weighing 286,13±6,26 g. Catgut implants, standardized in shape and size, were made of 2/0 catgut thread of 0.3 mm thick and 17 cm long, which was randomly rolled into compact balls with an area of 1 cm2, after which they were engrafted into the peritoneal cavity of rats. Morphological analysis of the findings of the study was carried out using the traditional anatomical and histological methods for obtaining serial paraffin slices of 4 μm thick (Microm HM 325), which were stained with hematoxylin-eosin.

Results: the findings of our study showed that the greater omentum and epididymal omentums are the acceptors of the xenogenic implant. In all cases, without exception, the catgut implant was fixed on the marginal zone of one or another omentum. Starting from day 3 of the experiment, a clearly pronounced response of the local immune system to the catgut implant was noted. Morphologically, it was visualized by the invasion of the blood vessels into implant and the appearance of continuous concentrated layer of immunocompetent cells, which were mainly represented by lymphoid and phagocytic elements, formed around the catgut thread and in consequent penetrating into the catgut thread.

Conclusion: following two weeks of staying in the tissues of one or another omentum, the catgut thread was far from complete rejection.

Keywords: Greater omentum; Peritoneum; Aseptic inflammation; Lymphoid Infiltration; Immunocompetent cells; Adipocytes; testis; Mucous membrane.

Introduction

The paper is presented within our study of the problem of experimental modeling of aseptic inflammation of the peritoneum (peritonitis). The analysis of the literature sources showed the existence of a large number of experimental models of peritonitis[1-3]. To date, peritonitis is considered as an inflammation of the peritoneum, represented by a complex of severe pathophysiological reactions with dysfunction of all organs and systems of the body as the response to the action of pathogenetic stimuli[4,5]. However, the role of the immune system, which is directly involved into inflammation, has not been fully elucidated[6-9]. Obviously, the leading role in this process belongs to cellular immunity, the effector elements of which are cytotoxic T-lymphocytes and macrophages, which is crucial for the development of aseptic inflammation[10-12].

Aseptic peritonitis should be understood as a reaction of the local immune system aimed at rejection of a foreign body, which, having antigenic properties, does not (unlike a pathogen) counteract the immune system[13,14]. It should be noted that the suture materials used in intracavitary surgical operations possess the above qualities[15-17].

Consequently, we decided (after a preliminary analysis of many types of the state-of-the-art suture materials) to use catgut, traditionally used in surgery, for antigenic stimulation of the immune system of the peritoneal cavity of animals, since it is not artificial, but a bioorganic suture, consisting mainly of collagen fibers of the submucous layer of the small intestine of sheep[18], that is, this material fully possesses xenogenic properties, which fully meets the objectives of our study. Therefore, the aim of the investigation was to study destructive changes in the catgut implant in the peritoneal cavity of albino male rats in experimental modeling of aseptic inflammation of the peritoneum.

Material and Methods

Catgut implants, standardized in shape and size, were made of 2/0 catgut thread of 0.3 mm thick and 17

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cm long, which was randomly rolled into compact balls with an area of 1 cm^2, after which they were engrafted into the peritoneal cavity of rats.

The study involved 15 albino mature male rats, weighing 286,13±6,26 g. The animals were kept in the standard conditions of the experimental biological clinic (vivarium) of the Poltava State Medical University, in compliance with the regulations for keeping experimental animals adopted by the Directive of the European Parliament and Council (2010/63/EU), the Order of the Ministry of Education and Science, Youth and Sports of Ukraine as of 01.03.2012 No. 249 "On Approval of the Procedure for Conducting Experiments on Animals by Scientific Institutions" and "General Ethical Principles of Animal Experiments", adopted by the Fifth National Congress on Bioethics (Kyiv, 2013), (Minutes No. 198 as of 21.10.2021 from the meeting of the Commission on Biomedical Ethics of the Poltava State Medical University[19,20].

The animals under ether anesthesia[21] (with observance of sterile conditions and relevant requirements for the ethics of experimental research on animals) alternately underwent a median laparotomy, 1.5 cm long, along the linea alba and catgut implant was accurately inserted through the incision. Subsequently, the edges of the wound were sutured with a polypropylene thread without antigenic properties.

In order to study the dynamics of destructive changes in the catgut implant in the peritoneal cavity of the experimental animals, all of them were assigned into three groups of five subjects in each group. Subsequently, the abdominal cavity of all animals was dissected alternately, according to the time interval of their euthanasia (by ether anesthesia overdose[21] following the 3, 7 and 14 days after the start of the experiment) on the dissecting device in the supine position, with the creation of a complete view of the internal organs in their natural relations[22].

Prior the further manipulations, at first, the entire contents of the abdominal cavity were subjected to gentle rinsing with warm 0.9% saline NaCl solution, and then irrigated from a syringe with 10% neutral formalin solution. Afterwards, the imaging was made using a digital camera directly in the carcass of the laboratory animal.

Morphological analysis of the findings of the study was carried out using the traditional anatomical and histological methods for obtaining serial paraffin slices of 4 μm thick (Microm HM 325), which were stained with hematoxylin-eosin. The method of plastination of tissue complexes in epoxy resin was also used, followed by the manufacture of polished slices, which were stained with 1% methylene blue solution[23-27]. The specimens were studied and documented using the Konus light microscope equipped with the Sigeta DCM-900 9.0MP digital microphotographic attachment with the Biorex 3 software adapted for these studies (serial number 5604). Morphometric characteristics of the tissue structures of the corresponding specimens were obtained using a system for visual analysis of histological specimens, as well the Sigeta X 1 mm/100 Div.x0.01 mm object micrometer, the scale of which (equal to 1 mm, where the smallest division corresponds to 10 μm) was applied on the corresponding micro image obtained at an equivalent magnification.

Statistical processing of the resulting data was carried out on a personal computer using the Prism 5 (version 5/03) and Microsoft Excel 2010 software packages, as well as methods of descriptive statistics and statistical analysis. Descriptive statistics are presented as mean ± standard error of the mean (M±m). Qualitative indicators were presented in the form of absolute values (n) and percentages (%).

The values of the studied parameters between the groups were compared using the Kruskal-Wallis test (One-Way ANOVA).

The differences were considered statistically significant at $p<0.05$.

Results

The findings of our previous study showed that both the greater omentum (40%) and its two homeomorphic derivatives of the visceral peritoneum, which we named the epididymal omenta, since they are associated with testicular appendages (60%), are the acceptors of the xenogenic implant in the form of a ball of catgut thread in the mature albino male rats[28]. Thus, according to our data, this animal species (unlike a human) has not one, but three omenta (the lesser omentum described in human anatomy manuals does not belong to similar formations)[28,29]. Generally, the majority of catgut implants are absorbed by two epididymal omenta (4 cases (44.44%) by the right epididymal omentum and 5 cases (55.56%) by the left one) p=0,3114.

Interestingly, in all cases (n=15), without exception, the catgut implant was fixed on the marginal zone of one or another omentum. It occurred already within three days of the experiment (Fig. 1).

At this point, the external morphological sign of engrafting of the implant within the marginal zone of the omentum is the formation of a serous coating on its anterior surface, which hides the external relief of the loops of the catgut thread. But the most apparent confirmation of the process of organic engrafting of the implant in one or another omentum is the invasion of blood vessels into it, which start from the vascular-fatty arcades of the greater omentum and the vascular-fatty tracts of the epididymal omentum (Fig. 1).

On day seven, increased vascularization of the implant was noted, which became the most prominent within 14 days. It was evident not only by an increase in the number of the afferent blood vessels, but also by their invasion into the implant with the formation of separate microvascular loops in it, conformal to the tortuosity of the catgut thread in the ball (Fig. 2).

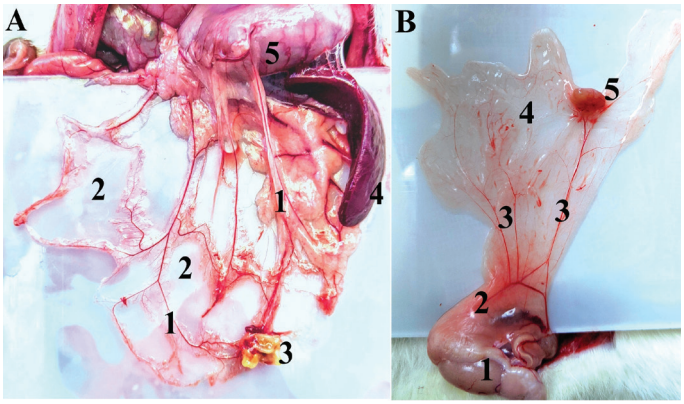

Figure 1. The implant adhered to the greater (A) and epididymal (B) omenta of albino male rats within three days of the experiment. Macro image. A – 2 × magnification, B – 2 × magnification
A: 1 – vascular-fatty arcades, 2 – serous-reticular arcs, 3 – invasion of blood vessels into the implant, 4 – spleen, 5 – stomach. B: 1 – testicle, 2 – the base of the omentum; 3 – radial vascular-fatty tracts; 4 – serous-reticular membrane; 5 – invasion of blood vessels into the implant.

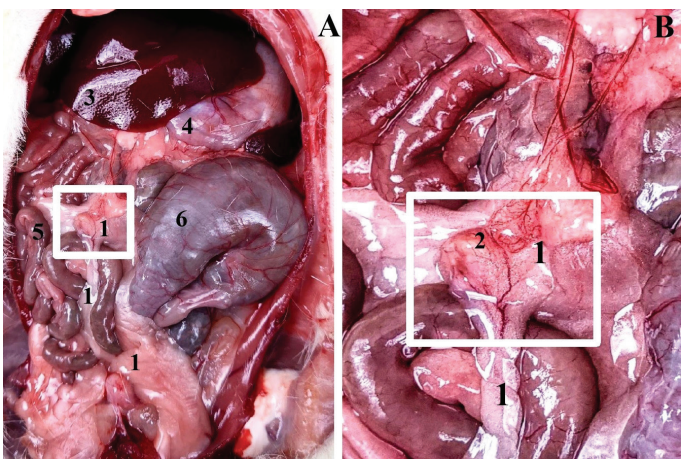

Figure 2. The overall view of the organs of the peritoneal cavity of a male rat within 14 days of the experiment. The white square outlines the catgut implant absorbed by the left epididymal omentum. Macro image. A – 2 × magnification B – 6 × magnification.
1 – epididymal omentum; 2 – blood vessel invaded into the catgut implant; 3 – liver; 4 – stomach; 5 – loops of the small intestine; 6 – cecum.

Moreover, the morphological findings revealed the response of the immune system in the catgut implant at the end of day three of the experiment. Visually, it was manifested by the presence of basophilic granularity, directly adjacent to the external surface of the catgut thread. At high magnification of the microscope, it was clearly seen that this granularity was a dense aggregation of lymphocytic elements, represented by the small forms of lymphatic cells, among which macrophages were scattered, which is characteristic of the cellular immune response (Fig. 3 A).

At the same time, in the marginal zone between the superficial layer of the catgut thread and the cells of the infiltrate, the closest contact of interaction occurred. However, no destructive changes of the thread were noted. Noteworthy, in the implant, the entire intermediate space between the loops of the catgut thread, with adjoining lymphoid infiltrates, was filled with adipose tissue, in which blood vessels of various calibers were localized (Fig. 3 B). Their branches,

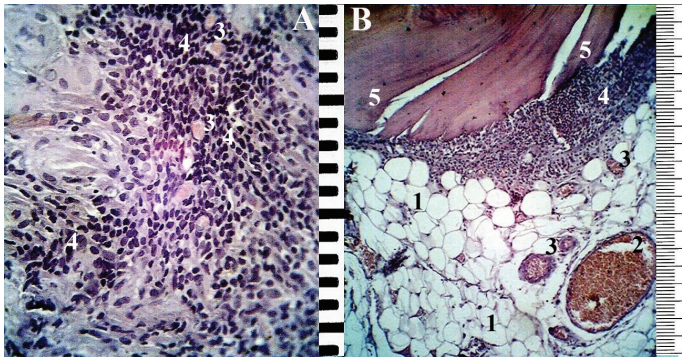

Figure 3. Cytoarchitectonics of the lymphoid infiltrate around the catgut thread (A) and tissue structures surrounding the catgut thread (B) formed in the implant within 3 days of the experiment. A – epoxy slice; methylene blue stain. Ocular lens 10× magnification. Objective lens 40× magnification. B – paraffin slice; H&E stain. Ocular lens 10× magnification. Objective lens 10× magnification. The smallest division in the metric scale is 10 μm.
1 – adipocytes of adipose tissue, 2 – afferent blood vessel, 3 – blood microvessels, 4 –lymphoid infiltrate, 5 – catgut thread at the initial stage of disintegration.

namely microvessels of the capillary type, reached the margins of lymphocytic infiltrates. We hypothesize that these circulatory communications provide with a constant delivery of new immunocompetent cells, represented by cytotoxic T-lymphocytes and monocytes, to the foci of infiltration.

So, within three days of the experiment, the implant was completely engrafted into the tissue environment of one or another omentum. This was evidenced, firstly, by the invasion of the adipose tissue together with blood vessels into the intermediate space between the loops of the catgut thread and, secondly, by the appearance of a continuous layer of lymphoid infiltrations around them, which indicated the beginning of the development of an immune response to the rejection of this xenograft.

This process became more prominent within 7 days of the experiment. At this point, in the implant, the lymphoid infiltrate, in which the lymphoid cells were previously chaotically distributed, in some places became structurally ordered. It was manifested by the appearance of the tortuous two-row trajectories, which were penetrated into the superficial disintegration of the catgut thread (Fig. 4).

It should be noted that within 7 days of the experiment, this phenomenon occurs in certain sites of the implant, affecting only the superficial layer of the catgut thread.

Following a week, namely on day 14 of the experiment, this process became more widespread in the implant and the reaction to the catgut thread, which underwent destruction in the form of disintegration of fibrous bundles, which were completely absorbed by the lymphoid infiltrate was more prominent (Fig. 5).

It is noteworthy that it is in this zone that an increased concentration of macrophages is noted, which utilize the detritus of the catgut thread. Apparently, the prolongation of this process is carried out due to the constant replenishment of lymphocytic infiltrates with new clones of activated immunocompetent cells.

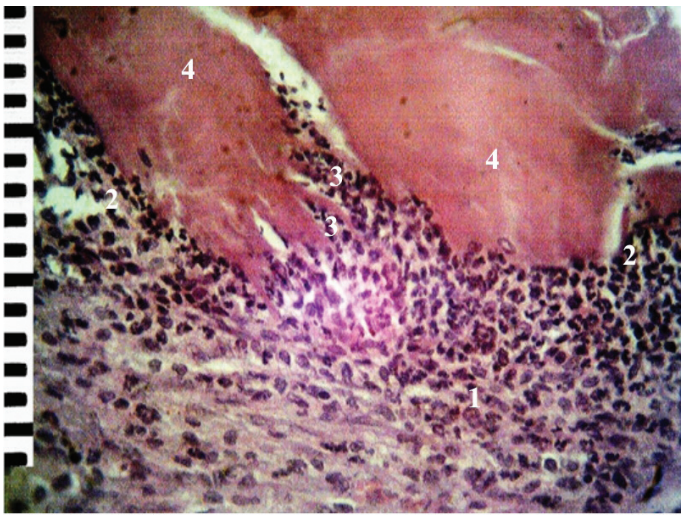

Figure 4. The zone of the destructive effect of lymphoid cells on the catgut thread on day 7 of the experiment. Paraffin slice; H&E stain. Ocular lens 10× magnification. Objective lens 40× magnification. The smallest division in the metric scale is 10 μm.
1 – the site of chaotic structure of lymphoid infiltrate; 2 – the site of well-ordered lymphoid infiltrate; 3 – the sites of invasion of lymphocytes into the disintegrated catgut thread; 4 – catgut thread.

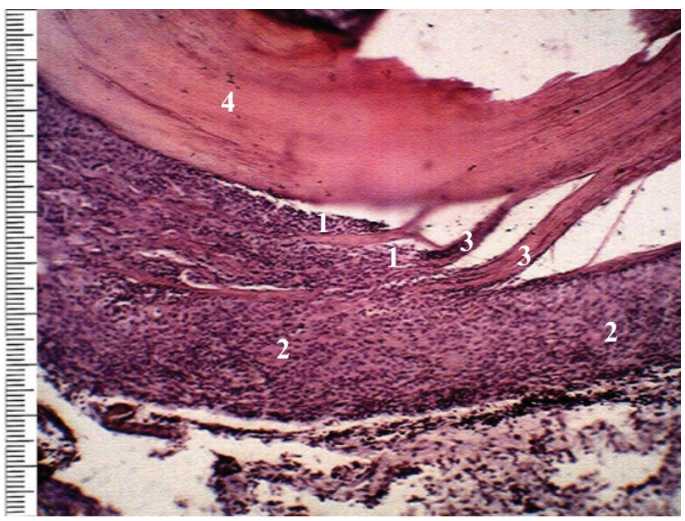

Figure 5. Disintegration of fibrous bundles of catgut thread in the implant on day 14 of the experiment. Paraffin slice; H&E stain. Ocular lens 10× magnification. Objective lens 10× magnification. The smallest division in the metric scale is 10 μm.
1 – migrating lymphoid elements from the intermediate zone of the implant; 2 – lymphoid infiltrate; 3 – disintegration of fibrous bundles of catgut thread; 4 – a loop of catgut thread.

Regarding the time of complete destruction of the catgut thread, the literature data are very contradictory; different periods of time are given: from 40 to 90 days[18,30-32]. Our study was not aimed at verification or clarification of these data.

Discussion

Peritonitis is an inflammation of the peritoneum and can be classified according to the underlying cause (primary and secondary), prevalence (local and diffuse), or the presence of infectious agents (septic and aseptic). Notwithstanding the successes in treatment of the disease, mortality from peritonitis and its complications is tending to increase[33,34].

To date, a laboratory rat is the most widely used animal model for simulating peritonitis and investigating the diseases of the gastrointestinal tract[35]. Interestingly, the anatomical structure of internal organs of laboratory rats is similar to human one[36].

Simulation of peritonitis can be made by several ways depending on the characteristics of reproduction of the purulent-inflammatory process in the abdominal cavity. It can be the technique of introduction of foreign bodies or chemical substances into the abdominal cavity, or bacterial contamination of the abdominal cavity with various cultures of pathogenic microorganisms or fecal suspension through a puncture or incision of the abdominal wall by perforation of any part of the gastrointestinal tract, as well as combined methods of modeling acute experimental peritonitis, which include elements of the above-mentioned methods in various combinations [3,37,38].

Liu *et al.* classify peritonitis, according to its cause, as aseptic peritonitis, bacterial peritonitis, peritoneal dialysis-associated peritonitis, and LPS (lipopolysaccharide)-induced peritonitis[39]. An experimental peritonitis model is created by intraperitoneal injection of LPS (10 mg/kg).

Shepitko in his study modeled experimental aseptic inflammation of the peritoneum in rats by intraperitoneal injection of 5 mg of λ-carrageenan (Sigma, USA) in 1 ml of physiological saline solution per animal, which caused acute aseptic inflammation. Subsequently, acute aseptic inflammation of the peritoneum caused by a single intraperitoneal injection of λ-carrageenan led to general changes in the membranes of the wall of the jejunum[40].

Syplyviy *et al.* also experimentally modeled aseptic disseminated peritonitis on Wistar mature albino rats by administration of 5 ml γ-carrageenan (Sigma, USA) per 1 ml of isotonic saline solution. On the basis of the conducted research, the staging of the course of experimental peritonitis with hemomicrocirculatory changes, characteristic for each stage, have been confirmed[41].

One of the main conditions for the development of experimental peritonitis is the reproducibility and uniformity of the development of the disease, which undoubtedly affects the findings of the conducted experimental study.

For experimental modeling of aseptic peritonitis, researchers performed an erroneous laparotomy in some cases or intestinal abrasion – namely cecum without perforation Surgical interventions were carried out in sterile conditions on laboratory animals[42,43].

Inflammation of the peritoneum in the absence of infectious agents (aseptic peritonitis) most often occurs in response to exposure of the peritoneum to sterile fluids (e.g., gastric juice, bile, or urine), pancreatic enzymes, or a foreign body. Microscopic and

macroscopic foreign bodies, including surgical glove powder, surgical materials (sutures, cotton swabs, surgical sponges), hair, and various objects (sticks, plant material, metal) can cause a granulomatous reaction[34,44].

The greater omentum is considered as a barrier to bacterial invasion and, as a result, can turn the source of a possible development of peritonitis into a local abscess[45,46]. It has been found that the structures of the greater omentum are capable of encapsulating foreign bodies[47-49] that was revealed during our study. However, the data presented in our publication on the adhesive properties of serous formations of the testicular appendages to foreign bodies in the abdominal cavity do not present in the literature, as well as comprehensive, systematic and morphometric characteristics of the above structures[28].

Conclusions

Thus, following two weeks of staying in the tissues of one or another omentum, the catgut thread was far from complete rejection. Nevertheless, the given period of the experiment was quite sufficient to visually trace the main features of the catgut implant after its absorption by one of the three omenta, which can be summarized in the form of the following conclusions:

Starting from day 3 of the experiment, a clearly pronounced response of the local immune system to the catgut implant was noted. Morphologically, it was visualized by the invasion of the blood vessels into it and the appearance of continuous concentrated layer of immunocompetent cells, which were mainly represented by lymphoid and phagocytic elements, formed around the catgut thread.

On day 7 of the experiment, lymphoid cells were arranged in the form of tortuous two-row trajectories, which, penetrating into the catgut thread, led to its disintegration into separate fibrous bundles, subjected to utilization by macrophages. This process of destruction became more prominent on day 14 of the experiment.

Consequently, the further development of the local response of cellular immunity will ultimately result in rejection of the foreign body.

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