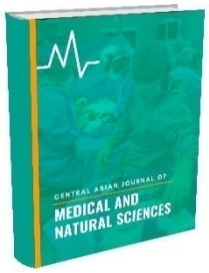
CENTRAL ASIAN JOURNAL OF MEDICAL AND NATURAL SCIENCES



Volume: 04 Issue: 04 | Jul-Aug 2023 ISSN: 2660-4159

http://cajmns.centralasianstudies.org

Experimental Substantitation of the Use of Laser Irraditation and Photodynamic Therapy in Diabetic Femoral Phlegmon

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Received 26th Jun 2023,
Accepted 26th Jul 2023,
Online 28th Aug 2023

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Resume: The article presents the results of experimental studies on the possibility of the formation of a purulent wound in laboratory rats and an assessment of the effectiveness of using traditional and new methods of healing. for this purpose, the authors conducted experimental morphological studies on 54 laboratory rats of the "Vistar" breed in the experimental department of the Academician V.V.Vakhidov "RSNPMCH". A model of diabetic phlegmon was used to perform an autopsy of the purulent-necrotic process of the thigh in rats using photodynamic therapy and laser irradiation. Studies have shown that the use of local laser exposure and photodynamic therapy accelerated the regression of inflammatory infiltration, complete cessation of purulent-necrotic discharge, wound cleansing and activation of regeneration processes.

Key words: experimental model, diabetic phlegmon, autopsy of the purulent - necrotic process, photosensitization, laser irradiation. the aim of the study is to improve the results of treatment of purulent lesions of the extremities in patients.

Models on various experimental animals are the most convenient for studying the pathophysiology of any disease. One of the simplest methods of creating a model of diabetes mellitus and hyperglycemia in an animal is the removal of the pancreas, partially or completely. To create a model of diabetes mellitus, chemicals are also used, as a result of which damage to the beta cells of the pancreas occurs and, as a result, diabetes mellitus develops [2,9]. To reproduce the model, several substances with diabetogenic activity are used: streptozotocin and alloxan, pirinuron, ditizon, dialuric acid, etc. [1,6].

For the local treatment of extensive and long-term non-healing wounds, many methods and wound coverings have been developed. Nevertheless, their wide variety indicates that there is currently no perfect method of treating extensive and long-term non-healing wounds in diabetes mellitus (DM), and this determines the relevance of the search for new methods of treatment [3,5,10]. The course of the wound process in DM is characterized by a long duration, complexity and high cost of treatment, and an ambiguous therapeutic prognosis. Standard methods do not always allow to achieve wound defect

healing and prevent amputation, which makes it urgent to search for new therapeutic approaches [4, 7, 8, 9].

Despite the development and introduction into practical healthcare of new technologies that minimize intra- and postoperative complications, however, there is no information in the literature about the development of new methods of treating purulent wounds in diabetes mellitus on an experimental model.

The aim of the study: to develop a method for the treatment of purulent-necrotic lesions of the soft tissues of the thigh in conditions of diabetes mellitus and to evaluate its effectiveness in experimental morphological studies.

Material and methods

Experimental studies were performed in the laboratory of experimental surgery of the State Institution "RSNPMC named after Academician V. Vakhidov" on white mongrel rats of both sexes weighing 230-280 gr. (table.1). Operations were performed under general anesthesia in accordance with the requirements for humane treatment of experimental animals (Strasbourg, 1986). The animals were kept in a vivarium equipped with supply and exhaust ventilation, a temperature regime of 21-22 ° C, in separate cages of 2 individuals. Nutrition was carried out with a balanced composition of carbohydrates, proteins, fats and vitamins.

Table 1 Distribution of animals by series of experimental studies

Series.	Groups.	1 days	7 days.	14 days.	30 days	Total.
1 series	1 group without treatment	3	3	3	-	9
	2 group treatment	3	3	3	-	9
2 series	1 group phlegmon in diabetes without treatment	3	3	3	3	12
	2 group - phlegmon with DM traditional. treatment	3	3	3	3	12
3 series	A new method for the treatment of phlegmon in diabetes	3	3	3	3	12
Total		15	15	15	9	54

Series of experiments:

1. Series: formation of a phlegmon model in the thigh area in rats.
2. Series: study of the features of the course of experimental phlegmon in rats in control and against the background of diabetes mellitus.
3. Series: development of a new method for the treatment of phlegmon with comparative studies of the results of treatment of diabetic phlegmon in rats in a traditional and new way.

To conduct objective studies, before forming an experimental model of soft tissue phlegmon in rats, a model of the development of diabetes mellitus was initially reproduced. For this purpose, a method of forming an experimental model of SD was used, proposed by a team of authors of the State Institution "RSNPMC named after Academician V.Vakhidov". The drug Alloxan was used as a pharmacological agent. Method and dosage of alloxan administration according to the proposed method: administration of alloxan tetrahydrate intraperitoneal solution at a dose of 170 mgr. per 1 kg of body weight. Alloxan was administered after daily fasting.

The first signs of diabetes manifested themselves in the form of a sharp increase in water consumption up to 120 ml, a sharp loss in weight, hair loss. In biochemical studies, whole blood and serum were

used. Several phases of changes in blood glucose were observed: the first phase – hyperglycemic, reaching a maximum during the first hours; the second – hypoglycemic, which mainly manifested itself during the first day, the third phase – the phase of persistent hyperglycemia (Table 2).

Table 2 Dynamics of glucose in the blood of experimental animals

Indicator	Control values for rats	Days of the experiment						
		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

A model of the formation of a purulent-necrotic process in the thigh region in rats

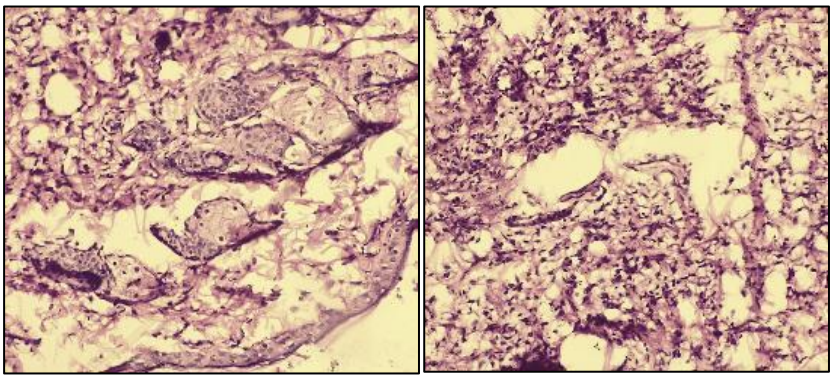
The formation of the phlegmon of the lower limb was carried out according to the following method. Under general anesthesia with sevoflurane vapors, an incision was made in the area of the b/3 thigh of the lower limb. Using the mosquito tool, a bed was formed distally in the subcutaneous space, where a gauze strip moistened with a solution of a bacterial mixture obtained from the lumen of the colon was placed. Dilution at the rate of 100 mg. of feces per 20 ml of saline solution. The skin wound is sutured with a nodular suture.

In the postoperative period, the animals received painkillers for 1 day in dissolved drinking water: Ipobrufen tablets at the rate of 500 mg. per 100 ml of water. In the following terms, anesthesia was not carried out.

During 3 days of observation, the animals remained active, moved freely around the cage, took food and water. In the area of the left thigh, starting from 2 days after the operation, the volume of the thigh began to increase. During palpation, soreness and fluctuation were noted due to the formation of a purulent cavity in the subcutaneous tissue.

Result and discussion

Morphological studies of the biopsy material showed that on day 3, partially necrotic epidermis, abundant round-cell infiltration (macrophages, lymphocytes) were detected in this zone, neutrophilic leukocytes, serous exudate and hemorrhages in the hypodermis are often found. The blood vessels are dilated and full-blooded. Diffuse leukocyte infiltration in the dermis and subcutaneous tissue, which push apart the connective tissue fibers and surround the sebaceous and sweat glands (Pic. 1).



Pic. .1. 3 days. Purulent-necrotic lesion of soft tissues in the area of the left thigh. Stained with hematoxylin and eosin. Ok10hOb40.

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

Pic. 2. Purulent discharge from the phlegmon of the thigh area

Pic.3. Purulent wound of subcutaneous tissue

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

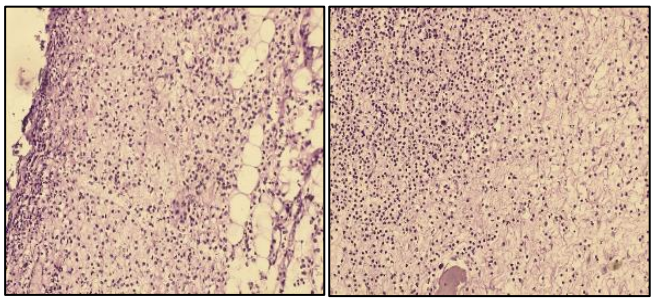
Later, during 7-9 days after the operation, spontaneous healing of the purulent-necrotic process occurred with the exit of a foreign body from the wound in the form of a gauze strip (Pic.5). The wound healed by secondary tension for 10-11 days after surgery (Pic.6).

Pic. 5. Wound healing process after opening and sanitation of the hip abscess

Pic. 6. Healing of a purulent hip wound in a control (healthy) group of animals

Formation of a purulent-necrotic process in the thigh area in rats against the background of diabetes mellitus. In the group of animals that were modeled diabetes mellitus by the administration of alloxan, a persistent increase in blood sugar levels occurred on 7-10 days. During this period, a purulent process was formed in the control group of rats in the area of the left thigh in the previously described way. After the operation, animal management was carried out in a standard way. On the 2nd and 3rd days after the operation, against the background of a slight increase in the volume of the limb and the formation of an abscess, a fatal outcome occurred in 3 animals. According to laboratory tests and autopsy, the progressive deterioration of the animals' condition was associated with an increase in septic condition and organ failure.

Microscopically, during these periods, the destruction of the dermis and hyperplasia of the epidermis were noted. There was pronounced edema, hemorrhages and diffuse infiltration of polymorphonuclear leukocytes in the subcutaneous tissue. Blood vessels are dilated and unevenly full-blooded (Pic. 7).



Pic. 7. 3 days. A purulent-necrotic lesion of soft tissues was formed in the area of the left thigh. Stained with hematoxylin and eosin. Ок10хОb40

The rest of the animals had a sluggish purulent process with the formation of necrotic tissues in the subcutaneous tissue and skin. Subsequently, the rats independently gnawed out necrotic tissues with the extraction of the gauze strip left in the wound. Independent healing of a 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 in a traditional and new way

In the third series of experiments, comparative studies of the effectiveness of a new method of treating purulent-necrotic wounds of subcutaneous tissue against the background of experimental alloxan diabetes mellitus were carried out.

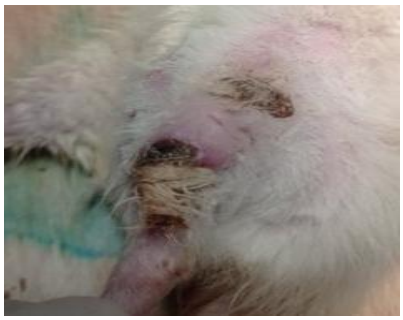
Against the background of the formation of a purulent process in the thigh area, surgical intervention was performed on 4-5 days after the operation.

The results of traditional treatment. In the control group of animals, the purulent wound was dissected under general anesthesia with sevoflurane vapor with sanitation, necrectomy and treatment with 3% hydrogen peroxide solution. Then the wound remained open and observation was carried out.

The next day after the opening of the abscess, the wound was covered with a purulent-necrotic plaque. There were no signs of granulation and wound cleansing.

On the 3rd day after the opening of the abscess, necrosis of the skin took place along the edges of the surgical wound, the latter gaped, without signs of a reduction in the area. The bottom of the wound was covered with a purulent-necrotic plaque (Pic. 8). On the 7th day after the operation, the wound was covered with a dense crust, after removal of which a meager amount of purulent discharge with necrotic tissues arrives. Granulating tissue appears in some places of the wound (Pic. 9).

Pic. 8. Control. Purulent-necrotic process in the hip area on the background of diabetes mellitus.



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

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

Results of treatment in an experimental group of animals. In the experimental group of rats, a new method of treatment was performed at the same time:

A method of surgical treatment of purulent-necrotic processes and phlegmon of the lower extremities against the background of diabetes mellitus: dissection of tissues and sanitation of purulent congestion, excision of necrotic tissues with the leaving of two-light transparent drainage tubes in pockets and cavities, characterized in that after removal of drains, the open wound is closed with a sterile polyethylene self-adhesive film (film for bandages), then, in the postoperative period, drip washing of the wound cavity is carried out through the installed drainage with a 0.1% solution of methylene blue for 1 hour under a pressure of at least 500 mm of water.st. at a rate of 60 drops per minute and 30 minutes after the start of washing through a film fixed over the wound, the wound area is irradiated with an Vostok-2 laser device in a defocused continuous mode, in the range of 630-660 nm, with a power of 120 MW, for 1 minute for each 2.5-3.0 cm2, while this procedure is performed 3-4 times a day for 3-5 days, and after the acute purulent inflammation subsides 2 times a day for 2-3 days and then 1 time a day for another 2-3 days.

Description of the operation. When examining the left lower limb, swelling and soreness in the area of the outer surface of the thigh are determined. An autopsy of the abscess was performed, while thick pus with an unpleasant odor and necrotic tissues were released. Treatment of the abscess cavity with a 3% solution of hydrogen peroxide. The wound surface is hermetically sealed using an optically transparent film. A catheter with a diameter of No. 19 French is left in the lower edge of the film. A needle was inserted through the upper corner of the coating and a 1% solution of methylene blue was slowly injected using a syringe for 2 minutes. Next, the wound was irradiated with red radiation with a power of 5 MW for 2 minutes. After 1 hour, the procedure was repeated in a similar way (Pic. 10-13).

Pic.10. Experience. Fixation of a transparent film on the area of the opened abscess of the thigh area

Pic. 11. Experience. Introduction of methylene blue solution through a catheter into the cavity of the abscess

Pic. 12. Experience. Washing of a purulent wound with a solution of methylene blue under a transparent film

Pic. 13. Experience. Treatment of a purulent wound with a laser with photosensitization with a solution of methylene blue.

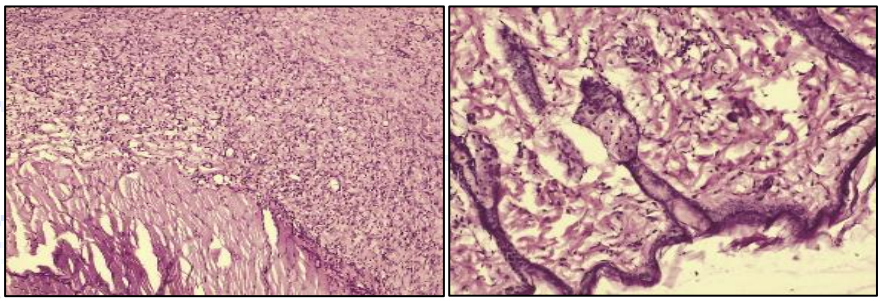
In the experimental group of animals, after the operation and wound rehabilitation, the rats actively moved around the cage, resisted when trying to pick up. On examination, the wound is covered with a dense crust, moderately painful on palpation. On the dressing under general anesthesia, after removing the crust, a small amount of cloudy, odorless liquid is released. The bottom of the wound is covered with fibrin, there are areas of marginal tissue necrosis. The wound has an oblong shape.

On the 3rd day after the operation, the condition of the animals differs little from healthy individuals. On examination, the wound was significantly reduced in size, covered with a dense crust. With palpation, it is slightly painful. On dressing under general anesthesia, the crust over the wound is removed with difficulty, the wound bleeds. The wound has significantly shrunk in size. The bottom of the wound with signs of granulation. Necrotic tissues were removed together with the removed crust (Pic. 14). On the 5th and 7th days after the operation, when examined, the wound is covered with a dense crust, slightly painful, the edges of the wound have shrunk (Pic.15).

Pic.14. Experience. Wound cleansing for 3 days after treatment

Pic.15. Experience. 5 days after the operation.

On the 5th day after the operation, a biopsy of tissues in the hip area was taken during treatment (Pic.16)



Picture 16. Experience. 5 days. Purulent soft tissue lesion in the left thigh area on the background of diabetes mellitus. The condition after treatment of a purulent wound with a laser with photosensitization with a solution of methylene blue. Stained with hematoxylin and eosin. Oк10xOb40.

The figure shows that the epidermis grows moderately, and there is swelling in the dermis layer due to inflammation of the connective tissue. Blood vessels are evenly distributed, sclerosed, there was no outpouring of blood in the tissues.

On the 9th-11th day after the operation, the crusts were removed from the wound with the formation of a scar on the site of the previously formed purulent-necrotic process.

Thus, the development of new methods for the treatment of purulent-necrotic soft tissue lesions on the background of diabetes is associated with the problem of creating a model of diabetes mellitus in experimental animals. The most optimal model is the formation of DM in rats using alloxan.

To assess the body's response to an acute infectious process in the soft tissues of the extremities, we have developed an experimental model of the phlegmon of the lower extremities in rats. To do this, a gauze turunda infected with intestinal contents was implanted into the subcutaneous tissue of rats. In

healthy rats, the purulent necrotic process was formed on the 3rd day after infection and was expressed in an increase in the volume of the limb, swelling and fluctuation due to the presence of pus in the subcutaneous tissue, signs of intoxication appeared in the form of decreased appetite and restriction of physical activity, leukocytosis. In these animals, the healing process took place in the form of rejection of a foreign body or gnawing out the abscess by the animal itself. No deaths were observed, however, the healing process was delayed up to 10-12 days.

The main experiment consisted in the formation of a purulent process in the soft tissues of the NK with concomitant diabetes mellitus and performing therapeutic manipulations in the control and experimental groups of animals. It was found that in rats that were not treated, the generalization of the process occurred in the form of sepsis with a fatal outcome in 30% of animals on 4-6 days. During the traditional treatment, which consisted in opening the abscess on the 3rd day and treatment with antiseptics, the generalization of the process in the form of sepsis and the death of 10% of animals was observed. In the remaining rats on the background of DM, the healing process was delayed up to 13-16 days with the formation of an abscess with a crust and the completion of the process in the form of a rough scar. At the same time, normalization of the level of white blood cells did not occur even after 30 days.

In the control group of animals, the traditional method of treating purulent-necrotic lesions of the soft tissues of the limb was used. Against the background of experimental diabetes mellitus, the purulent-necrotic process was often complicated by sepsis with a fatal outcome of animals. Morphological changes after treatment of skin phlegmon in the control group were characterized by: hyperplastic epidermis, diffuse leukocyte infiltration of subcutaneous tissue. The blood vessels are dilated and unevenly full-blooded. Integumentary epithelium with dystrophic changes, serous exudate in hypoderm. Minor leukocyte infiltration in subcutaneous tissue, swelling of the connective tissue stroma, sebaceous and sweat glands are hyperplasized. Blood vessels are dilated.

We have developed and experimentally tested a treatment method based on sufficient aeration of purulent congestion by a donor of singlet oxygen and oxygen radicals (methylene blue) under the influence of radiation in the red spectrum (630-660 nm), which effectively suppresses bacterial growth.

In this group of animals, on the 3rd day after the formation of the phlegmon, the purulent cavity was opened, treated with an antiseptic -0.1% solution of methylene blue, followed by irradiation with laser radiation in the spectrum of 640-660nm, which had a pronounced antimicrobial photodynamic effect. As a result of treatment, there were no deaths in the experimental group of animals. Wound healing took place with the formation of a crust on 2-3 days after the opening of the abscess and complete healing within 8-10 days and normalization of the level of leukocytes in the blood.

Morphological changes on the 3rd day of the development of the purulent-necrotic process in the thigh area in rats against the background of diabetes mellitus: destruction of the epidermis and dermis was noted, necrotic masses with a large number of neutrophil leukocytes were at the bottom of the wound. Pronounced edema, hemorrhages and diffuse infiltration by polymorphonuclear leukocytes, serous exudate and hemorrhages in the hypodermis were noted at the bottom and edges of the wound. The blood vessels are dilated and full-blooded. It was found that inflammatory infiltration spread to all layers of the dermis and subcutaneous fat. In some observations, the formation of cavities filled with purulent-necrotic masses was noted. On the 5th day after treatment with a new method, an improvement in the condition of animals and the nature of the wound was observed. Purulent discharge stopped, the wound was covered with a crust. Morphologically, the epidermis is moderately proliferating. There is swelling in the layers of the dermis due to inflammation of the connective tissue. Blood vessels are evenly distributed, sclerosed. The process of hemorrhage and the progression

of necrosis is not observed in the tissues. The bottom of the wound is cleared of fibrinous overlays and the process of tissue regeneration is traced.

Comparative macroscopic, histological, biochemical and bacteriological studies have made it possible to establish the effectiveness of a new method of treating purulent-necrotic soft tissue lesions on the background of diabetes mellitus:

- requires less bandages, not burdensome and painless;
- constant irrigation of the wound leads to the leaching of necrotic tissues and pus;
- Methylene blue, being an antiseptic, under the action of laser radiation becomes a donator of oxygen radicals, providing a photodynamic effect that is detrimental to all types of pathogenic microflora.

In all groups of animals with experimental DM, normalization of blood sugar levels did not occur, even after the complete subsiding of the purulent-necrotic process of soft tissues.

CONCLUSIONS

1. Morphological studies during the formation of a purulent-necrotic process in the thigh area in rats on day 3 showed that necrotic epidermis, infiltration, serous exudate and hemorrhages in the hypodermis were detected in the affected area, while against the background of diabetes mellitus, microscopically marked destruction of the dermis, pronounced edema, hemorrhage, and also diffuse infiltration by polymorphonuclear leukocytes.
2. Experimental morphological studies conducted on the model of purulent-necrotic soft tissue lesions in conditions of diabetes mellitus have shown that the use of local laser exposure and photodynamic effect in the treatment of these destructive processes accelerate the regression of inflammatory infiltration of all layers of the dermis and subcutaneous fat, shortening the period until the complete cessation of purulent-necrotic discharge and cleansing the wound from fibrinous overlays, reduction of local tissue edema and activation of regeneration processes.

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