TRANSLATED FROM RUSSIAN

RESEARCH OF THE IMPLANTATION AREA (IMPLANTATION TEST)

1. The local action of the material after implantation (implantation test) was researched in accordance with GOST R ISO 10993.10-99 “Medical devices. Study of biological action of medical devices. Part 6. Research of the local action after implantation”.

The experimental animals were kept in accordance with GOST R ISO 10993.2-99 “Medical devices. Study of biological action of medical devices. Part 2. Regulations on protection of animals”.

Samples
The material is submitted in a sterile form. “ARGIFORM” is filled in disposable injection plastic syringes, which are tipped and vacuum-sealed in individual blister packaging. Each set consisting of a blister packaging with a syringe and an injection cannula which are packed into a separate branded carton box. The marking, description and the trademark are printed on the boxes.

2. Description and results of the research

A histological study of rabbit’s jumping joint tissues after an intraarticular injection of “ARGIFORM” material was carried out.

2.1. Description of the experiment

The experiments were made on 20 rabbits. The 1 ml amount of material was injected into the articular cavity of right jumping joint, and left intact joint served as a control. The rabbits were removed from the experiment on the 1^{st}, 3^{rd}, 7^{th}, 10^{th} and 14^{th} days and in 1, 3, 6, 12 and 14 months after injection. The joint was dissected; tissues were taken from the synovial membrane and articular cartilage, fixed in 70% ethanol solution and embedded into paraffin, then preparations were taken for the histological and histochemical study. Sections with 4-5 microns thickness were stained with hematoxylin-eosin, with picric acid and fuchsine according to Van Gieson, with toluidine blue for acid glycosaminoglycans (GAG), with PAS reaction for glycoproteins and with Brachet reaction for RNA. The tissues of intact symmetric joint were taken for the control.
For cytological study smears of joint content were done (synovial liquid and synovial liquid with the material). Smears were fixed in a 96% ethanol solution, stained according to Romanovsky-Gimza; the percent ratio of cells of different types was calculated. Cytological study performed at the 1^{st} day till 6^{th} month of experiment and the histological one performed at the 1^{st} day till 14^{th} month after material injection.

2.2. Results

2.2.1 Macroscopic examination

In the course of dissection of intact joints, a small amount of translucent synovial liquid with moderate viscosity is found in their cavities.

The synovia of rabbits has an adipose structure.

The articular cartilage is thin, whitish; its surface is smooth and brilliant.

In 1 day after material injection the articular cavity contained a translucent substance in which the material and synovial fluid constitute one substrate.
The viscosity of this substance is higher than that of the pure synovial liquid and equal to the initial tested material.

The synovia does not have hyperemia, has a usual structure; the cartilaginous lamina is not changed.

In 3 days the articular cavity also contains a viscous and translucent substance.
Absence of blushing of this substance, as well as the absence of hyperemia and oedema of synovia points to the absence of inflammatory reaction.

In 7-14 days the amount of material in the articular cavity diminishes, its viscosity decreases.
The synovia and cartilage still do not have any sign of pathological changes.

In 1-14 months the content of articular cavities where the material had been injected do not differ from the content of intact contralateral joints.
The same may be said about the synovia and the cartilage.

2.2.2. Cytological and histological study

In 1-3 days after injection the material preserved its homogeneity, but from the 7^{th} day processes of vacuolization and fibrillation intensified, and that is the evidence of the beginning of material lysis.

The resorption of the material is generally made by synoviocytes. Normally, synoviocytes (parietal cells of synovial membrane) consist of two populations: A-cells of macrophage origin and B-cells of fibroblast genesis.

According to the data of cytological study (table #1), phagocytosis of the material begins already at the 1st day, intensifies at the 7-14th days and it is made generally by A-cells migrated to the articular cavity, and in a significantly smaller degree by macrophages of hematogenous origin (from blood monocytes).

The phagocytizing cells which are presented at intact joints only by 0.7% of cells, on the 1-14th day after material injection constitute more than 70% of all cells. Then their content diminishes, but even in 6 months there are still 21.4% of these cells.

At all experimental terms the phagocytizing synoviocytes significantly exceed the phagocytizing macrophages of hematogenous origin.

The quantity of neutrophils in the smear, which normally constitute 0.7% of all cells, in 1-3 days after material injection increases insignificantly up to 3.3 and 3.1%.

This points to the fact that the aseptic inflammatory reaction of the synovia to material injection is very weakly expressed and is quickly leveled: in 14 days the neutrophils constitute 1.3% and in 1 month 0.9%.

The content of the lymphocytes practically does not change.

The quantity of non-phagocytizing macrophages increases insignificantly only from the 1st day to the 7th day.

Table #1

Percentage of cells in the smears of joint synovia

Cells	Control	Experiment						
		1 day	3 days	7 days	14 days	1 month	3 months	6 months
1.Non-phagocytizing synoviocytes	91.8 %	7.8%	2.8%	19.8%	22.4%	51.2%	64.5%	70.8%
2. Monocytoid macrophages	2.7%	8.1%	4.1%	4.0%	3.2%	8.3%	3.4%	2.9%
3. Phagocytizing cells (total percentage)	0.7%	76.3%	85.8%	70.4%	70.1%	34.9%	27.1%	21.4%
3a. Synoviocytes	0.7%	58.2%	74.2%	62.3%	57.9%	27.8%	22.9%	18.3%
3b. Macrophages	0%	18.1%	11.6%	8.1%	12.2%	7.1%	4.2%	3.1%
4. Lymphocytes	4.0%	4.5%	4.2%	3.1%	3.0%	4.7%	4.2%	4.0%
5. Neutrophils	0.7%	3.3%	3.1%	2.7%	1.3%	0.9%	0.8%	0.9%
Quantity of cells in the field of vision	2.5 units	16.6 ед.	18.4 units	8.5 units	6.5 units	5.4 units	2.9 units	2.7 units

After material injection the total cells amount of smears increases significantly: in 1-3 days it amounts to 16.6 and 18.4 cells accordingly, and at intact joints, in average, 2.5 cells in view.

This growth takes place mainly owing to the phagocytizing synoviocytes. To the 7^{th} and 14^{th} days the cells amount decreases (8.5 and 6.5 accordingly), and to the 1-3^{rd} months it becomes normal which is attributed to termination of material phagocytosis.

3. Conclusion on the implantation test during an intraarticular material implantation

The histological and histochemical researches confirm and supplement the cytological data.

In 1-3 days no noticeable inflammatory changes are observed in the synovial membrane. There is no edema or vascular reaction which is characteristic of synovitis. The neutrophilic infiltration is minimal. The tissue reaction to the injection of the material consists of a nidal hyperplasia of synoviocytes, basically at the expense of the macrophage A-cells which participate in the material phagocytosis.

On the 3^{rd} day small areas of the thickened synovial layer (the parietal stratum) are formed, which increase in the volume to the 7^{th} day. Material resorption and foamy cells formation take place in these areas. However, the inflammatory reaction does not intensify.

During later terms (from 10 days to 3 months) the structure of the synovial membrane in most cases gradually becomes fully normal, but some of the animals have small hyperplasia areas of synoviocytes layer with resorption of the material. In 6 months at one animal there were founded small areas of fibrosis of the parietal stratum of the synovial membrane. In 14 months no changes of the synovial membrane were found, in comparison with the control joints.

It is necessary to note, that histochemical study during all terms did not reveal any changes of RNA content and synthesis of acid glycosaminoglycans (GAG) in the synoviocytes which is the evidence of a full safety of their biosynthetic function.

Anatomical or histochemical features of the articular cartilage did not differ from those the intact joints during all time of the observation. Taking into consideration the fact that nutrition of the cartilaginous tissue is partly carried out through diffusion of substances from the synovial liquid, we may conclude that the absence of dystrophic changes into cartilage is the evidence of the fact that articular cavity gel injection does not affect cartilage metabolism.

Thus, intraarticular injection even of a large dose of material “ARGIFORM” does not lead to development of an inflammatory process in the synovial membrane (synovitis) or a dystrophic process of the cartilaginous tissue. The tissue reaction to the material injection into a joint is minimal and basically consists of hyperplasia and moving of A-type synoviocytes which gradually resorb the material in the articular cavity (macrophage origin).
The material, evidently, forms with the synovial liquid a complex compound, which does not worse the metabolism of the articular tissues.

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