

MORPHOLOGICAL AND HISTOLOGICAL STUDIES OF EFFECTIVENESS VOLUME FORMING PREPARATIONS

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Abstract. *The morphological studies carried out determined the range of issues to be solved in the experimental part of the work, on the basis of which we studied: fixation of the volume of the forming drug introduced into the subcutaneous part of the abdomen of mice, cellular infiltration and the condition of the tissues around the drug, germination of blood vessels and the formation of granulation tissue around the drug.*

A histological experimental study showed that the volume-forming preparation "Polyacrylamide gel + Bioplant" was well fixed in the submucosal layer, on the 20th day of the experiment a thin layer of granulation tissue appeared around the preparation and the germination of its boluses by vessels, and on the 30th day the formation of a capsule around the preparation was noted.

Keywords: *anal incontinence, volume-forming device, morphology.*

Introduction. The morpho functional features of the recto anal zone in patients with anorectal malformations have been and remain the object of study by many researchers dealing with the problem of these severe malformations. A sufficient number of scientific works are devoted to these studies, which highlight issues related to organic changes in the retaining apparatus of the rectum after surgical correction of these malformations, leading to such a formidable complication as fecal incontinence [1, 4, 5, 7, 9, 10].

Congenital combined anomalies, intraoperative errors and injuries to the obturator apparatus of the rectum are the causes of low basal pressure in the anal canal due to insufficiency of the internal anal sphincter and manifest themselves in the form of postoperative anal incontinence in children both in the immediate and long-term periods of observation. Morpho functional inferiority of the recto anal zone in children with anorectal malformations requires reconstructive corrective surgical interventions, and if they are ineffective, in order to increase the basal pressure in the anal canal and restore the continence function, the use of safe volume-forming drugs for injection into the submucosal layer of the anal canal [2, 3, 6, 8, 11].

Target. Determination and confirmation of the effectiveness of the volume of forming drugs used in the treatment of post-operative anal incontinence.

Materials and methods. An ex- perimeter study was carried out on the basis of an agreement between the Tashkent Pediatric Medical Institute (Professor of the Department of Faculty Pediatric Surgery of the Tashkent Pediatric Medical Institute, Doctor of Medical Sciences, Aliev M.M.) and the Institute of Bioorganic Chemistry at the Academy of Sciences of the Republic of Uzbekistan (Deputy Director of the Institute of Bioorganic Chemistry Academician A.S. Turaev).

In the clinical and experimental part of the work we performed, morphological studies concerned the study of changes in the subcutaneous part of the abdomen in 30 laboratory mice

with a body weight of 20-22 g, after the introduction of an injection volume of the forming preparations “Noltrex” produced by CJSC Bioform Scientific Center (Moscow, Russia), as well as locally produced preparations “Polyacrylamide gel + Bioplant”, manufactured in the biomodelling laboratory of the Institute of Bioorganic Chemistry of the Academy of Sciences of the Republic of Uzbekistan (Tashkent).

The morphological studies carried out determined the range of issues to be solved in the experimental part of the work, on the basis of which we studied: fixation of the volume of the forming drug introduced into the subcutaneous part of the abdomen of mice, cellular infiltration and the condition of the tissues around the drug, germination of blood vessels and the formation of granulation tissue around the drug.

Experimental animals were divided into 2 groups:

Group I (control), animals in which the synthetic material “Noltrex” was injected into the subcutaneous part of the abdomen in a volume of 1.0 ml.

II (comparison), animals in which “Polyacrylamide gel + Bioplant” was injected into the subcutaneous part of the abdomen.

The timing of morphological studies corresponded to 10, 20 and 30 days of observation. The distribution of laboratory animals by experimental period is presented in Table. 1.

Table 1.

Morphological study according to the timing of the experiment

No.	Experimental groups	Timing of morphological studies and number of animals		
		10 days	20 days	30 days
1	I. -group-control (n =15)	5	5	5
2	II. -comparison group (n =15)	5	5	5

After administration of the drug and the deadline for conducting morphological studies was reached, pieces of tissue from the subcutaneous part of the abdomen were selected and fixed in a 12% neutral formaldehyde solution, dehydrated and embedded in paraffin. Then the sections were stained with hematoxylin-eosin and histomorphological examination was carried out. In parallel with morphological studies, the animals underwent ultrasound scanning in order to establish the location of the injected drug and determine the volume of boluses formed around it.

Ultrasound examination was carried out in dynamics according to the periods of observation in the experiment.

During ultrasonic control, the longitudinal, transverse and diagonal dimensions of the bolus were measured with the calculation of the proper volume of administered drugs. The results of the ultrasound study showed that in group I (control) animals that were injected with the synthetic volume-forming drug “Noltrex” on days 10-20-30 after its administration, the volume of the drug decreased slightly from 0.94 cm³ to 0.86 cm³.

In animals of group II (comparison), which were injected with “Polyacrylamide gel + Bioplant,” the volume of the drug increased from 0.88 cm³ to 1.14 cm³ (Table 2.)

The obtained ultrasound data allowed us to conclude that a good result was observed in animals of group II, which were administered the combined use of the drugs “Polyacrylamide gel + Bioplant,” and in animals of group I, which were administered the drug “Noltrex,” a satisfactory result was obtained.

Table 2.

Dynamics of ultrasonic testing to determine the location of the implant

No.	Groups animals	Volume of the drug during initial administration (cm ³)	Volume of administered drug in d and nam (cm ³)		
			10 days	20 days	30 days
1	I. -group (n = 15)	1.0	0.94±0.18	0.76±0.10	0.86 ± 0.44
2	II. -group (n = 15)	1.0	0.88±0.06	1.03±0.15	1.14 ± 0.63

Microscopic studies have shown that on the 10th day after the administration of injection drugs “Noltrex”, as well as with the combined administration of drugs “Polyacrylamide gel + Bioplant”, a formation in the form of a bolus with clear boundaries is determined in the subcutaneous part of the abdomen of animals (Fig. 1-2.)

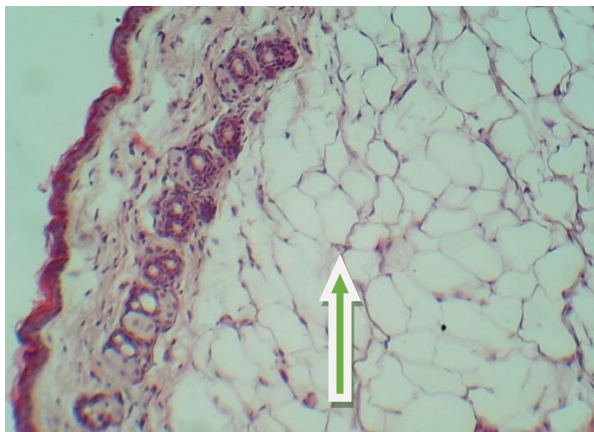
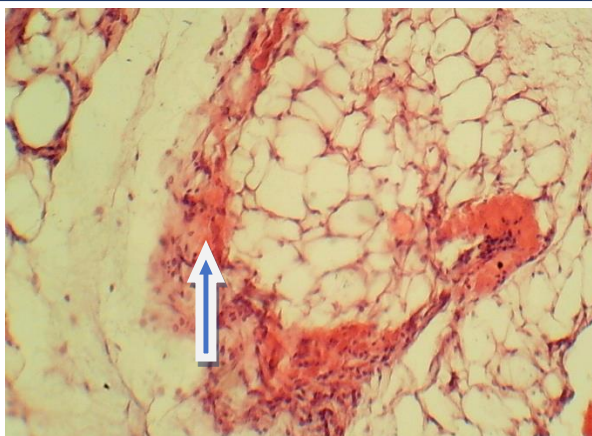


Fig.1. Formation of a bolus in the subcutaneous part of the abdomen after administration of the drug “Noltrex” on the 10th day of the experiment (PTS 10x20).

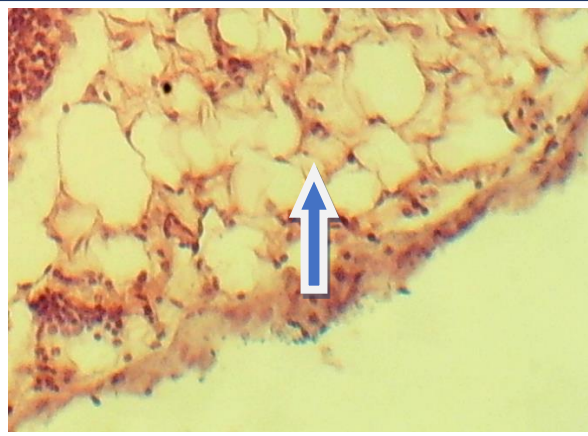


Rice. 2. Formation of a bolus in the subcutaneous part of the abdomen after the combined administration of the drugs “Polyacrylamide gel + Bioplant” on the 10th day of the experiment (PTS 10x20).

It should be noted that during visual inspection, thickening was observed in the area of drug administration at all periods of the experiment, while on the 20th day of the experiment it was established that the drugs were stored in the subcutaneous area, their boundaries did not change, and their germination into other underlying layers was not observed. On the 30th day of the experiment, intense cellular infiltration was noted in the control group of animals that were injected with the drug “Noltrex” (Fig. 3). When administering the “Polyacrylamide gel + Bioplant” preparations, less pronounced cellular infiltration was noted, while damage to the layers of the skin and underlying tissues was not observed (Fig. 4).



Rice. 3. Cellular infiltration at the border of the bolus, after administration of the drug "Noltrex", 30 days of the experiment (PTS 10x20).



Rice. 4. Cellular infiltration at the border of the bolus, after administration of the drug "Polyacrylamide gel + Bioplant" (PTS 10x20).

In this group of (comparison) animals, on the 20th day, the formation of a connective tissue granulation capsule around the injected drug, as well as germination of blood vessels, was noted (Fig. 5).



Rice. 5. Formation of a connective tissue capsule around the gel bolus after administration of the "Polyacrylamide gel + Bioplant" preparations.

Results and discussion. The experimental studies carried out clearly showed that after the administration of injectable drugs "Noltrex", as well as the combined administration of drugs "Polyacrylamide gel + Bioplant" on the 10th, 20th and 30th days of the experiment, there are no damaging, locally irritating effects of the drugs under study. It should be noted that after the administration of these injectable drugs into the subcutaneous area of the abdomen of laboratory mice, at all times of the experiment a thickening in the form of a bolus is noted, which does not grow into the surrounding skin tissue. The advantage of the combined use of "Polyacrylamide gel + Bioplant" drugs is the formation of a stable connective tissue granulation capsule around the gel bolus, which prevents migration and resorption of the drug from the site of its administration. Thus, the morphological study showed that the administered drugs had no fundamental differences

in their structure. In turn, this serves as confirmation of the above-mentioned ability of the administered volume-forming drugs to form a stable connective tissue granulation capsule around the bolus. The data obtained in the experiment on mice allowed us to proceed to the second stage of morphological research, which consisted of introducing the drug “Polyacrylamide gel + Bioplant” into the submucosal layer of the anal canal in rabbits. The purpose of this study was to determine the effectiveness of the volume-forming preparation “Polyacrylamide gel + Bioplant” when introduced into the submucosal layer of the anal canal in rabbits.

An experimental study was carried out on 10 rabbits under ether anesthesia. The volume-forming preparation “Polyacrylamide gel” + “Bioplant” in an amount of up to 2.0 ml was injected transdermally into the submucosal layer of the anal canal to a depth of 1.0-1.5 cm, directly above the level of the external anal sphincter until the walls of the anal canal are completely closed, while the projections of the administered drug were considered to be the approximate points “3, 6, 9, 12” on the clock dial. The next day after administration of the drug, the size of the gel boluses and the patency of the anal canal were determined. Ultrasound examination measured the volume of the drug administered the day before and assessed its location, determining the volume-forming boluses around it.

A morphohistological study of the area of the injected drug was studied on days 10, 20 and 30 of the experiment, after euthanasia of the animals. In this case, tissue was collected in a single block, which included the distance from the anus to the internal sphincter of the anal canal. The extracted material was assessed macroscopically and microscopically. For histological examination, the material was fixed in a 12% neutral formalin solution, dehydrated and embedded in paraffin. Then the sections were stained with hematoxylin-eosin and histomorphological examination was carried out. Macroscopic studies showed that boluses of the administered drug “Polyacrylamide gel + Bioplant” in the submucosal layer of the anal canal retained their original sizes, migration of the drug and its resorption were not detected. It should be noted that the injected volume-forming drugs do not subject to special structural changes in the surrounding tissues, even when they are administered in close proximity to the rectal mucosa.

Conclusion. A histological experimental study showed that the volume-forming preparation “Polyacrylamide gel + Bioplant” was well fixed in the submucosal layer, on the 20th day of the experiment a thin layer of granulation tissue appeared around the preparation and the germination of its boluses by vessels, and on the 30th day the formation of a capsule around the preparation was noted. This circumstance allowed us to conclude that the volume-forming preparation “Polyacrylamide Gel + Bioplant” introduced into the submucosal layer of the anal canal is capable of effectively creating gel boluses, due to which the patency in the anal canal narrows, and this in turn contributes to the correlated parameters of basal pressure in it, which is necessary for the treatment of postoperative anal incontinence. The experimental morphological and histological studies carried out allow us to confirm the feasibility of using volume-forming preparations in complex surgical treatment of postoperative anal incontinence and recommend them as the method of choice.

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