

Studying The Activity Of In Vitro Antiseptics Decasan, Furacillin and Chlorhexidine Bigluconate Against Hospital Strains of S. Aureus, E. Coli, Klebsiella Spp., P. Melaninogenica Separated from the Bed of the Soleus Muscle

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Abstract: A prospective analysis of the case histories of 38 DM patients who underwent shin amputation in the Department of Wounds and wound infection at the A.V. Vishnevsky National Research Center for Surgery of the Ministry of Health of the Russian Federation during 2017-2019 was performed. All the studied patients were taken biological material during the operation and the comparative antimicrobial activity of the antiseptics decasan, chlorhexidine bigluconate and furacillin was studied against strains of S. aureus, E. Coli, Klebsiella spp., P. melaninogenica. The analysis of the obtained results revealed that 0.02% decasan solution exhibits high antimicrobial activity in relation to aerobic and anaerobic hospital strains of microorganisms.

Key Words: critical ischemia, diabetes mellitus, diabetic foot syndrome, limb amputation, antimicrobial activity, decasan.

Introduction

Peripheral artery disease is a common manifestation of systemic atherosclerosis associated with a high risk of developing cardiovascular complications and deaths and leading to a significant restriction of physical activity of patients and a decrease in their quality of life [8]. Diabetes mellitus (DM) is a risk factor for the development of peripheral artery diseases. Patients with DM are at a higher risk of developing peripheral artery diseases (PAD) compared to the general population [1,7,11]. According to epidemiological studies, the prevalence of ZPA in the group of patients with DM reaches from 8 to 40% [2].

The prevalence of critical ischemia of the lower extremities (CILE) is 500-1000 cases per 1 million population per year (about 150 thousand cases per year) [3,14]. The risk of developing CILE in patients with DM is 10-20 times higher compared to patients without diabetes [2,7]. Also, 70% of PAD are asymptomatic due to the presence of diabetic polyneuropathy [4,12].

Unfortunately, the frequency of amputations in critical ischemia of the lower extremities does not tend to decrease and is performed in 25% patients with obliterating vascular diseases [5,16]. The need for high amputation reaches the level of 52-95% within 3 years from the onset of CILE [6,13] and is accompanied by an overall mortality rate of 10-40% to 71% [9] over the next 2-3 years. According to a number of authors, the mortality rate in the perioperative period after amputations ranges from 15 to 43.7%, and in some age categories exceeds 50% [10]. The high percentage of mortality is primarily due to purulent-necrotic and cardiovascular complications that occur after amputations [5,15]. Thus, the number of postoperative purulent-necrotic complications from the amputation stump of the limb reaches an alarming size – 20-65% [2,11]. Even a high amputation at the level of the upper third or middle third of the thigh is accompanied by an extremely high level of purulent-necrotic complications, which, in turn, significantly increase the postoperative mortality, the duration of inpatient and subsequent outpatient treatment and the associated material costs [2,10]. Currently, there is no single effective system for predicting the clinical course of the postoperative period in patients who have undergone lower limb amputations [3,4].

All these data indicate that this problem is unresolved and it is advisable to further develop new tactical approaches to the treatment of this category of patients to reduce the frequency of disability, mortality and postoperative complications.

Purpose of the work- was to improve the results of surgical treatment of patients with critical lower limb ischemia with DM by determining the antimicrobial activity of antiseptic solutions of decasan, furacillin and

chlorhexidine bigluconate against hospital strains of *S. aureus*, *E. coli*, *Klebsiella* spp., *P. melaninogenica* isolated from the bed of the soleus muscle.

Materials And Methods

In the experiment, the bactericidal effect of decasan was studied in comparison with antiseptic solutions of furacillin and chlorhexidine bigluconate.

Taking into account that during the bacteriological study of the bed of the flounder muscle in patients in the comparison group, in most cases, *S. aureus* was detected from aerobic microorganisms, the study was carried out relative to this microorganism.

The antimicrobial activity of decasan against hospital microorganisms was determined in the microbiology laboratory at the Bukhara State Medical Institute according to the "Methodological recommendations for the experimental (preclinical) study of medicines for the local treatment of purulent wounds" [Dotsenko et al., 1989]. As a dense nutrient medium, Muller-Hinton agar was used, which is used in international practice to determine the antibiotic sensitivity of microorganisms. As test cultures, we used clinical strains of gram-positive and gram-negative microorganisms isolated from patients of the purulent surgery department who underwent amputation at the level of b/3 of the lower leg and were hospitalized for critical ischemia of the lower extremities against the background of diabetes mellitus. The microbial suspension of the daily culture of microbes was prepared according to the turbidity standard and corresponded to 10⁸ CFU in 1 ml of the suspension. The study conducted a comparative study of the antimicrobial activity of Decasan with antiseptic solutions widely used in surgical practice for the local treatment of purulent wounds: chlorhexidine bigluconate and furacillin. The activity of antiseptics was studied in relation to the most frequently isolated pathogens of purulent-inflammatory processes in surgical patients. Among gram-positive microorganisms, it was *S. aureus*, the gram-negative flora was represented by the following bacterial species: *E. coli*, *Klebsiella* spp., from the group of anaerobic bacteria – *P. melaninogenica*. The total number of strains is 40, with 10 strains in each group. The diameter of the growth retardation zones was measured with an accuracy of 1 mm, using a caliper.

The antimicrobial activity was evaluated according to the following criteria :

- a growth retardation zone with a diameter of up to 10 mm or its absence indicates that the microorganisms are not sensitive to the drug introduced into the well;
- the growth delay zone with a diameter of 11-16 mm indicates a low sensitivity of the culture;
- the growth delay zone with a diameter of 17-20 mm is considered as an indicator of the sensitivity of microorganisms;
- the growth delay zone with a diameter above 20 mm indicates a high sensitivity of microbes. The research data are presented in Table 1.

Table 1.
Comparative antimicrobial activity of antiseptic solutions to aerobic microorganisms

Microorganisms	Microbial growth retardation zone (mm)		
	Furacillin	Chlorhexidine	Decasan
<i>S. aureus</i>	17,7±0,36	36,4±0,87	45,2±0,56
<i>E. coli</i>	18,1±0,43	35,8±1,50	43,4±0,41
<i>Klebsiella</i> spp.	12,5±0,47	40,6±0,52	47,9±0,60

Note: if the growth delay zone is less than 10 mm, the drug is inactive



Fig. 1. The growth delay zone of *S. aureus* to the decasan solution, 45 mm.

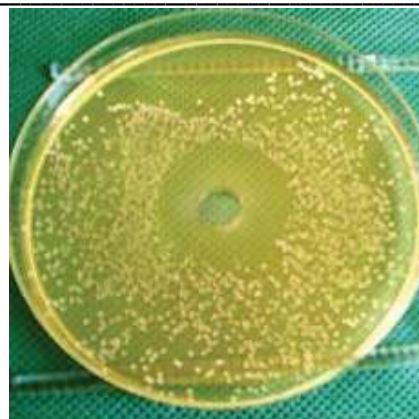


Fig. 2. The growth delay zone of *S. aureus* to the chlorhexidine solution, 36 mm.

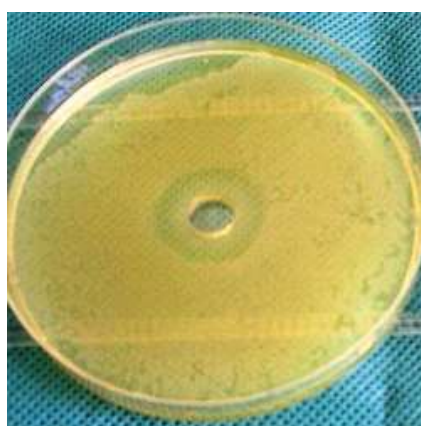


Fig. 3. The growth delay zone of *S. aureus* to the solution, furacillin 17 mm.

Analyzing the results obtained, it can be noted that the antiseptic Decasan has a high antimicrobial activity against *S. aureus*, *E. coli*, *Klebsiella* spp., i.e., both gram-positive and gram-negative microorganisms (Fig. 1, 2, 3). All types of tested microorganisms were sensitive to Decasan. The growth delay zone of 45,2 mm corresponds to the minimum inhibitory concentration of the antiseptic $5.65 \pm 0,29$ micrograms/ml (serial dilution method). The advantage of the antimicrobial activity of decasan against *S. aureus* was revealed in comparison with the preparations of furacillin and chlorhexidine bigluconate.

Table 2.

Comparative activity of antiseptics decasan, chlorhexidine bigluconate and furacillin against strains of *S. aureus*, *E. Coli*, *Klebsiella* spp. isolated from the bed of the flounder muscle (MIC)

MIC *, Antiseptic solutions	Furacillin	Chlorhexidine	Decasan
<i>S. aureus</i>	25,49±2,03	18,32±1,21	5,65±0,29
<i>E. coli</i>	42,1±1,73	12,3±1,45	9,2±1,45
<i>Klebsiella</i> spp.	40,7±2,64	15,8±1,04	10,3±1,14

Note * - minimum inhibitory concentration, mcg / ml

The bactericidal effect of chlorhexidine and furacillin on *S. aureus* was 3.24 and 4.5 times lower than that of decasan, and the effect of chlorhexidine and furacillin on *E. coli* strains was 1.34 and 4.58, *Klebsiella* spp. was 1.53 and 3.95 times lower than decasan, respectively ($p < 0.001$).

Thus, experimental studies have revealed that 0.02% decasan solution has a higher efficacy than chlorhexidine and furacillin against strains of *S. aureus*, *E. coli*, *Klebsiella* spp. isolated from the bed of the soleus muscle of patients who underwent amputation at the level of b/3 of the lower leg for DFS with critical ischemia of the lower extremities.

A comparative study of the antimicrobial activity of decasan, furacillin and chlorhexidine in relation to *P. melaninogenica* was carried out by taking biological material from the bed of the flounder muscle in patients with SDS with critical lower limb ischemia to determine the qualitative and quantitative composition of the wound microflora and its sensitivity to the antiseptics decasan, furacillin, chlorhexidine. To isolate anaerobic bacteria, we used the method proposed by V. I. Kocherovets et al. (1996). The initial material for microbiological studies was exudate from the bed of the flounder muscle of patients with DFS with critical ischemia of the lower limb. The cups with the crops were placed in a micro-aerostat of domestic production MK-752, which was filled with a three – component gas mixture (nitrogen – 80%, carbon dioxide – 10% and hydrogen-10%).

The sensitivity of anaerobic bacteria was determined by the method of standard wells in agar under anaerobic conditions. The culture media were cooled to 40°C, infected with the appropriate test culture and poured 20 ml into Petri dishes, then dried in a thermostat for 30 minutes at 37°C. The investigated antiseptic was placed in the well of the seeded medium. The cups were kept for 1 hour at room temperature to eliminate fluctuations in the time between the introduction of the antiseptic and the start of temperature control. Then the cups were incubated in a thermostat at (36+1)°C for 48 hours. After the incubation period, the growth inhibition zones of the test strains were measured.

As noted above, in most cases, during the bacteriological study of the bed of the flounder muscle in patients of the comparison group, *P. melaninogenica* was most often isolated from anaerobes, so the activity of antiseptics was determined specifically for this microorganism. The research data are presented in Table 3.

Table 3.
 Comparative antimicrobial activity of antiseptic solutions in relation to *P. melaninogenica*

Microorganism	Microbial growth retardation zone (mm)		
	Furacillin	Chlorhexidine	Decasan
<i>P.melaninogenica</i>	5,7±0,29	38,9±0,92	46,2±0,73

Analyzing the results obtained, it can be noted that the antiseptic Decasan has a high antimicrobial activity against *P. melaninogenica*. Furacillin is not active against anaerobes, chlorhexidine is less active than Decasan, which showed the highest activity.

Table 4.
 Comparative activity of antiseptics decasan, chlorhexidine bigluconate and furacillin against an anaerobic strain of *P. melaninogenica* (MIC)

MIC* Antiseptic solutions	Furacillin	Chlorhexidine	Decasan
<i>P.melaninogenica</i>	51,4±1,76	17,6±2,01	7,85 ± 0,95

Note * - minimum inhibitory concentration, mcg / ml

The bactericidal effect of chlorhexidine and furacillin on *P. melaninogenica* was 2.24 and 6.54 times lower than that of decasan, respectively.

The minimum inhibitory concentration of the antiseptic Decasan is significantly lower (7.85 ± 0.95 mcg / ml) than that of furacillin and chlorhexidine, which indicates a higher antimicrobial activity.

Table 5.

Comparative antimicrobial activity of antiseptic solutions on aerobic and anaerobic microorganisms

Microorganism	Microbial growth retardation zone (mm)		
	Furacillin	Chlorhexidine	Decasan
S. aureus	$17,7 \pm 0,36$	$36,4 \pm 0,87$	$45,2 \pm 0,56$
E. coli	$18,1 \pm 0,43$	$35,8 \pm 1,50$	$43,4 \pm 0,41$
Klebsiella spp.	$12,5 \pm 0,47$	$40,6 \pm 0,52$	$47,9 \pm 0,60$
P.melaninogenica	$5,7 \pm 0,29$	$38,9 \pm 0,92$	$46,2 \pm 0,73$

Table 6.

Comparative activity of antiseptics decasan, chlorhexidine bigluconate and furacillin against strains of S. aureus, E. Coli, Klebsiella spp. isolated from the bed of the flounder muscle.

MIC* Antiseptic solutions	Furacillin	Chlorhexidine	Decasan
S. aureus	$25,49 \pm 2,03$	$18,32 \pm 1,21$	$5,65 \pm 0,29$
E. coli	$42,1 \pm 1,73$	$12,3 \pm 1,45$	$9,2 \pm 1,45$
Klebsiella spp.	$40,7 \pm 2,64$	$15,8 \pm 1,04$	$10,3 \pm 1,14$
P.melaninogenica	$51,4 \pm 1,76$	$17,6 \pm 2,01$	$7,85 \pm 0,95$

Note * - minimum inhibitory concentration, mcg / ml

Thus, experimental studies conducted in vitro showed that 0.02% decasan solution exhibits high antimicrobial activity against aerobic and anaerobic hospital strains of microorganisms isolated from the bed of the soleus muscle of patients who underwent amputations at the level of b/3 of the lower leg for SDS with critical ischemia of the lower extremities. The obtained results became the basis for choosing a 0.02% decasan solution as the main means of sanitizing the bed of the soleus muscle during surgery and in the postoperative period in order to prevent suppuration of wounds.

Conclusions:

1. In case of critical ischemia of the lower extremities, amputation at the level of the lower leg according to Mitish-Svetukhin is the optimal method.
2. The analysis of the microflora of the flounder muscle and the wound bed contributes to the correct approach when choosing antibacterial therapy and antiseptic solutions for the rehabilitation of the wound bed.
3. Experimental studies have shown that 0.02% decasan solution exhibits high antimicrobial activity against aerobic and anaerobic hospital strains of microorganisms.
4. In case of amputations of the lower limb below the knee joint, the rehabilitation of the wound bed with modern antiseptic solutions significantly reduces postoperative purulent complications.

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