International Journal of Pharmaceutical Science Invention
ISSN (Online): 2319 – 6718, ISSN (Print): 2319 – 670X
www.ijpsi.org Volume 5 Issue 7 ǁ November 2016 ǁ PP. 45-49

Treatment of Purulent Wounds Immobilized Antiseptics

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Abstract: *An experimental study of the wound healing abilities of immobilized forms of m and x lorgeksidinairamistinabigluconate on various bases in the treatment of purulent wounds. Results of the study showed the benefits of using a combination of m with m etronidazolomiramistinaized and immobilized on sodium carboxymethylcellulose and the preparation of chlorhexidine digluconate with m etiluratsilom immobilized on polymethylsiloxanepolyhydrates compared with ointment "Aevomekol."*
Keywords: *chlorhexidine, miramistin immobilized form, purulent wounds.*

I. Management

In recent years, the problem of the treatment of wound infections is not only the stop discharges a current, but also becoming more acute. In the first decade of the 21st century in Western Europe, wound infection among all surgical patients occurs in 15-20% of patients [1.2], in the second decade in Russia it increased to 35-45% [3,4]. The increase in the prevalence of nosocomial infections due to injuries of various etiology: acute purulent inflammation of the soft tissues, chronic trophic wounds, post-surgical wounds, and others [7,8]. In the arsenal of doctors there is a huge range of treatments pus wounds, but in recent years the microflora of wounds and its biological properties have undergone significant changes, manifested rapid loss of sensitivity to modern antibiotics [9,10]. Currently, one of the most effective antiseptics are aqueous solutions of chlorhexidine and miramistin. However, aqueous solutions of preservatives in readjustment of wound healing diluted and inactivated for 3-6 hours. These facts necessitate development of new combinations based antiseptics immobilized capable prolonged release the active substance for 24 hours in the wound, which increases their activity and reduces the frequency of dressing, which injure the surface of the wound and delay the process of restoring the integrity of the soft tissue.

II. Objective

To study wound healing activity immobilization forms miramistin andchlorgeksidinadigluconate and comparative aspect with the officinal ointment "Levomekol."

III. Materials and Methods

The material for the study is based on drugs whose composition is designed the team Kursk gov't p-governmental Medical University.

Composition 1:
Chlorhexidine digluconate 0.5% - 30.0 grams
Methyluracil - 2.0 grams
Polymethylsiloxanespolyhydrate - 70.0 grams
Composition 2:
The solution Miramistin 0.01% - 100.0 grams
Metronidazole - 1.0 grams
Sodium carboxymethylcellulose - 4.0 grams

As a comparison, selected the most frequently used in medical practice for the treatment of septic wounds ointment "Levomekol." To solve this problem have been conducted in experimental studies and in vitro vivo.

In experiments in vitro studied spectrum antimicrobial ointment "Levomekol" and immobilized forms of x and m lorgeksidinabigluconateiramistina. It was performed in 6 parallel studies of each test specimen. Determination spectrum antimicrobial action of the agents in the experiments carried out by the

method of diffusion in agar solid culture media using test microorganism strains St. Aureus ATCC 6538- P, you. Cereus ATCC 10702, E. Coli ATCC 25922, Proteus vulgaris and Pseudomonas aeruginosa ATCC 9027, Candida albicans ATCC 885-653.

Experiments in vivo carried out on 180 white male rats breed "Wistar". For the study were selected animals weighing February 18, 0 ± 5, 12 g without any external signs of disease, passed quarantine vivarium Medical University KSMU Russian Ministry of Health. Eksperiment carried out in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, 18.03.1986). The study protocol approved by the Regional Ethics Committee for Kursk State Medical University of the Russian Health Ministry. All animals were kept under identical conditions on a standard diet.

Animals were anesthetized under aseptic conditions purulent wound modeled by the following procedure. On the shaved area of the wool treated with antiseptic excised back skin with subcutaneous fat measuring 16x16 mm. The resulting wound was administered gauze ball, containing 1 billion. Microbial bodies overnight culture St. aureus ATCC 6538-P and E. coli ATCC 25922, and then the wound sutured. After 48 hours after modeling abscess formed with all the characteristic signs of inflammation. After removing the stitches the wound edges bred, gauze removed, pus evacuated.

Experimental animals were divided into three groups: the comparison, 1st trial and 2nd trial. The distribution of animals in groups is shown in Table 1.

Table 1: The distribution of animals in groups of experiments

Groups	Method of treatment	Number of animals
Comparisons	Using the officinal ointment "Levomekol"	60
1st of pytnaya	Treatment with the use of chlorhexidine digluconate with metiluratsilom immobilized by polymethylsiloxanepolyhydrate	60
2nd Experiment	Treatment with m and iramistin from metronidozolimmobilizovanny sodium carboxymethylcellulose	60
Total:		180

In the control group animals produced daily debridement with 3% solution of hydrogen peroxide and the imposition of haze napkins with officinal ointment "Levomekol."

The 1st pilot performed daily debridement with 3% solution of hydrogen peroxide and the imposition of haze napkins with an immobilized form of chlorhexidine bigluconate. In the 2nd test group animals produced daily debridement with 3% solution of hydrogen peroxide and the imposition of haze napkins with immobilized form miramistin. Dressings experimental animals in all the series is done once a day, every day for 14 days. The course of wound healing in experimental animals were evaluated planimetric, microbiological, histological methods. Logging performance and removal of animals from the experiment performed on the 1st, 3rd, 5th, 8th, 10th and 15th day from the start of treatment. When purulent wound planimetry assessed the dynamics of the area and reduce the rate of healing.

During a standard bacteriological examination determined the wound colonization (cfu / 1g tissue) by seeding infiltrate the wound in Petri dishes with solid nutrient medium (agar). Histological study micropreparations wounds made on the 1st, 3rd, 5th, 8th, 10th day after the start of treatment of experimental animal breeding experiment. The fence material was performed by excision of soft tissue area of the bottom and the adjacent edges of the wound edge. Taken material immediately fixed in 10% neutral formalin solution, followed by wiring in ascending alcohols and fill in paraffin according to standard procedure. Prepared paraffin sections stained with hematoxylin and eosin. In assessing the histological sections drew attention to the severity of inflammatory reactions, time of appearance of granulation tissue, the occurrence of an edge epithelialization as well as the structural completeness of the newly formed epithelium.

When morphometric study of histological preparations in sections at X400 magnification on a selected area within the wound defect under leukocyte-fibrinous scab counted fibroblasts, granulocytes, lymphocytes and macrophages to 100 cells, the results were expressed as a percentage. When cytology defined cellular composition of infiltrate on the 3rd, 5th, 8th, 10th day. For objectification of wound healing process cell index was calculated by the formula. The cells were placed in the numerator, characterized reparative processes, and the denominator - the inflammatory process. The smaller the index, the more severe the inflammation of the wound. Statistical processing study results was performed using ANOVA techniques. Calculates the average value of quantitative indicators (M) and the average error of the mean (m). The distribution of symptoms was determined by the Shapiro-Wilk test criteria. The significance of differences was assessed by the criterion of Newman-Keylsa.

IV. The results of their Own Research

Results of the study of the spectrum of antimicrobial action of drugs studiedare presented in Table 2.

Table 2: AntimicrobialActivity drugs against hospital strains of microbes in the degree of growth retardation, mm (method of standard rings) (M ± m)

Name of agent	**Levomekol**	**Chlorhexidine**	**Miramistin**
St. aureus ATCC 6538-P	30,2 ± 4,79	29,5 ± 2,25	28,5 ± 1,87
You. cereus ATCC 10702	21,7 ± 3,01	22, 7 ± 3,05	^{27,0} ± 2,19 2,3
Coli ATCC 25922 E.	26,5 ± 5,01	28,9 ± 1,12	29,2 ± 1,47
Proteus vulgaris	26,2 ± 5,56	26,2 ± 2,42	24,7 ± 1,03
Pseudomonas aeruginosa ATCC 9027	26,2 ± 4,58	24,3 ± 2,58	25,5 ± 2,59
Candida albicans ATCC 885-653	11,7 ± 2,07	10,6 ± 2,33	^{27,7} ± 1,63 2,3

Note: ^{1} - $p \leq 0.05$ compared with ointment "A evomekol" with immobilized form chlorhexidine;
^{2} - $p \leq 0.05$ compared with ointment "Levomekol" with immobilized form miramistina;
^{3} - $p \leq 0.05$ when compared with the immobilized form miramistin immobilized form chlorhexidine

From the analysis of the data presented in Table 2, it follows that we have developed drugs possess high antimicrobial activity against all the strains examined test. When compared with the ointment "Levomekol" statistically significant differences with the immobilized form of chlorhexidine has not been revealed, but immobilized form miramistina statistically significantly superior and in the zones of growth inhibition and ointment "Levomekol" and gel form of chlorhexidine against you. Aureus ATCC 10702 and Candida albicans ATCC 885-653. And similar experimental wounds in all animals were comparable in terms of its area (252,4 ± 4,85 mm $^{2)}$. The findings of the experiment data of planimetric meth do are presented in Table 3.

Table 3: The dynamics of the area and the rate of wound healing (M ± m)

Groups	Index	Terms observation day			
		3 (n = 50)	5 (n = 40)	10 (n = 20)	15 (n = 10)
Comparisons	Percentage reduction in wound area	21,2 ± 4,84	44,9 ± 3,52	78,4 ± 3,07	88,9 ± 2,13
	The rate of wound healing,% / day.	10,5 ± 0,51	12,0 ± 0,69	10,1 ± 0,54	2,0 ± 0,12
1st Experiment	Percentage reduction in wound area	35,6 ± 2,64 ^{1}	54,0 ± 2,44	91,2 ± 1,20 ^{1}	99,7 ± 0,10 ^{1}
	The rate of wound healing,% / day.	16,5 ± 0,47 ^{1}	9,9 ± 0,40	6,9 ± 0,42 ^{1}	1,2 ± 0,25
2nd Experiment	Percentage reduction in wound area	30,9 ± 4,36	52,5 ± 3,39	88,9 ± 2,29 ^{2}	99 5 ± 0.05 ^{2}
	The rate of wound healing,% / day.	12,5 ± 1,43 ^{3}	11,1 ± 1,03	^{12,9} ± 1,21 2,3	1,4 ± 0,30

Note: ^{1} - $p \leq 0.05$ when compared groups a comparison with the 1 st experimental group;
^{2} - $p \leq 0.05$ when compared groups compared to 2 minutes of the experimental group;
^{3} - $p \leq 0.05$ when compared 1st experimental groups of 2nd experimental group.

With the passage of time in all groups there was an increase percentage of wound area reduction. Statistically significant differences between the 1st experimental group and group comparison of observed practically during the whole period of the experiment, and between 2 nd experimental group and the comparison group - only starting with the 10-day observation period. In the experimental group statistically significant differences were found.

The rate of healing in the 1st experimental group was maximal at 1-3 days interval (16,5 ± 0.47% / day), which was significantly higher than the values in the other groups, then the rate decreased gradually, indicating that the maximum activity of the drug in the first phase of the wound healing process.

In turn, the rate of healing in about two minutes the test group was consistently high throughout the observation period, indicating that the activity of the drug in the first and second phase of wound healing.

Analysis of the results of microbiological studies of injuries is shown in Table 4.

Table 4 : Dynamics of microbial contamination of the wounds (CFU per 1 g tissue) (M ± m)

Groups	(CFU per 1 g tissue)				
	1 day	3 days	5 days	8 days	10 days
	n = 10 (in each study)				
Comparisons	14,7 ± 1,09h10 ^{7}	19,2 ± 2,55h10 ^{6}	16,6 ± 1,29h10 ^{5}	15,5 ± ^{4} 0,38h10	7,3 ± 0,60h10 ^{4}
1st Experiment	14,5 ± ^{7} 2,13h10	13,2 ± ^{6} 1,83h10	12,3 ± ^{5} 1,91h10	9,3 ± 1,12h10 $^{4(1)}$	1,1 ± 0,27h10 $^{4(1)}$
2nd Experiment	14,6 ± 1,95h10 ^{7}	13,4 ± 2,84h10 ^{6}	12,9 ± 1,57h10 ^{5}	9,0 ± 2,15h10 $^{4(2)}$	1,2 ± 0,35h10 $^{4(2)}$

Note: ^{1} - $p \leq 0.05$ when compared groups comparison with 1st trial;
^{2} - $p \leq 0.05$ when compared groups comparison with a 2nd pilot;
^{3} - $p \leq 0.05$ when compared 1st experimental groups of 2nd experimental group.

In all groups, the microbial contamination of wounds on day 1 averaged 14, 6 ± 1, 72 x 10 ^{7} CFU / g. With the passage of time in all groups, there was a decrease microbial contamination of wounds. No statistically significant differences between the experimental group and the comparison group used yli observed starting

from 8-day observation period, indicating a high decontamination activity study medication. In the experimental groups no significant differences were found.

Histological studies of all groups in the first days after the wound defect modeling the entire surface of the wound was covered with massive fibrinopurulent masses, which revealed a large number of dead white blood cells. Underlying tissues greatly swollen and infiltrated by polymorphonuclear leukocytes and macrophages at various stages of differentiation, bundles of collagen fibers loosened and separated from each other foci of infiltration. Blood and lymph vessels dilated. Swelling of the tissues and infiltration in combination with impregnating the red blood cells spread beyond the wound defect over the entire thickness of the dermis and hypodermis passed on.

After 3 days after modeling purulent wounds CPA Group sake of compari- son the surface of the wound is covered with a scab. Under the scab - granulation tissue infiltrated by granulocytes. The swelling of the dermis and fiber. In the 1st test group directly on the wound beneath the preparations showed a thick layer infiltrate composed overwhelmingly of granulocytes. In about 2 minutes the test group the wound was covered with fibrin, fuzzy granulating shaft infiltrated polymorphonuclear leukocytes. Present areas of the Grand Prix at the translational tissue. In the dermis and hypodermis infiltration and mild swelling phenomenon.

On day 5, observations Group comparing the wound is covered with leukocyte-necrotic scab, scab by granulation tissue, epithelialization signs absent. Deep sections of the dermis more swollen. In the 1st test group proliferative processes were better than in the other groups. However, in some preparations to observe the reaction of macrophages, which manifested an increased number of polymorphonuclear - leukocytes in the infiltrate. In a 2nd test group granulation tissue and fibrin covered quite clearly demarcated granulating shaft. The young granulation tissue observed pronounced neoangiogenesis processes. Granulation tissue infiltrated with neutrophils, lymphocytes and macrophages.

H and 8 day the comparison group on the surface of the wound leukocyte necrotic scab is present in part. The bottom of the wound performed full granulation tissue rich in blood vessels. Fibroblasts are connective tissue of various forms of process, arranged strands, surrounding blood vessels. In the 1st test group gistopreparation noted in the presence of edema in the central parts of the wound defect, but on the periphery - well-formed epithelial shaft. A few preparations to the center of the wound shaft continued in the epidermis layer having a two-layer organization. In a 2nd test group in the surface layers of the wound defect stored only small areas of granulation tissue. Located beneath the newly formed connective tissue is well vascularized, there are signs of an edge epithelialization of the wound.

On day 10 Group the comparison is the formation of epithelial tree on the border of the wound defect. Granulation tissue is clearly demarcated from the intact dermis and infiltrated leukocytes. All gistopreparation 1st experimental groups mentioned granulation coating the epidermis, which is composed of 2 layers of cells. Derivatives of the epidermis absent across the area of the wound defect. In about 2 minutes the test group well-marked signs of epithelialization of the wound. Infiltration of the surface layers of the dermis is preserved. The newly-formed connective tissue as well vaskulyrization on, no signs of edema. Reactive changes are less pronounced. Lots regenerated epithelium without obvious morphological changes.

In order to identify the distinctive features of the process of reparative regeneration in the experimental groups compared us were vestigated cross sections of experimental wounds with their surrounding tissues and skin and muscle morphometry and held, the result s are presented in tables 5.

Table 5: Dynamics composition infiltrate wound during treatment (M ± m) in% (n = 10)

Indicators	Groups	Timing of treatment per day			
		3-i	5th	8th	10th
Fibroblasts	Comparisons	31,9 ± 1,17	32,2 ± 0,94	43,3 ± 1,96	51,4 ± 0,57
	1st Experiment	32,4 ± 2,29	42,8 ± 1,36 [1]	55,4 ± 2,32 [1]	60,8 ± 2,18 [1]
	2nd Experiment	27,0 ± 1,92	35,3 ± 1,25 [3]	54,3 ± 2,12 [2]	65,1 ± 2,07 [2]
Macrophages	Comparisons	20,6 ± 1,51	21,4 ± 1,26	18,4 ± 1,51	14.7 ± 1.64
	1st Experiment	16,4 ± 0,75 [1]	12,2 ± 0,58 [1]	10,6 ± 0,68 [1]	8,2 ± 0,49 [1]
	2nd Experiment	23,1 ± 1,66 [3]	18,5 ± 1,35 [3]	[14,6] ± 1,58 2,3	9,1 ± 1,37 [2]
Lymphocytes	Comparisons	17,5 ± 1,27	19,1 ± 2,13	16,5 ± 2,42	15,4 ± 1,58
	1st Experiment	19,6 ± 0,51	16,0 ± 0,45	15,2 ± 0,58	15,5 ± 1,00
	2nd Experiment	[24,8] ± 2,25 2,3	24,8 ± 2,89 [3]	15,0 ± 3,37	12,3 ± 2,26
Granulocytes	Comparisons	32,1 ± 1,91	29,2 ± 1,66	24,4 ± 2,01	20,4 ± 0,97
	1st Experiment	33,0 ± 2,28	30,8 ± 1,55	20,6 ± 1,36	16,8 ± 0,86 [1]
	2nd Experiment	[24,9] ± 2,74 2,3	[21,4] ± 1,26 2,3	16,1 ± 1,45 [2]	[13,5] ± 1,27 2,3
Cell Index	Comparisons	1,06 ± 0,034	1,10 ± 0,027	1,51 ± 0,041	1,85 ± 0,027
	1st Experiment	0,93 ± 0,017 [1]	1,18 ± 0,022	1,85 ± 0,036 [1]	2,14 ± 0,031 [1]
	2nd Experiment	1,01 ± 0,014 [3]	1,16 ± 0,021	[2,22] ± 0,025 2,3	[2,87] ± 0,042 2,3

Note: [1] - $p \leq 0.05$ when compared groups comparison with 1st trial;
[2] - $p \leq 0.05$ when compared groups comparison with a 2nd trial;
[3] - $p \leq 0.05$ when compared 1st test group with 2 minutes of the experimental group

The direct process of treatment in all groups x is an increase in number of fibroblasts compared to macrophages, lymphocytes and granulocytes. Statistically significant more fibroblasts over other cellular elements first of all noted in the 1 st experimental group (5 hours), which indicates a high regenerative activity of chlorhexidine bigluconate in the first phase of wound healing process. However, since 8 days maximum values of fibroblasts were observed in about two minutes the test group compared to the other groups. In addition, macrophage (towards lymphocytes) and simultaneous increase in lymphocyte numbers (over macrophages) before going on for 1 second experimental group (3 hours) in about two minutes the test group - 3- 5 hours, and the comparison group - for 8-10 hours.

On the 3rd, the 5th day of the experiment there were no statistically significant differences between the groups in the dynamics of cell index. An integral component of wound healing process (cell index) in the first experimental group, 8-10 th day was higher than in the control group by 1.2 times. H and 8th day of the experiment the cell index in the 2nd experimental group exceeded 1.5 Compere group and 1.2 times the 1st experimental group, and on the 10th day, respectively 1.55 and 1.3 times. The dynamics of the cell index in the experimental groups testifying tons of high regenerative activity of the study drugs and the earlier change of phases of wound healing.

V. Discussion

Modern studies of wound healing process have shown that the loss of sensitivity of microorganisms to the development of drug resistance to modern antibiotics due to the formation of biofilm microbial agents around [11]. The biofilm formed by bacteria and fungi, is a thin layer of polymer, which provides a reliable protection against the effects of antibiotics on microorganisms, antiseptics, factors of the immune system [12]. Therefore, the effectiveness of modern antiseptics depends on their ability to destroy the biofilm formed by microorganisms [13,14]. One of the drugs effectively deplete biofilm microorganisms is chlorhexidine, a biguanide derivative having two chlorine compound. The mechanism of antimicrobial action is that adsorbed on the surface of microbial cells chlorhexidine structure gives the cell membrane, causing intracellular protein chlorination which leads to death of the microorganism. Mirimistin - cationic surfactant antiseptic relates to quaternary ammonium compounds. More effectively destroy the biofilm microorganisms than hlorgeskidin which is only effective against gram positive and gram negative bacteria. Thereforemiramistin has a very broad spectrum of antimicrobial activity that includes not only aerobic and anaerobic microorganisms, fungi, viruses, hospital strains resistant to antibiotics. Thus, the results of planimetric, bacteriological and cytological studies of septic wounds indicate a more pronounced positive effect readjustment wounds immobilization forms of chlorhexidine and bigluconatemiramistin than standard ointment "Levomekol." The use of antiseptic gel base has a number of advantages: they are easy to apply, for a long time remain on the surface due to good adhesion, have very low volatility, reduce the number of dressings.

VI. Conclusions

1. The immobilized form of chlorhexidine and bigluconatemiramistina t possess strong anti-inflammatory and antimicrobial action, biologically inert, accelerate the healing of purulent wounds.
2. C persons preparing the proposed composition of the drug is optimal to obtain the maximum therapeutic effect, simple and accessible to health facility pharmacy chains.

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