THE HISTOLOGICAL STUDY OF THE EFFECT OF GELS WITH SILVER NANOPARTICLES AND GLUCOSAMINE ON PURULENT WOUND HEALING

L.Bulyga, Ya.Butko, Yu.Laryanovska
National University of Pharmacy

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The search for new drugs to treat purulent wounds is one of the urgent problems of modern medicine. Most local wound healing drugs at the pharmaceutical market of Ukraine do not meet all modern medical and biological requirements for effective treatment of the wound process, and there is a need in expansion of their range. Modern developments related to the use of silver nanoparticles for treatment of wounds have several advantages, such as the multilevel antibacterial effect, delayed resistance, low toxicity, anti-inflammatory and reparative properties. The aim of our work was the histological study of the effect of gels with silver nanoparticles and glucosamine on healing the skin with a purulent necrotic wound in rats. The results have shown that the gels with glucosamine or silver nanoparticles and their combination applied to animals have better histological parameters of recovery of damaged tissues than those of the reference drug – “Dermazin” cream. A newly formed tissue has the most complete character in the wound of animals treated with the sample of the gel with silver nanoparticles and glucosamine. It confirms the rationality of the combination proposed and the absence of antagonism between the active ingredients. Therefore, for further pharmacological studies the combined gel containing glucosamine and silver nanoparticles is promising in order to develop a local drug of a new generation to treat infectious surface wounds.

Treatment of purulent wounds is one of the problems of modern medicine. It is connected with the numerous domestic, industrial and military injuries, traffic accidents, natural disasters, as well as the increasing number of postoperative complications [2, 4]. According to medical statistic approximately 30% of patients have postoperative infectious complications [2].

In the treatment of wounds and infectious complications, along with surgical treatment and systemic therapy, the local treatment is also important. Most local wound healing drugs at the pharmaceutical market of Ukraine do not meet all modern medical and biological requirements for effective treatment of the wound process, and there is a need in expansion of their range. For example, antibiotics included in the majority of wound healing drugs adversely affect granulation (especially tetracycline; aminoglycosides (gentamycin) and chloramphenicol to a lesser extent); they are characterized by the rapid development of resistance, inhibit the local flora. Among antiseptics iodopiron and silver nitrate (18 and 25%, respectively) slightly inhibit development of granulation. Chlorhexidine has the most negative effect on the reparative processes in the wound [1, 3, 6, 7].

The use of achievements of nanotechnologies opens up prospects for optimizing reactive changes and reparative processes that develop in the site of the tissue damage. Modern developments related to the use of silver nanoparticles for treatment of wounds have several advantages, such as the multilevel antibacterial effect, delayed resistance, low toxicity, anti-inflammatory and reparative properties [2, 7, 8, 9].

Materials and Methods

The study of pharmacological properties of the gels with the different composition (the gel with glucosamine, the gel with silver nanoparticles and a combined gel with silver nanoparticles and glucosamine) was carried out on the model of purulent necrotic wounds (Table). This model allows studying several types of the pharmacological activity for the local drugs for treating wounds, such as antimicrobial, anti-inflammatory, reparative.

“Dermazin” cream (manufacturer – Salyutas Pharma GmbH, Germany, batch SR0680) containing 1% of silver sulfadiazine and indicated for the treatment of purulent wounds was chosen as a reference drug [2].
In the experiment 40 male albino rats weighing 260-300 g were used. They were divided into 8 groups: Group 1 – intact control (IC); Group 2 – peak of pathology (PP, rats were decapitated after the wound festering); Group 3 – control pathology (CP); Group 4, 5, 6, 7 – rats treated with gels 1, 2, 3, 4; Group 8 – animals treated with the reference drug. The skin necrosis was caused by introduction of 1.0 ml of 10% calcium chloride solution subcutaneously. In 3 days after injection the formation of necrosis foci was observed, then microbial contamination of wounds was carried out (P. aeruginosa – ATCC-27853 and S. aureus – ATCC-29213). Treatment with the gels containing nanosilver/reference drug under study was carried out within 9 days from the third day after infection with a causative agent (after the occurrence of pus on the ulcer surface). For the histological study all of the above samples of the skin were selected as follows: in natural healing – on day 3 (with pathology development) and on day 11 after infection, when treating with the gels or the reference drug – on day 11 after infection. Samples were cut from the skin defect site and the neighbouring areas of the healthy skin. All tissue material was fixed in the neutral 10% formalin solution and poured in paraffin. Sections were made on a sliding microtome and stained with haematoxylin and eosin [2, 4]. The microslides were examined under a Micros 400 microscope. Microphotography of microscopic images was made by a Nicon Col Pix 4500 digital camera. The pictures were processed on a Pentium 2.4 GHz PC using Nicon View 5. The quality assessment was performed by such healing characteristics as the size and depth of the defect, the condition of a newly formed tissue in it (the presence and depth of layers, severity of fibroblast proliferation, the signs of fibre formation), the level of epithelialisation.

**Results and Discussion**

The results of histological studies have shown that on day 3 after infection of necrotic wounds a widespread deep defect on the rat’s skin is seen on microslides. The multilayered sanious haemorrhagic necrotic crust tightly covers the surface of the defect. The defect is filled with a mixture of modified destructive residues of connective tissue fibres of the dermis and muscle fibres that are densely infiltrated, and the foci of festering are observed. There is also the cellular infiltrate in the subcutaneous adipose tissue, the layer of muscle fibres and the underlying loose fibrous tissue. The process of inflammation is advanced. There are visible “petrified” fibres, giant cells, thrombosed blood vessels in the defect (Fig. 1 a, b, c).

In the control pathology group on 11 day after infection a purulent necrotic wound has little changes in depth and size (Fig. 1 d, e, f, g). The crust on the surface is less dense, sometimes with visible stratified remnants. The diffused cellular infiltration of the subcutaneous fat tissue, the layer of muscle fibres and the underlying fibrous tissue is preserved. The sites of necrosis are seen. At the edge of the defect hypertrophy of the epidermis is preserved, and there is edema of the papillary dermis. The marginal epithelialisation is absent.

After 9-day treatment with the gel containing glucosamine (gel 1) there are no skin defects in 40% of rats. The epidermal layer is completely restored. In the site of the dermis a loose fibrous tissue with a moderate cellular content is seen. In 20% of the animals there

| Drug   | The main active substances                        |
|--------|--------------------------------------------------|
| Gel 1  | Glucosamine (1.0%) + PVP (2.0%)                  |
| Gel 2  | PVP (Ag 0.164% – 0.1%) + PVP (2.0%)              |
| Gel 3  | Glucosamine (1.0%) + PVP (Ag 0.164% – 0.1%) + PVP (up to 2.0%) |

Table: The composition of the gels containing silver nanoparticles

Notes: 1) On day 3 after infection, a – a multilayer crust on the surface of ulcer, necrosis of the connective tissue fibres of the dermis (x 100); b – the abundant inflammatory infiltrate of the subcutaneous fat and muscle tissue (x 200); c – destructive fibres with the altered tinctorial properties. Haematoxylin-eosin, x 250, 200. 2) On day 11 after infection; d – under the stratified crust there is a visible collagen matrix in the state of protein coagulation; e – sites of tissue detritus in the underlying tissues; f – the site with “petrified” remnants of fibres.

Fig. 1. The histological study of purulent necrotic wounds in the control pathology group.
are open ulcerative defects. The collagenic fibres of the reticular dermis of the sites located near the defect are damaged (Fig. 2 a, b). The rest 40% of cases the ulcer is large and deep, covered by a thick crust, it is filled about ⅔ with the cellular detritus containing less degraded leukocytes. In the depths the initial signs of creation of the granulation tissue are observed. The rest of the rats had ulcerative defects.

After treatment of purulent necrotic wounds using the gel with silver nanoparticles (gel 2) in 60% of rats there is no defect of the skin on microslides. In the site of the former ulcer the epithelial layer is slightly thicken. The newly formed fibrous tissue has the high cell content; sometimes small accumulations of small white blood cells are observed in it. Sometimes there are sites of destructive fibres under the scar and the dermis on the neighbouring healthy areas of the skin in the underlying tissues (Fig. 2 c, d).

In the group of rats treated with the gel with silver nanoparticles and glucosamine (gel 3) in 40% of the animals the ulcerative defect has been completely healed. In its place a soft scar is seen (Fig. 2 e, f). In 20% of the rats there is a small defect. In other 40% of the animals the open ulcerous defects that are large in size have been found; they are covered by a narrow scab, under which a significant amount of immature granulation tissue, or its small sites at the bottom are observed. In some rats there is cellular infiltration (varying by the intensity) of the complex of tissues underlying to the dermis with impregnations of a few giant cells and “petrified” fibres.

After treatment with the reference drug the complete healing of the defect is not observed in any rats. The ulcerative surface of some animals is covered by the crust other animals have a clean surface. In most cases the defect is filled with the immature granulation tissue, in which there are visible remains of died “petrified” fibres, giant cells. In some rats the marginal epithelisation of the surface is well visible (Fig. 2 g, h, i). In 20% of the animals there is an abscess in the deep layers of the defect. In the sites of the healthy skin that are marginal with ulcers there is the thickened epidermis with a visible acanthosis, parakeratosis, and in the dermis the isolated sites of damaged fibres with giant cells nearby can be observed. In the tissues surrounding the dermis there are visible cell infiltrates.

Thus, the gels with glucosamine or silver nanoparticles and their combination under study applied to animals with purulent necrotic wounds have better histological parameters of recovery of damaged tissues than those of the reference drug – “Dermazin” cream. By histological evaluation gel 2 (silver nanoparticles) and gel 3 (with silver nanoparticles and glucosamine) have revea-
led the most positive effect on healing of the infected ulcers. However, by characteristics a newly formed tissue has the most complete character in animals treated with the sample of gel 3. It confirms the rationality of the combination with silver nanoparticles and glucosamine proposed and the absence of antagonism between the active ingredients. Consequently, by the effect on maturation and quality of the newly formed tissue when healing wounds the drugs under study can be arranged as follows: gel 3 > gel 2 > gel 1 > “Dermazin” cream.

CONCLUSIONS

1. On the model of the infected wound in rats the histological studies have shown that all gels with glucosamine or silver nanoparticles and their combination contribute to healing of infected wounds more than those that heal naturally. By maturation and quality of a newly formed tissue the drugs under study can be arranged as follows: gel 3 > gel 2 > gel 1 > “Dermazin” cream.

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ного лечения раневого процесса, что требует расширения их номенклатуры. Современные разработки посвящены использованию наночастиц серебра для лечения ран, которые имеют ряд преимуществ: многоуровневый антибактериальный эффект, медленное развитие резистентности, низкий уровень токсичности, противовоспалительные и репаративные свойства. Целью этой работы было гистологическое исследование влияния гелей с наночастицами серебра и глюкозамином на заживление кожи в условиях гнойно-некротической раны у крыс. Результаты исследований показали, что у животных с ранами, которым наносили исследуемые гели с глюкозамином или наночастицами серебра и их комбинацию, имеются гистологические показатели восстановления поврежденных тканей лучше, чем у препарата сравнения крема «Дермазин». Наиболее полноценный характер имела новообразованная ткань у животных, которым наносили образец геля с наночастицами серебра и глюкозамином, что подтверждает рациональность предложенной комбинации и отсутствие антагонизма между действующими веществами. Итак, комбинированный гель, содержащий глюкозамин и наночастицы серебра, является перспективным для дальнейшего фармакологического исследования с целью разработки местного средства нового поколения для лечения инфекционных поверхностных ран.