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## **Comparison of markers of skin inflammation after injections of polylactic acid and threads based on polylactic acid**

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### **Abstract**

Aging processes lead to the tremendous changes in every tissues of human and skin is not an exception. Externally observable aging changes of the skin are caused by involutionary processes, which are happening on all levels, from skin to bones. The development of methods of prevention and treatment, which are aimed at fighting age-related changes in the skin and activating regenerative processes, is a live issue and has great scientific and practical significance.

One of the important factors of skin aging is involutinal changes occurring in the dermis. Currently, injections, based on polylactic acid are actively used as collagen stimulants in aesthetic medicine. Polylactic acid is a hydrophobic, biodegradable polymer derived from lactic acid. However, the mechanism of effect of polylactic acid on the components of the dermis, as well as the morphohistochemical status of the implant itself and the effect on markers of skin inflammation remains insufficiently understood and are actively discussed in the literature.

We carried out biochemical studies of rat skin in order to study the effect of polylactic acid on markers of skin inflammation. To assess the condition of the skin of rats, after the administration of the studied drugs, the following was determined:

- marker of inflammation - the activity of neutrophilic elastase;
- factor of the state of cell membranes - the activity of lysosomal acid phosphatase;
- the state of nonspecific antimicrobial protection - the activity of lysozyme.

The conducted study allows us to conclude about the relative safety of preparations based on polylactic acid, the subdermal administration of which after 60 days shows minor signs of inflammation and practically does not affect the nonspecific antimicrobial protection of the skin of laboratory rats.

**Key words: polylactic acid; skin; markers of skin inflammation; skin cell membrane factors; connective tissue; collagen; involutive skin changes; skin aging; anty-age.**

## **INTRODUCTION**

Polylactic acid is a hydrophobic, biodegradable polymer made from lactic acid. Polylactic acid belongs to the family of alpha hydroxy acids, it is a popular material in medicine, which is obtained from potato and corn starch through polymerization and fermentation.

This synthetic biodegradable polymer does not cause rejection, completely resorbable and eliminated from the body naturally [1]. It is widely used in cardiology, dentistry, arthrology. However, the mechanism of the effect of PLLA polylactic acid (poly-L-lactic acid) on the components of the dermis, the effect on markers of skin inflammation, the morphohistochemical status of the implant itself remain unexplored and are actively discussed in the literature [2, 3, 4].

Research by G.M. Mogilnaya with co-authors "On morphological transformations of implants from polylactic acid with its subdermal localization" showed that polylactic acid activates the effect of neocollagenogenesis in different zones in different ways: for example, the capsule surrounding the implant is characterized by an increase in its thickness; for the dermis located above the implant, the activation of collagen synthesis was established by the effect of thickening of the collagen fibers themselves and an increase in their number, and in the implant area, due to an increase in the number of cells from the periphery to the center and the appearance of collagen fibrils between them. (5)

Polylactic acid is used in aesthetic medicine in various forms:

- In the form of a sterile powder, which is diluted with sterile water for injections (sometimes with the addition of 2% lidocaine solution), and is injected with a needle or cannula subdermally; it consists of polylactic acid of porous structure with globular particles (compact spherical tertiary structures) and powdery additives (mannitol, carboxymethylcellulose). Depending on the manufacturer, the size of these particles varies is located in the range of 40-80 micrometers.

- In the form of sterile finished fibers inserted into a carrier needle for subcutaneous administration.

The main purpose of using drugs based on polylactic acid is to stimulate the growth of new connective tissue fibers.

Currently, various injections based on polylactic acid are presented on the world market, which provides an aesthetic medicine specialist with a wide choice.

**PURPOSE OF THE WORK:** to study the effect of subdermal injections of various polylactic acid drugs on markers of skin inflammation, factors of the state of cell membranes and nonspecific antimicrobial protection.

**MATERIALS AND METHODS:** The experiment was carried out on 34 female white laboratory rats, with an average weight of 324 g, 8 months old. The animals were divided into five groups, depending on the type of drug they were injected with:

1. Control group, animals were injected with 0.1 ml of sterile 0.9% saline solution
2. Sculptra (150 mg PLLA) diluted in 9 ml of water for injection with the addition of 1 ml of 2% lidocaine (PLLA concentration 15.0 mg / ml) was injected in 0.1 ml of the finished solution.
3. Composition "GANA V + HA", powder GANA V (210 mg PLLA) in dilution with 15 ml of stabilized HA 5mg / ml (concentration of PLLA 14 mg / ml & HA 5mg / ml), 0.1 ml of the prepared solution was injected.
4. GANA V 520 (210 mg PLLA) diluted in 14 ml of water for injection with the addition of 1 ml of 2% lidocaine (concentration of PLLA 14 mg / ml)
5. Noble lift threads (PLLA single N, 27G 38mm-52mm)

Liquid drugs were injected into the paravertebral region, 1 cm lateral to the spine, using a syringe with a diluted solution, an 18-22G needle; prepared PLLA threads were installed paravertebrally at the same distance directly with a prepared needle with PLLA thread.

When working with animals, we were guided by the Law of Ukraine "On the Protection of Animals from Cruelty" (No. 1759-VI of December 15, 2009), taking into

account the rules of the European Convention "On the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes".

The animals were withdrawn from the experiment 60 days after administration of drugs under thiopental anesthesia (40 mg / kg). Shaved areas of the skin (3.0 by 1.5 cm<sup>2</sup>) were picked out in the area of drug administration. The skin was frozen prior to analysis.

Skin homogenates were prepared at the rate of 75 mg / ml 0.05 M Tris-HCl buffer pH 7.5, and the supernatant obtained after centrifugation at 2500 rpm for 30 min was used for biochemical studies. To assess the condition of the skin of rats after administration of the studied drugs, the following was determined:

- a marker of inflammation - the activity of neutrophilic elastase;
- factor of the state of cell membranes - the activity of lysosomal acid phosphatase;
- the state of nonspecific antimicrobial protection - the activity of lysozyme.

The acid phosphatase activity (pH 4.8) was determined according to the method of Bessey et al. in the modification of Levitsky using the substrate of n-nitrophenyl phosphate. Under the influence of tissue phosphatase, n-nitrophenol is cleaved from the substrate, which has a yellow color in an alkaline medium, the intensity of which is proportional to the activity of the enzyme and was determined spectrophotometrically. The enzyme activity was expressed in  $\mu\text{cat} / \text{kg}$  of tissue [6, 9].

The elastase activity was assessed by the Visser and Blout method according to the hydrolysis of the synthetic substrate N-t-BOC-L-alanine-p-nitrophenyl ester (Sigma, Germany). Under the action of elastase, n-nitrophenol is cleaved from the substrate, giving a yellow color, the intensity of which is proportional to the activity of the enzyme and was recorded spectrophotometrically. Elastase activity was expressed in  $\mu\text{cat} / \text{kg}$  [7, 9].

Determination of the activity of lysozyme in the skin of rats was carried out using a bacteriological method based on the ability of lysozyme to degrade the substrate of the culture of bacteria *Micrococcus lysodeikticus*. When lysozyme is exposed to the substrate, its clearing is observed, which is recorded spectrophotometrically. The degree of clearing is proportional to the activity of lysozyme, which was expressed in units / kg of tissue [8].

Statistical processing of the results was carried out using the parametric Student's T-test. Differences with a significance level of at least 95% ( $p < 0.05$ ) were considered significant.

## **Results**

The table summarizes the results of biochemical analysis of the skin of experimental animals after administration of the drugs of research. The criteria for assessing the safety of

the investigated drugs – determining of the markers of inflammation of the acid phosphatase and elastase activity. The indicators of antimicrobial protection in the skin of rats were also evaluated.

Table - Indicators of lipid peroxidation, inflammation and antimicrobial protection in the skin of rats after the use of the studied drugs

Drug	Acid phosphatase activity, $\mu$ -cat / kg	Elastase activity, $\mu$ cat / kg	Lysozyme activity, units / kg
Control group	13,2 $\pm$ 1,0	11,7 $\pm$ 0,84	5,21 $\pm$ 0,24
«Sculptra»	11,8 $\pm$ 0,91 p > 0,4	13,2 $\pm$ 0,92 p > 0,5	4,73 $\pm$ 0,26 p > 0,2
Composition «GANA V + HA»	11,7 $\pm$ 0,85 p > 0,4 p1 > 0,8	10,6 $\pm$ 0,82 p > 0,4 0,05 < p1 < 0,1	4,48 $\pm$ 0,22 p < 0,05 p1 > 0,6
«GANA V»	14,3 $\pm$ 0,92 p > 0,6 0,05 < p1 < 0,1	12,4 $\pm$ 0,91 p > 0,7 p1 > 0,5	4,02 $\pm$ 0,18 p < 0,01 p1 < 0,05
Threads PLLA	13,8 $\pm$ 0,98 p > 0,8 p1 > 0,2	15,9 $\pm$ 1,10 0,05 < p < 0,1 p1 > 0,8	4,75 $\pm$ 0,24 p > 0,4 p1 > 0,8

Note. p - reliability of differences to the indicator in the intact group (norm), p1 - reliability of differences to the indicator in the comparison group (Sculptra).

Breaking of the integrity of the membranes of lysosomes, containing a large number of destructive enzymes, in particular acid phosphatase, is considered to be the starting stage of inflammation. Thereby, acid phosphatase was selected as a safety marker of the drugs of interest. The analysis revealed a constant level of this indicator in all cases, both in relation to normal values in the intact group (p > 0.4-0.8) and in relation to the Sculptra preparation (p1 > 0.1-0.2). The above results indicate the absence of a damaging effect of the investigated drugs on the membranes of animal skin cells.

As an exception, we can consider the drug GANA V, after the application of which the activity of acid phosphatase tended to slightly increase in comparison with the preparation Sculptra (0.05 < p1 < 0.1).

Elastase is a powerful destructive proteolytic enzyme, the main source of which is segmented neutrophils. In addition to the destructive effect, elastase activates procollagenase, converting it into the active form of the enzyme - collagenase, which significantly aggravates the destructive processes. Our studies have shown that administration of Sculptra did not affect elastase activity in rat skin (p > 0.5).

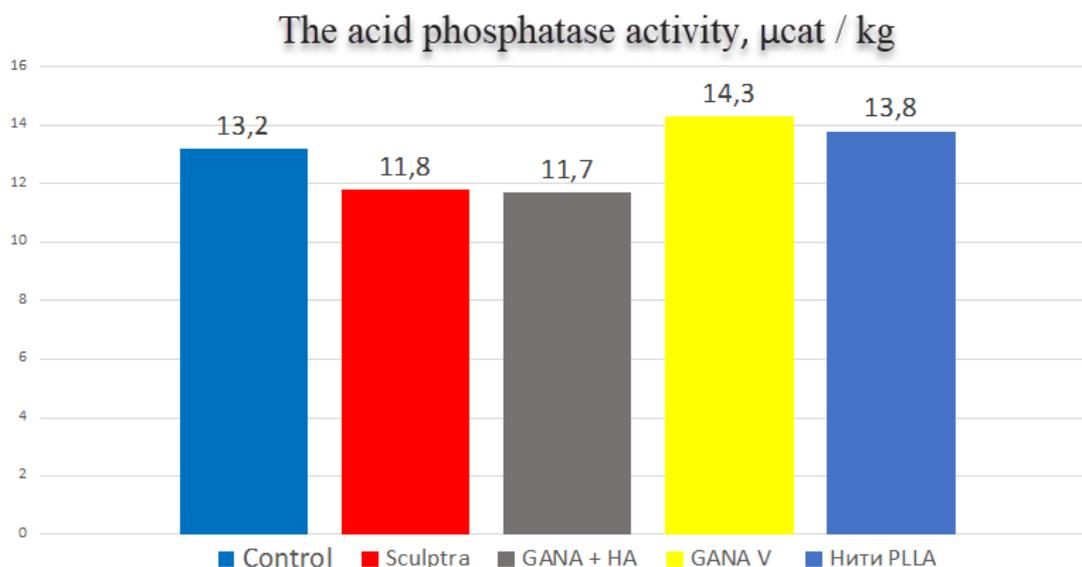


Fig. 1. Acid phosphatase activity

The use of the composition "GANA V + stabilized HA 5 mg / ml" even contributed to a slight decrease in this marker of inflammation, which indicates the anti-inflammatory efficacy of the composition "GANA V + stabilized HA 5 mg / ml", possibly due to the presence of hyaluronic acid in the composition. The use of GANA V had no significant effect on elastase activity in animal skin. After insertion of PLLA threads, elastase activity tended to increase compared to normal values ( $0.05 < p < 0.1$ ), which may indicate a longer period of connective tissue activation.

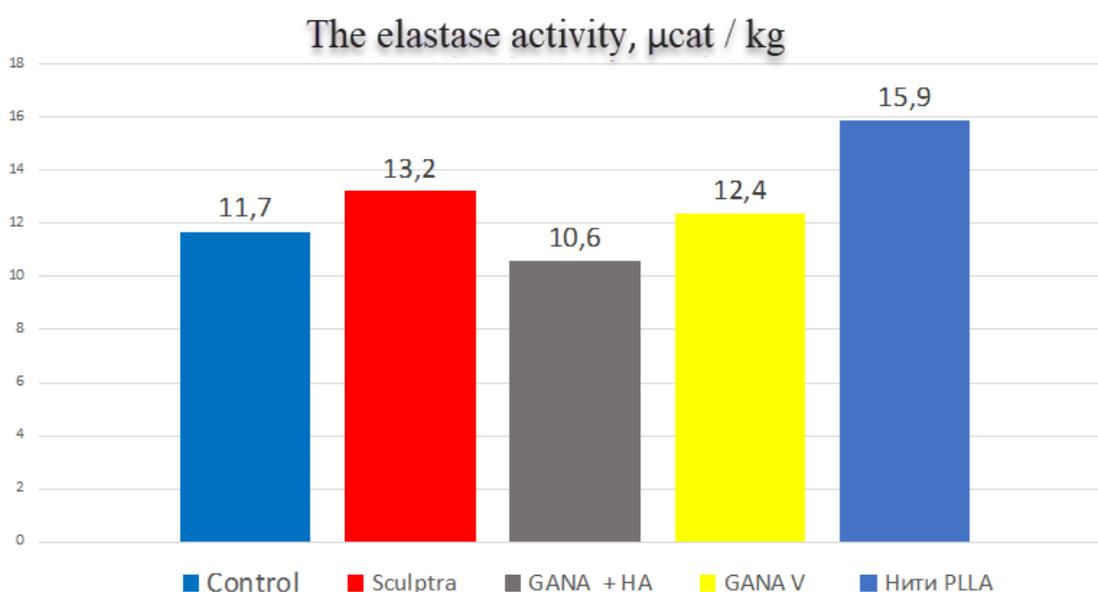


Fig. 2. Elastase activity

Lysozyme, an enzyme capable of destroying bacteria and viruses, as well as activating phagocytic leukocytes and the production of immunoglobulins, plays a key role in the system of antimicrobial protection of mucous membranes and skin integuments. A change in the content of lysozyme in the skin may indicate either an increase in antimicrobial protection or a weakening of it, and also indicates the level of the adaptive response of the skin. As established by our analysis, the activity of lysozyme did not undergo significant changes in the skin of rats, which were injected with Sculptra ( $p > 0.2$ ) and PLLA threads ( $p > 0.4$  and  $p > 0.8$ ). After the introduction of the composition "GANA V + stabilized HA 5 mg / ml", the degree of antimicrobial protection of the skin was reduced by 14.0% ( $p < 0.05$ ), and after the application of GANA V - by 22.8% ( $p < 0, 01$ ).

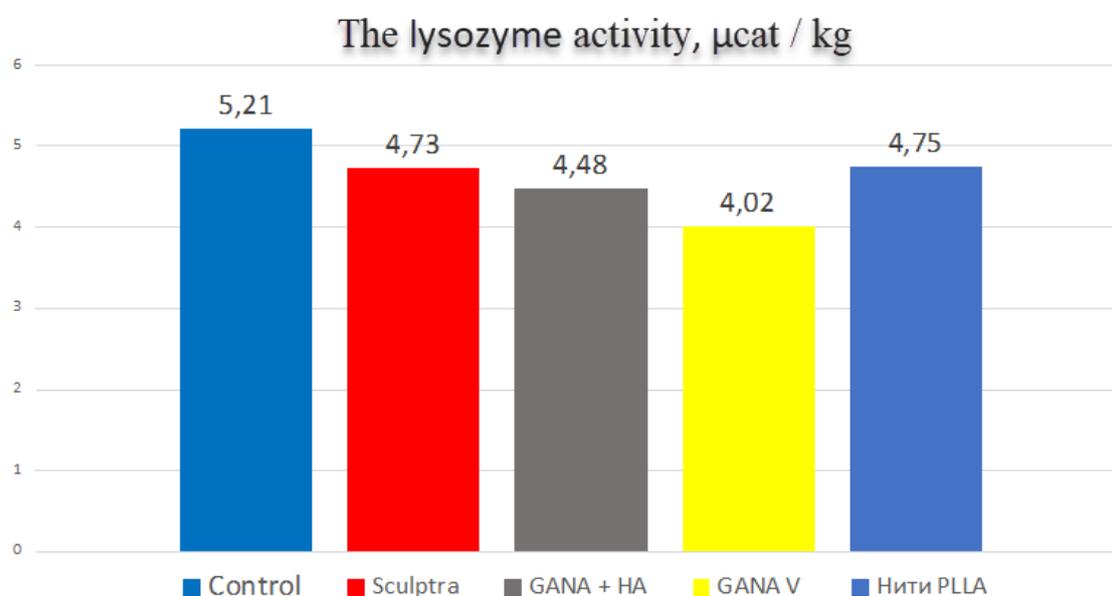


Fig. 3. Lysozyme activity

#### Discussion and conclusions:

The biochemical analysis of the skin of laboratory rats after the administration of the studied drugs allows us to make the following conclusions:

1. After application of the Sculptra preparation after 60 days, no signs of inflammation (acid phosphatase and elastase activity) and a decrease in antimicrobial protection (lysozyme activity) were detected in the skin of rats.

2. The injection of the composition "GANA V + stabilized HA 5 mg / ml" was, in some degree, anti-inflammatory, possibly due to the content of hyaluronic acid, since the

activity of neutrophil elastase tended to decrease, both in relation to the level in the skin of control rats group and in relation to the group after administration of the Sculptra drug. At the same time, the activity of antimicrobial protection was slightly reduced.

3. The action of the "GANA V" preparation is similar to the action of the "GANA V + stabilized HA 5 mg / ml" composition in terms of the degree of antimicrobial activity of the skin; indicators of inflammation - with a tendency to increase lysosomal acid phosphatase, which may be associated with the characteristics of this drug.

4. The use of PLLA filaments caused an increase in neutrophil elastase compared with normal levels in animal skin, but did not differ much from Sculptra. The acid phosphatase activity and the degree of antimicrobial protection were normal.

The conducted study allows us to conclude that there are biochemical changes after a single injection of polylactic acid into the skin of rats 2 months after the start of the experiment, which may indicate the preservation of the activity of the administered drugs during these periods, which is more expressed in PLLA threads.

The obtained data can serve as an experimental justification for a better understanding of the effects after the injections of drugs based on polylactic acid into the skin, however, further research is needed (including research in clinical settings and course injections) to study its efficacy and safety.

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