EXPERIMENTAL VALIDATION OF POLYHYDROXYALKANOATES BASED BIODEGRADABLE SUTURE MATERIAL USE

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The availability of monofilaments made by 3- hydroxybutyric acid polymer melt extrusion (polyhydroxybutirate, PHB) in surgery has been investigated. The applicability of PHB suture filaments for sealing muscle-fascial wounds, one-row enteroenteroanastomosis applying has been proved. At the formation of one-row enteroenteroanastomosis there were no PHB retention sutures detected in the specimens after 100 days of observation, all the anastomoses being well-fixed. There were no complications in the form of early or delayed malfunctions, anastomosites, bowel obstruction or adhesion registered in the abdominal cavity. When studying the anastomosis zone histologic specimens after 100 days of observation a developing granulation tissue was found out.

Key words: (polyhydroxybutirate, PHB, suture filaments, muscle-fascial and intestinal sutures, one-row enteroenteroanastomosis, tissue reaction, resorbable suture material.

Introduction

The penetration of a surgical needle and filament through tissues causes damage and cell death. The presence of a foreign body (filament) promotes the macrophage excess accumulation in the inflammatory tissue, and this feature retains not less than 3-5 days. During this time no morphological features of reaction to separate synthetic suture materials are practically noticeable. The specificity of reaction to various kinds of suture materials starts manifesting itself in 10-15 days only. The inflammation degree and character dependency on the applied suture material kind in the surrounding tissues is mentioned by many authors.

The invalidity of intestinal anastomoses – is one of the most frequent causes of early postoperative complications and lethality in surgical gastroenterology. The given complication occurs in 1, 5-3% of stomach and duodenum operations, in 2, 8-8, 7% of small and in 4-32% - large bowel operations. That is why the creation of optimal conditions for suture and gastrointestinal tract anastomoses healing – is the principal reserve of improving the nearest results in the patients operated on their stomach and intestinal tract.

Thus, when applying an intestinal suture, the defining moments are technical features of fistulization and characteristics of the suture material, which should cause the minimal tissue reaction of the intestinal wall.

Materials and methods

The monofilaments are made of the highly purified 3-hydroxybutyric acid polymer (polyhydroxybutirate, PHB) samples synthesized on the technology of the Institute of biophysics of RAS. The filaments are obtained by means of the PHB melt extrusion, using a laboratorial autonomic extrusion machine Brabender® 19/25 D (Germany). The physical and mechanical properties of the filaments are defined in a universal electromechanical tensile-testing machine Instron 1122 (Great Britain) with the extension rate of 100 mm/min. The filaments had the diameter of 0, 15-0, 17 mm (metric dimension 2), the absolute strength of 300 MPa, the elasticity modulus of 3GPa and a high mechanical strength in conditions of static and cyclic stressing (up to 100 MPa). The filaments are used for sealing muscle-fascial wounds and one-row enteroenteroanastomosis applying. The animal experiments are performed by the authority and in accordance with the Research Programme approved by

the Committee of the Institute of Biophysics of RAS on Bio-Ethics and Ethical Committee of KrasSMA named after Pr. V.F. Voino-Yasenetsky. The experimental animals (Vistar-line rats raised in the nursery of the Institute of Cell Biology and Genetics of RAS) were given inhalation anaesthesia and a 2 cm long vertical section of skin and muscle was performed on the right huckle in aseptic conditions; three PHB sutures (total length of 3, 0-3, 5 cm) were applied to the muscle; the skin was sealed with silk. In the group of comparison (control) a surgical gut, metric dimension 2, "Catgut 0, 41101" trademark (HELM PHARMACEUTICALS GMBH, Germany), was used. The possibility to use PHB filaments to apply an intestinal suture was tested on both sex non-pedigree dogs weighing from 12 to 20 kg. The animals were divided into 2 groups. The first group or the group of comparison included 8 animals, which an "end-to-end" enteroanastomosis with the help of a one-row serous-muscular-submucosal U-bend suture was performed on. As a suture material a wide-spread Vicril 3.0 with an atraumatic needle was used. The second group under research consisted of 9 animals, which an analogous anastomosis with the help of PHB filaments was performed on.

The study of total tissue reaction to PHB filament was carried out by histological methods. To that effect the tissue fragments in the locus of filament implantation were taken; the material was fixed in 10%-formaline and put into wax; 5-10 cm thick sections were made of the blocks and were analyzed, using Image Analysis System "Carl Zeis" (Germany); the intensity and duration of the inflammation, the fibrous capsule formation dynamics round the filament and its cellular composition were evaluated. The activity of cellular elements was judged on their average quantity per high power field at the analysis of 15 visual fields. The definition of fibrous capsule (FC) thickness and fibroblast rowness (FR) in it was carried out on the morphometric method of V.P. Yatsenko (Yatsenko and co-authors, 1986).

Results and discussing

The PHB filaments, as analogous to catgut, safely held the muscle-fascial section wound edges together for the entire postoperative period. The wound repair in all the experimental animals occurred by primary intention. The microscopic state in the PHB filaments implantation site on the 7^{th} day after the operation was characterized by an insignificant tissue edema and single fine necrotic zones round the implanted filaments. The filaments were mainly surrounded by macrophages and lymphocytes, also neutrofils and fibroblasts. In 2 weeks the inflammation sings diminished, an insignificant tissue puffiness round all the implants being preserved; leukocytic cells still occurring in the inflammation zone; the beginning of fibrous capsules formation round the implants being registered. The tissue reaction round the PHB filaments concerning the inflammatory force was considerably less manifested compared to the reaction to catgut. In 4 weeks after the operation the thickness of fibrous capsules round the PHB filaments made 172, 23±13, 64 um, that was much less than that in the catgut implantation site. The number of active macrophages with a great amount of excrescences and cellular lysosomal structures (up to 11-12 per high power field) kept on growing. The capsules were represented by fibroblasts and collagen fibers, which started forming into batches. In 8 weeks the microscopic state of tissues in the experimental and control filaments implantation zone remained practically without changes, as well as the capsules' thickness and their cellular composition did. In the zone surrounding the filaments a great number of active macrophages was still registered. The FC thickness achieved 514.21+12.01 um round catgut in spite of its destruction features in this very term. The collagen fiber batches in the FC round catgut were much thicker and took practically the capsule's total volume. 16 weeks after the implantation a significant thinning of the capsules round the PHB filaments was registered, their average thickness being reduced up to 54, 09±3, 28 with the fi-

broblast rowness (FR) at the level of 4, 64±0, 37; the number of active macrophages in the tissues being adjacent to the implant still remained at the high level. The FC thickness achieved 514.21+12.01 um round catgut in spite of its destruction features in this very term. The collagen fiber batches in the FC round catgut were much thicker and took practically the capsule's total volume. In 24 weeks after the operation a further involution of the fibrous capsules round the PHB, the CT reduced thereat up to 33, 73±2, 05 um accordingly. Fully formed collagen fibers prevailed in the capsules, active phagocyting macrophages still being present. In the catgut implantation site, in spite of its active destruction (by 4 months in hadn't been detected in the tissues), solid capsules remained unchanged. In 16 and 24 weeks after the implantation of catgut the CT made 342, 00±9, 68 и 272, 14±4, 11 um accordingly.

A further tissue state monitoring in the animals, which were implanted the PHB experimental filaments, testified that there were no strong changes in the implanted filaments' state taken place. 9 months after the operation there were no negative occurrences in the zone of implantation registered. The capsule's thickness round the filaments in separate animals made 20-40 um. The implants were surrounded by sound tissues of newly formed fibers, which were centered round the polymer filament. In 12 months there was practically no fibrous capsule round the implants registered. In the close proximity to the polymer filament, circumferentially and also in the adjacent tissues, a considerable amount of mono- and polynuclear macrophage cells was still registered. In this term there was no negative reaction of the tissues to a foreign body registered in spite of the polymer filament presence in the animals' muscle tissue.

The morphological methods of research of tissues in the zone of intestinal anastomosis included a macroscopic description and histologic characteristic of preparations. Macroscopcally (according to the data of autopsy) the effusion presence, adhesive process intensity in the free abdominal space, appearance of enteroenteroanastomosis, its patency, cicatrical changes presence in the zone of intestinal sutures application were evaluated in 100 days.

At the autopsy all the anastomoses were patent and well-fixed, there were no signs of local and extended peritonitis registered in any of the animals. In all the first group animals there was a moderate adhesive process involving the omentum and mesostenium with the presence of solid sagittal commissures detected in the anastomosis area. In two animals there was a moderately expressed cicatrical deformation registered in the anastomosis application area. Vycril sutures were clearly visualized.

In the animals of the second group the adhesive process was considerably less manifested. Macroscopically in the area of anastomoses there was an insignificant thickening of the intestinal wall registered, there was no cicatricial deformation in the intestinal suture performance area fond out, the PHB filaments were not visualized.

Similar results were got at the analysis of morphological preparations of anastomoses zones in the animals of both groups. At the anastomosis level a developing granulation tissue represented by the vessels of capillary type, fibroblasts, epithelial, plasmatic cells, lymphocytes, eosinophils and single leukocytes were defined. There was no intestinal walls' layers deviation detected.

Thus, the results of experimental studies of PHB filaments for the performance of a manual serous-muscular-submucosal U-bend intestinal suture found out the lack of peritonitis and anastomosis malfunction signs, insignificantly expressed formation of commissures in the intervention area, lack of inflammatory response of the intestinal wall to the filament. It allows taking a favourable view of the PHB filaments application preliminary results for the intestinal suture formation and requires further studies.

The work has been carried out with a financial support from the RAS Presidium

Program “Fundamental Sciences – to medicine” (Project 12.5).

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