

# Antimicrobial Effect of Led Radiation on Microflora Sensitized with Methylene Blue in Strangulated Abdominal Hernias

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**Abstract** The widespread development of photodynamic therapy (PDT) in recent decades and the successful introduction of the technique into clinical practice for the treatment of inflammatory processes of various localizations [5] and the discovered bactericidal effect of PDT [2] allow, in our opinion, to use the PDT method for the prevention of wound purulent-inflammatory complications in strangulated abdominal hernias. PDT is based on reactions in which a photosensitizer (PS) transfers its energy to molecular oxygen, which passes into an active singlet form (anion radical) and induces cytotoxic reactions based on oxidation [5]. Methylene blue (MB) can be used as a PS. The absorption spectrum of MB is 500 nm - 700 nm, with a maximum at 664 nm, which allows it to be used in combination with LED radiation with a wavelength of  $640 \pm 20$  nm. It is known that they have already been used in ENT diseases [3], in purulent surgery [4], and in peritonitis [1]. Important factors in favor of using MB are its low cost and availability. In the available literature, we have not found any works on the use of MB for the purpose of preventing wound purulent-inflammatory complications in strangulated abdominal hernias.

**Keywords** Strangulated abdominal hernia, Antimicrobial action, LED radiation, Sensitized methylene blue

## 1. Introduction

The aim of our study was to develop a method for preventing early purulent-inflammatory procedures for strangulated hernias using photodynamic effects on microflora.

Isolation of pure cultures was performed using the traditional method of mechanical separation on the surface of a dense nutrient medium. The number of microorganisms per 1 cm<sup>2</sup> was calculated based on the number of colonies grown on the dish, taking into account the dilution, and expressed in CFU/cm<sup>2</sup>. A certified domestically produced LED lamp FDU-1 served as a radiation source (wavelength -  $640 \pm 20$  nm, output radiation power - 200 mW/cm<sup>2</sup>). The main culprits of postoperative purulent-inflammatory complications are several microbes, among which the priority in occurrence is: *Staphylococcus aureus* - 28%; *Staphylococcus epidermidis* - 6%; *Escherichia coli* - 46%. The species composition of the microflora of hernial fluid, isolated from patients with strangulated hernias of groups I and II, was the same. In the clinical part of the work, we conducted a comparative analysis of the results of surgical treatment of 225 patients with strangulated abdominal hernias. All patients with strangulated abdominal hernias were divided

into two groups: I (main) - 127 patients who received intraoperative LED irradiation in the range of  $640 \pm 20$  nm of the surgical field in combination with preliminary photosensitization of the microflora of the surgical field with a 0.05% solution of MB (2018-2020), while the irradiation power density was 200 mW / cm<sup>2</sup>, the energy density was 25-35 J / cm<sup>2</sup>, and also locally for hemo- and lymphostasis from the edges of the surgical wound, as well as the prevention of seromas, a new domestic local hemostatic "Hemosponge" collagen was used. For PDT, we used a domestically produced FDU-1 apparatus; treatment of 98 patients in group II (control) was carried out traditionally, i.e. The surgical field was sanitized with a furacilin solution - 5:1000. In the control group, the age of patients ranged from 31 to 79 years. The average age was  $64.7 \pm 9.7$  years. There were 45 men (45.9%), 53 women (54.1%). There were 23 patients over 70 years old (23.5%).

Table 1 presents patients by type of strangulated hernia.

In the main group of patients, 0.05% MB solution was prepared ex tempore under sterility for photodynamic sanitation of the surgical field. After removal of the hernial sac, before plastic surgery of the hernial orifice, after hemostasis, a napkin soaked in the MB solution was placed on the wound and left on the wound for 5 minutes. Then the napkin was removed and dried. LED radiation was directed at the wound at a distance of 5 cm from the surgical field

for 3-5 minutes. Subsequently, a wound culture was taken and plastic surgery of the hernial orifice was performed; according to indications, alloplasty with the Esfil lightweight mesh was performed. For the purpose of hemo- and lymphostasis, at the end of the operation, a new domestic collagen hemostatic, Hemosponge, was poured onto the plastic area. The wound was sutured layer by layer.

**Table 1.** Distribution of patients in the main group and comparison group by type of hernia

Type of hernia	Main		Control		Total
Inguinal	58	45,7%	39	39,8%	97
Umbilical	29	22,8%	31	31,6%	60
Femoral	5	3,9%	5	5,1%	10
P/o ventral	35	27,6%	23	23,5%	58
Total	127	100%	98	100%	225

## 2. Results of the Research and Their Discussion

In the first series of experiments, the effect of different doses of LED irradiation with a wavelength of  $640 \pm 20$  nm on the microflora of hernial fluid in patients with strangulated hernias was studied. The time parameters of irradiation were 1, 3, 5, 10 min, and the energy parameters were 200 mW/cm<sup>2</sup>. It was found that LED radiation, even with a power density of 200 mW/cm<sup>2</sup>, with an irradiation duration of 3-5 min, did not have an antibacterial effect.

At the second stage of our research we studied the antimicrobial activity of MB solutions in concentrations of 1.0, 1.0.01 and 0.001% in relation to the most common microflora of hernial fluid of patients with strangulated hernias at exposure of 1, 2, 3, 4, 5 minutes. It was found that MB in concentrations of 0.1 - 0.001% do not have an antimicrobial effect. When staining microbial cells with the indicated dyes, only 1%, 0.01% and, to a greater extent, 0.05% MB solutions at 3-minute exposure stain microbial cells, which are clearly visible at subsequent immersion microscopy. All this allowed us to choose a 0.05% MB solution as a potential photosensitizer, which we used in our further work. In the next series of experiments, the effect of LED radiation generated by the FDU-1 device (power density of 200 mW/cm<sup>2</sup>, irradiation duration of 3-5 min) on the microflora of hernial fluid of patients with strangulated hernias pre-sensitized with MB (0.05% solution, exposure 3-5 min) was studied. FDU-1 is a certified device approved for clinical use, the radiation power density is 200 mW/cm<sup>2</sup>. We used FDU-1 for the first time to prevent purulent-inflammatory wound complications in strangulated hernias. When using a 0.05% dye solution, at a power density of 200 mW/cm<sup>2</sup> in combination with LED radiation, irradiation time from 3 to 5 min, a pronounced antimicrobial effect on the microflora of hernial fluid of patients with strangulated hernias was observed. The number of CFU/ml decreased from  $10^4$  in the control to  $10^2$  after irradiation. This decrease

was directly dependent on the duration of irradiation.

The results of our experimental studies suggest that the use of sanitation of the surgical field with a 0.05% solution of the MB photosensitizer (exposure 3 minutes) and FDU-1 (power density - 100 mW/cm<sup>2</sup>, irradiation time - 3-5 min) after removal of hernial fluid and hernial sac in compliance with the rules of antisepsis will allow already at this stage of treatment to reduce the degree of canal infection by 2-3 orders of magnitude, reduce the number of wound purulent-inflammatory complications, and therefore improve the results of treatment of strangulated abdominal hernias. In the clinical part of the study, we analyzed the results of the use of PDT sanitation of the surgical field with MB. Table 2 presents the nature of postoperative complications in the main group and in the placement group. It follows from the table that in the main group of 127 patients with strangulated hernias, early complications were observed in 8 (6.3%) patients, in the comparison group - in 16 (16.3%) patients. In patients with PP hernial orifice plastic surgery using the Esfil lightweight mesh, the most common complication was seroma, which occurred in 4 (3.1%) cases. Hematoma was detected in 2 (1.6%) patients in the postoperative period. Wound suppuration was observed in 2 (1.6%) patients. No wound infiltrates or marginal skin necrosis were observed.

**Table 2.** Early postoperative wound complications among patients in the main and control groups

Type of complication	Main group n=127 (PDT with MB)		Control group n=98 (furacilin 1:5000)	
	Abs.	%	Abs.	%
Seroma	4	3,1±1,6	7	7,14±2,6
Hematoma	2	1,6±1,1	1	1,02±1,0
Wound suppuration	2	1,6±1,1	5	5,10±2,2
Infiltrate	-	-	2	2,04±1,4
Marginal skin necrosis	-	-	1	1,02±1,0
Total	8	6,3±2,2*	16	16,3±3,8

Note: \*- reliable compared with the control group indicators (\*- P < 0.05).

Among 98 patients in the control group, seroma was observed in 7 (7.1%) patients, hematoma was detected in 1 (1.02%) patient, and wound suppuration in 5 (5.1%), infiltrate in 2 (2.04%) and marginal skin necrosis in 1 (1.02%). It should be noted that the decrease in seroma formation in the main group by more than 2 times, and wound suppuration by more than 3 times is associated, firstly, with the use of "Hemosponge" in combination with the antiseptic miramistin for intraoperative seroma prevention, and secondly, only local use of electrocoagulation for hemostasis in the main group. The "Hemosponge" provided almost instant hemo- and lymphostasis in the surgical wound, and miramistin in the hemosponge created an antibacterial environment for 5-6 days, due to the slow biodegradation of the hemosponge. In the control group, hemostasis was carried out exclusively by electrocoagulation, and the collagen "Hemosponge" was not used.

A relatively large number of seromas (7.1%) in plastic surgery using a PP mesh in the on lay position is explained by extensive tissue dissection, with prolonged capillary bleeding, lymphorrhea, cellular tissue burn during electrocoagulation, contact of the mesh prosthesis with subcutaneous fat, features of the tissue response to the introduction of synthetic material, the timing of mesh ingrowth by connective tissue and is not associated with the development of infectious complications. In the main group, due to the use of a hemosponge, a drug that provides instant hemostasis and lymphostasis, while practical electrocoagulation is not used. Moreover, covering the PP mesh with an inert composite (hemosponge) reduces the "foreign body-tissue" reaction.

We studied the microbial landscape of the hernial sac contents, as well as a biopsy of the tissues around the hernial sac. In patients with strangulation of the greater omentum, it was found that the level of contamination of the effusion in the hernial sac and tissues around the hernial sac remained below the critical level -  $10^5$  microbial bodies/g.

The contamination of the effusion in the hernial sac in case of strangulation of the small intestine, when the strangulated hernia was often accompanied by intestinal obstruction in patients in our observations, exceeded the critical level 2-4 hours after strangulation. The critical level is 109 microbial bodies/g. However, the contamination of the tissues around the hernial sac remained at a significantly lower level and reached the critical level after 6-8 hours. Based on the microbiological studies of hernial fluid and tissue biopsy samples around the hernial sac conducted in the clinic, it was established that with strangulation of various abdominal organs, the critical level of bacterial flora develops after 6 hours from the onset of strangulation. Based on the studies conducted in the clinic, the indication for prosthetic plastic surgery in patients with strangulated postoperative ventral hernias is up to 6 hours after strangulation. However, the use of PDT sanitation in combination with a local hemostatic agent allowed us, if there were indications (large sizes of the hernial orifice, weakness of the muscular-aponeurotic complex), to perform allohernioplasty to eliminate ventral hernias even under infection conditions, i.e. when the critical level was 109 microbial bodies/g. In the main group, allohernioplasty for strangulated ventral hernias was performed by us in 2 of 35 patients, and in the control group - in 16 of 23. However, the use of PDT and collagen "Hemosponge" for the prevention of wound complications in the absence of intestinal gangrene with phlegmon of the hernial sac allowed us, if there were indications, to perform allohernioplasty of ventral hernias in 21 patients with a period of intestinal strangulation of more than 6 hours. Postoperative wound complications in the main group of patients after allohernioplasty were observed in 3 patients (seroma in 2, hematoma in 1), and in the control group, in terms of strangulation of more than 6 hours, postoperative wound complications were noted in 8 patients (seroma in 4, hematoma in 2, wound suppuration in 1, infiltrate in 1).

Thus, a comparative analysis shows that the use of PDT in combination with a hemosponge for strangulated postoperative hernias reduces the number of postoperative complications

in the early postoperative period by more than 2.5 times, even with strangulation periods of more than 6 hours after allohernioplasty in patients with strangulated postoperative ventral hernias.

The nature of systemic complications did not differ significantly between the main group and the comparison group.

Table 3 shows the percentage data on the total number of early postoperative wound complications for each type of hernia.

**Table 3.** Frequency of early postoperative wound complications among patients with different types of hernias

Type of hernia	Main group		Control group	
	Abs.	%	Abs.	%
Inguinal	2	1,6±1,1	4	4,1±2,0
Umbilical	2	1,6±1,1	5	5,1±2,2
Femoral	-	-	1	1,0±1,0
Ventral	4	3,1±1,5	6	6,1±2,4
Total	8	6,3±2,2*	16	16,3±3,8

Note: \*- reliable compared to the control group indicators (\*-P<0.01)

When comparing the incidence of postoperative wound complications in various types of hernias and different methods of plastic surgery, it was found that the proportion of complications is higher due to ventral hernias, while they are in no way associated with the use of alloplasty. As follows from the table, it was possible to reduce the number of postoperative complications due to the use of PDT in combination with a hemosponge. The longest stay of patients in bed was required after herniotomy for ventral hernias, especially after allohernioplasty. In this group of patients, we were able to reduce the length of stay from  $11.8 \pm 0.72$  to  $9.4 \pm 0.32$  bed days. The use of PDT in combination with a hemosponge allowed us to significantly reduce the length of stay of patients in the hospital from  $10.2 \pm 1.6$  to  $6.3 \pm 1.1$ . Reducing the number of days a patient spends in hospital brings not only economic benefits – a reduction in the cost of providing treatment to the patient, a reduction in the total number of days of incapacity for working citizens – but also social benefits.

### 3. Conclusions

1. In strangulated abdominal hernias, the hernial fluid is infected with facultative anaerobic microflora. The dominant species are *Staphylococcus aureus* - 28%; *Staphylococcus epidermidis* - 6%; *Escherichia coli* - 46%. A high degree of infection of the hernial fluid is noted after 6 hours of strangulation, in the main intestinal loop with an increase in the frequency of *Escherichia coli*.
2. MB solutions (0.05%) and LED radiation (wavelength  $640 \pm 20$  nm, output power density 200 mW/cm<sup>2</sup>, energy density 25-35 mW/cm<sup>2</sup> with exposure from 3 to 5 minutes), with an irradiation area of 10 cm<sup>2</sup>

separately do not have antimicrobial activity against the facultative anaerobic microflora of hernial fluid.

3. The radiation generated by the FDU-1 LED lamp with a wavelength of  $640\pm 20$  nm, a power density of 200 mW/cm<sup>2</sup> with an exposure of 3-5 minutes, an energy density of 25-35 mW/cm<sup>2</sup> has antimicrobial activity against the facultative anaerobic microflora of the hernial fluid of patients with strangulated hernia, sensitized with a 0.05% solution of MB for 3-5 minutes, which is manifested by a decrease in the number of CFU/ml of the total microflora of the surgical wound.
  4. The use of the developed method of photodynamic intraoperative wound sanitation using MB made it possible to reduce wound postoperative purulent-inflammatory complications from  $16.3\pm 3.8\%$  to  $6.3\pm 2.2\%$ , and to reduce the average length of a patient's stay in hospital from  $10.0\pm 0.67$  to  $7.3\pm 0.77$  bed-days.
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