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DEVELOPMENT OF A RAT MODEL OF DIABETIC FEMORAL PHLEGMON FOR EVALUATING THE EFFECTIVENESS OF TRADITIONAL AND INNOVATIVE TREATMENT METHODS

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RELEVANCE

Models on various experimental animals remain the most convenient tool for studying the pathophysiological mechanisms of various diseases. One of the simplest ways to create a model of diabetes mellitus and hyperglycemia in animals is partial or complete pancreatectomy. Also, chemical agents that cause damage to pancreatic beta cells, which leads to the development of diabetes, are used to model diabetes mellitus. In this context, such diabetogenic substances as streptozotocin, alloxan, pirinuron, ditizon, dialuric acid and others are used.

Many methods and wound coverings have been developed for the local treatment of extensive and difficult-to-heal wounds. However, their diversity indicates that today there is no perfect method for treating such wounds in diabetes mellitus. This highlights the need to find new therapeutic approaches. The course of the wound process in diabetes is characterized by the duration, complexity and high cost of treatment, as well as the uncertainty of the prognosis. Standard methods do not always lead to successful wound healing and amputation prevention, which makes the search for new treatment strategies relevant.

Despite the development and introduction into clinical practice of new technologies that minimize intra-and postoperative complications, the literature still lacks data on the development of innovative methods for treating purulent wounds in diabetes in experimental models.

THE AIM OF THE STUDY was to develop a method for treating purulent-necrotic lesions of the soft tissues of the thigh in conditions of diabetes mellitus and to evaluate its effectiveness in experimentalmorphological studies.

MATERIAL AND METHODS

Experimental studies were conducted in the laboratory of experimental surgery of the State Institution “Vakhidov Russian National Research Medical Center” on mongrel white rats of both sexes weighing 230-280 g (Table 1).Surgical interventions were performed under general anesthesia in accordance with international standards of humane treatment of experimental animals (Strasbourg, 1986). The rats were kept in a vivarium with supply and exhaust ventilation at a temperature of 21-22 °C, placed in separate cages of two individuals. The animals were fed a balanced diet of carbohydrates, protein, fat, and vitamins.

Table 1
Distribution of animals by series of experimental studies

Series	Group series	1 day	7 days	14 days	30 days	Total
1 series	1 group without treatment	3	3	3-9	-	9
	2 group treatment	3	3	3-9	-	9
2 series	1 group of phlegmon in DM without treatment	3	3	3	3	12

	2 grof scp-phlegmon in DM tradic. Treatment	3	3	3	3	12
3 series	A new way to treat flegmons in DM	3	3	3	3	12
Total		15	15	15	9	54

Series of experiments:

The experiment consisted of several episodes:

1. Series 1: Formation of a model of phlegmon in the femoral region in rats.
2. Series 2: Study of the features of the course of experimental phlegmon in rats in the control group and against the background of diabetes mellitus.
3. Series 3: Development of a new method of treating phlegmon and conducting comparative studies of the effectiveness of traditional and new approaches in the treatment of diabetic phlegmon in rats.

The first signs of diabetes were a sharp increase in water intake up to 120 ml per day, significant weight loss and hair loss. Whole blood and serum were used for biochemical studies. Several phases of changes in blood glucose levels were identified: the first phase — hyperglycemic, reaching a peak during the first hours; the second phase-hypoglycemic, most pronounced during the first day; the third phase-the stage of persistent hyperglycemia (Table 2).

Table 2

Dynamics of blood glucose in experimental animals

Indicator	Control values for rat	Experiment days						
		1st	3rd	5th	7th	14th	30th	45th
Glucose	2,3±0,2	3,7±0,05	10,7±0,04	10,2±0,12	9,4±0,02	9,7±0,05	9,4±0,04	10±0,07

Model of formation of purulent-necrotic process in the femoral region in rats

The formation of phlegmon of the lower limb was carried out according to the following method. An incision was made in the upper third of the lower thigh under general anesthesia using sevoflurane vapors. Using a surgical instrument of the “mosquito” type, a subcutaneous bed was formed in the distal direction, where a gauze strip soaked in a solution of a bacterial mixture obtained from the lumen of the colon was placed. The mixture was prepared at the rate of 100 mg of feces per 20 ml of saline solution. The skin wound was sutured with a knotted suture.

In the postoperative period, the animals received an analgesic during the first day. To do this, ibuprofen tablets were added to drinking water at the rate of 500 mg per 100 ml of water. In the following days, pain relief was not performed.

During the three days of observation, the animals remained active, moved freely around the cage, and consumed food and water. In the area of the left thigh, starting from the second day after surgery, an increase in the volume of the limb was observed. Palpation showed soreness and fluctuation, which indicated the formation of a purulent cavity in the subcutaneous tissue.

RESULT AND DISCUSSION

Morphological studies of the biopsy material on the third day showed that in the affected area there was a partially necrotic epidermis, significant round cell infiltration, including macrophages, lymphocytes, and neutrophilic leukocytes. Serous exudate and hemorrhages were found in the hypodermis. The blood vessels were dilated and full-blooded. Diffuse leukocyte infiltration was

observed in the dermis and subcutaneous tissue, which parted the connective tissue fibers and surrounded the sebaceous and sweat glands (Fig.

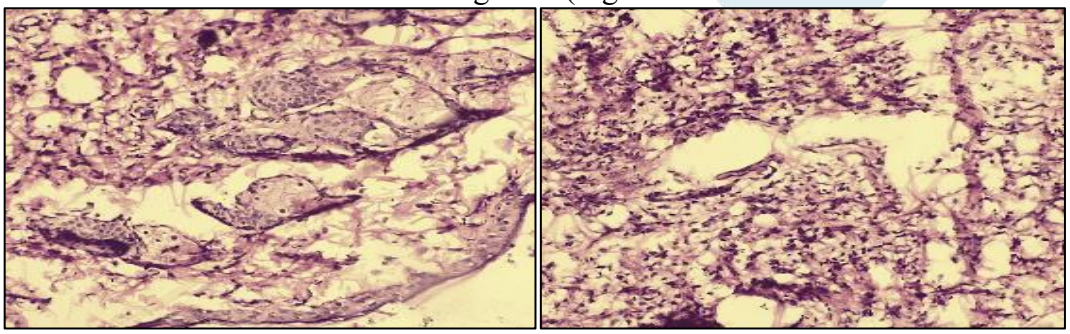

Fig..1. 3 days. Purulent-necrotic soft tissue lesion in the left thigh area. Hematoxylin and eosin staining. Ok10hOb40.

On 3-4 days after the formation of an abscess in the left thigh area, a breakthrough of purulent discharge from the area of the sutured skin wound was observed in rats (Fig. 2-3-4-4).

Fig. 2. Purulent discharge from phlegmon of the thigh

Fig.3. Purulent wound of subcutaneous tissue

Fig. 4. Purulent-necrotic process in the thigh area

Later, within 7-9 days after the operation, spontaneous healing of the purulent-necrotic process occurred with the release of a foreign body in the form of a gauze strip from the wound (Fig.5). The wound healed by secondary tension on days 10-11 after surgery (Fig. 6).

Fig. 5. Wound healing process after opening and sanation of the abscess of the thigh

region Fig. 6. Healing of a purulent thigh wound in the control (healthy) group of animals

Formation of purulent-necrotic process in the femoral region in rats with diabetes mellitus. In the group of animals that were modeled for diabetes mellitus by alloxan administration, a

persistent increase in blood sugar levels occurred on days 7-10. During this period, the control group of rats developed a purulent process in the left thigh area in the previously described way. After the operation, the animals were managed in a standard way. On the 2nd and 3rd days after surgery, 3 animals had a fatal outcome due to a slight increase in the limb volume and abscess formation. According to laboratory tests and autopsies, progressive deterioration of the animals ' condition was associated with an increase in septic conditions and organ failure.
Microscopically, during these periods, destruction of the dermis and hyperplasia of the epidermis were noted. Severe edema, hemorrhage, and diffuse infiltration with polymorphonuclear leukocytes were observed in the subcutaneous tissue. The blood vessels are dilated and unevenly full-blooded (Figure 7).

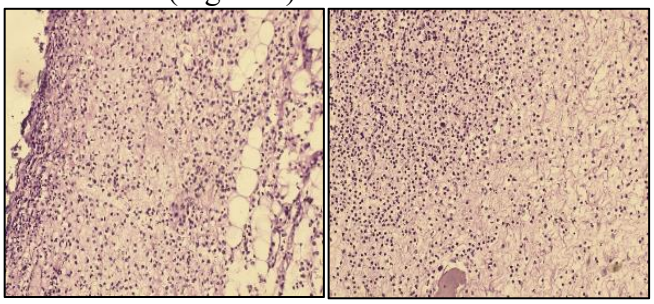

Fig. 7. 3 days. Purulent-necrotic soft tissue lesion was formed in the left thigh area. Hematoxylin and eosin staining. Ok10hOb40.

In other animals, a sluggish purulent process was observed with the formation of necrotic tissues in the subcutaneous tissue and skin. Subsequently, the rats independently gnawed out necrotic tissues with the extraction of a gauze strip left in the wound. Independent healing of the purulent wound occurred only in 1 of the control animals on the 14th day after surgery.

Comparative studies of the results of treatment of diabetic phlegmon in rats by traditional and new methods

In the third series of experiments, comparative studies of the effectiveness of a new method for treating purulent-necrotic wounds of subcutaneous tissue against the background of experimental alloxan diabetes mellitus were conducted.
Against the background of the formation of a purulent process in the hip area, surgical intervention was performed on 4-5 days after the operation.

Results of traditional treatment. In the control group of animals, the purulent wound was dissected under general anesthesia with sevoflurane vapors with sanitation, necrectomy, and treatment with 3% hydrogen peroxide solution. The wound was then left open and monitored.
On the next day after opening the abscess, the wound was covered with purulent-necrotic plaque. There were no signs of granulation or wound cleansing.
On the 3rd day after opening the abscess, there was skin necrosis along the edges of the surgical wound, the latter gaped, without signs of area reduction. The bottom of the wound was covered with purulent-necrotic plaque (Fig. 8). On the 7th day after the operation, the wound was covered with a dense crust, after which a meager amount of purulent discharge with necrotic tissues arrives. Granulating tissue appears in some places of the wound (Fig. 9).

Figure 8. Control. Purulent-necrotic process in the hip region on the background of diabetes mellitus.

Figure 9. Control. Necrosis of the skin on the 7th day after the formation of an abscess and traditional treatment on the background of DM

On the 11th day after opening and sanation of the wound in rats with diabetes mellitus, the wound is again covered with a dense crust, which does not separate from the edges and bottom of the wound. When the discharge is pressed, there is no fluctuation. There is a reduction in the area of the wound.

Results of treatment in the experimental group of animals.

The method of surgical treatment of purulent-necrotic processes and phlegmon of the lower extremities in diabetes mellitus includes dissection of tissues, sanitation of purulent congestion, and excision of necrotic areas. Two-light transparent drainage tubes are installed in the cavities and pockets. A special feature of the method is the use of a sterile polyethylene self-adhesive film to close the wound after installing drains. In the postoperative period, drip washing of the wound cavity is performed through drainage with a 0.1% solution of methylene blue. The procedure is continued for 1 hour under a pressure of at least 500 mm of water at a rate of 60 drops per minute. 30 minutes after the start of washing, the wound is irradiated with a laser device Vostok-2. Irradiation is carried out through the film in a defocused continuous mode with a wavelength of 630-660 nm and a power of 120 MW. Irradiation lasts 1 minute for every 2.5-3.0 cm2 of the wound surface. The procedure is performed 3-4 times a day for 3-5 days. After reducing acute purulent inflammation, the frequency of procedures is reduced to 2 times a day for 2-3 days, and then to 1 time a day for another 2-3 days.

CONCLUSIONS

1. Morphological studies conducted on the third day after the formation of a purulent-necrotic process in the femoral region in rats showed that necrotic epidermis, infiltration, serous exudate, and hypodermic hemorrhages were observed in the affected area. In diabetic patients, microscopic studies revealed more pronounced changes: destruction of the dermis, sharp edema and hemorrhage in the subcutaneous tissue, as well as diffuse infiltration by polymorphonuclear leukocytes.
2. Experimental morphological studies on the model of purulent-necrotic lesions of the soft tissues of the thigh in diabetes mellitus have shown that the use of local laser exposure and photodynamic effect accelerates the regression of inflammatory infiltration in all layers of the dermis and subcutaneous adipose tissue. These methods help to reduce the time to complete cessation of purulent-necrotic discharge, clean the wound from fibrinous overlays, reduce local tissue edema and activate regeneration processes.

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