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Miramistinum and Metronidazole in the Local Treatment of the Experimental Purulent Wounds.

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ABSTRACT

The article is devoted to the examination of the wound-healing ability of immobilized forms of Miramistinum and Metronidazole, based on a sodium salt of carboxymethylcellulose. The research was made on an experimental model of a purulent wound and Levomecol was used for comparison. During the experiment antimicrobial activity of made drugs was evaluated and the planimetric assessment of the process of epithelialization of the wound's surface, bacterial load, morphometric examination of histological drugs for wounds and were made. The results of the research showed the benefits of combination Miramistinum and Metronidazole, immobilized on a sodium salt of carboxymethylcellulose, compared with the drug Levomecol.

Keywords: the treatment of purulent wounds, sodium salt of carboxymethylcellulose, Miramistinum, Metronidazole, Levomecol, modeling of a purulent wound.

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INTRODUCTION

The actual problem of the modern surgery is the problem of a treatment of purulent wounds, what is connected with the prevalence of the wounds of different etiologies (acute purulent inflammatory processes of soft tissue, chronic trophic wounds, including diabetic foot, postoperative wounds, etc.), high mortality, high material costs of a treatment [1-4]. According to the facts of some authors purulent complications range from 35% to 45% of all surgical diseases. Among them, the proportion of in-hospital infection rates from 12% to 22%, and the mortality reaches 25% [5, 6]. The doctors have a great number of methods for treating purulent wounds [7, 8]. However, despite of the development and the introduction of new methods of treatment of purulent wounds, the use of a method of treatment of wounds under the bandage is main now because of its availability, simple use and economic benefits [9].

METHODOLOGY

The material for investigation was the drugs, which composition was developed by the collectives of the department of pharmaceutical technology and operative surgery and topographic anatomy of Kursk State Medical University.

The composition 1 (MirNaCMC): The solution of Miramistinum 0.01% - 100.0 grams (g), Sodium salt of carboxymethylcellulose– 4.0 g.

The composition 2 (MirMetNaCMC): The solution of Miramistinum 0.01% - 100.0 g, Metronidazole – 1.0 g, Sodium salt of carboxymethylcellulose– 4.0 g.

In experiments in vitro (the zones of growth inhibition were determined by disc method) the antimicrobial spectrum of drugs was examined, concerning strains St. aureus ATCC (American Type Culture Collection) 6538-P, Bac. cereus ATCC 10702, E. coli ATCC 25922, Proteus vulgaris and Pseudomonas aeruginosa ATCC 9027, Candida albicans ATCC 885-653.

In vivo experiments were carried out on 144 white male rats of Wistar breed, the animal of 180.0 ± 20.0 g. weight without any signs of the disease were selected for the examination. All animals were kept under equal conditions, on a standard diet, access for food and water was free. A purulent wound of 15x15 millimeters (mm) size according to the methodology of P.I. Tolstyh was modeled for animals under anesthesia [10], the wound was infected by 1 billion suspension of culture of St. aureus ATCC 6538-P and E. coli ATCC 25922. On the 3rd day after modeling all animals had abscess with all signs of inflammation.

Experimental animals were divided into 4 series: 1) Daily the wound cleaning was made by 3% hydrogen peroxide solution (H_2O_2) for animals of the control group. 2) Daily the wound cleaning by 3% H_2O_2 and a gauze bandage with Levomekol were made for animals of the group of comparison. 3) Daily the wound cleaning was made by 3% H_2O_2 and putting the bandage with the drug of the composition 1 in the group of MirNaCMC. 4) Daily the wound cleaning was made by 3% H_2O_2 and putting the bandage with the drug of the composition 2

in the group of MirMetNaCMC. The bandaging of experimental animals was made once a day, every day for 14 days.

The method of L.N. Popova [10] was used for an objective assessment of wound healing rate with changes of its area, so the area of wounds and the percentage of its reduction and the speed of wound's healing were determined. The microbiological research included quantitative determination of microorganisms (bacterial load) in the dynamics of 1 g of tissue of wound infiltration by the dilution method of biopsy material, followed by counting colony-forming units (CFU), grown on solid culture media.

The morphometric examination of infiltration was performed on the 3rd, 5th, 8th and 10th day from the beginning of the treatment. The count of 100 cells was made in the selected region of the wound (fibrocytes, fibroblasts, macrophages, lymphocytes, granulocytes), the cellular composition was expressed in percentage. The statistical analysis of the research results was performed by the method of one way ANOVA (Analysis Of Variance). The average value of quantitative indicators (M) and the standard deviation (sigma) were calculated. The distribution of signs was determined by the Shapiro-Wilk test. The accuracy of differences was assessed by Dunnett test, Newman-Keuls test.

The main part

The results of the research of the spectrum of antimicrobial activity, based on the drugs of different composition, immobilized on a sodium salt of carboxymethylcellulose, concerning to test strains St. aureus ATCC 6538-P, Bac. cereus ATCC 10702, E. coli ATCC 25922, Proteus vulgaris and Pseudomonas aeruginosa ATCC 9027, Candida albicans ATCC 885-653 are presented in Tables 1 and 2.

Table 1: The spectrum of antimicrobial activity of developed immobilized drugs (M±sigma)

Experimental composition		Miramistinum 0,01% solution (1)	Levomecol (2)	MirNaCMC (3)	MirMetNaCMC (4)
St. aureus	The zone of growth inhibition, mm	5.7±0.82	30.2±4.79	25.3±1.03	28.5±1.87
Bac. cereus		9.2±1.17	21.7±3.01	22.5±1.87	27.0±2.19
E. coli		9.8±1.17	26.5±5.01	24.5±1.38	29.2±1.47
Proteus vulgaris		5.5±1.05	26.2±5.56	12.3±1.86	24.7±1.03
Pseudomonas aeruginosa		9.0±1.41	26.2±4.58	25.0±1.10	25.5±2.59
Candida albicans		11.7±1.86	11.7±2.07	26.7±1.75	27.7±1.63

Table 2: The accuracy of differences (p) of the data presented in Table 1 (using Newman-Keuls test).

Compared groups	St. aureus	Bac. cereus	E. coli	Proteus vulgaris	Pseudomonas aeruginosa	Candida albicans
2 and 1	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p>0.05
2 and 3	p<0.05	p>0.05	p>0.05	p<0.05	p>0.05	p<0.05
2 and 4	p>0.05	p<0.05	p>0.05	p>0.05	p>0.05	p<0.05
4 and 1	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05
4 and 3	p<0.05	p<0.05	p>0.05	p<0.05	p>0.05	p>0.05
3 and 1	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05

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From the analysis of the data, presented in Tables 1 and 2, can be concluded that drugs, immobilized on a sodium salt of carboxymethylcellulose, possess antimicrobial activity against all examined test strains. When compared with 0.01% Miramistinum, it was found out that developed drugs in all zones of growth inhibition were different significantly in all investigated test strains.

The combination of MirNaCMC was significantly inferior to the drug Levomecol by the zones of growth inhibitions of the following test strains: St. aureus ATCC 6538-P, Proteus vulgaris, but surpass in the zones of growth inhibitions of Candida albicans ATCC 885-653.

The combination of MirMetNaCMC surpassed in the zones of growth inhibitions to the combination of a sodium salt of carboxymethylcellulose and Miramistinum concerning to test strains St. aureus ATCC 6538-P, Bac. cereus ATCC 10702, Proteus vulgaris, and significantly surpassed the drug Levomecol concerning Bac. cereus ATCC 10702 and Candida albicans ATCC 885-653, that says about a high antimicrobial activity of the combination of MirMetNaCMC.

The planimetric method was used for the examination of the wound healing activity: the area of wounds and the percentage of its reduction and the speed of wound's healing were determined were determined. The results of the experiment of the planimetric method are shown in Tables 3 and 4.

Table 3: The dynamics of the changes of wounds area of experimental animals in the process of the treatment (M±sigma)

Series	Indicant	3 days	5 days	10 days	15 days
		n=30	n=24	n=12	n=6
Control	Wound area (mm^2)	223.4±6.41	175.8±11.52	114.5±7.98	69.0±6.52
	Percentage of wound reduction (%)	12.2±3.62	31.0±5.41	55.0±3.69	72.8±2.87
Levomecol	Wound area (mm^2)	197.7±11.63[1]	138.2±8.35[1]	54.2±7.69[1]	27.8±5.17[1]
	Percentage of wound reduction (%)	21.2±4.84[1]	44.9±3.52[1]	78.4±3.07[1]	88.9±2.13[1]
MirNaCMC	Wound area (mm^2)	163.8±27.25[1,2]	89.1±21.91[1,2]	12.2±4.62[1,2]	1.25±0.51[1,2]
	Percentage of wound reduction (%)	35.5±10.91[1,2]	64.9±8.44[1,2]	95.2±1.77[1,2]	99.5±0.19[1,2]
MirMetNaCMC	Wound area (mm^2)	173.4±27.22[1,2]	119.5±21.12[1,2,3]	26.2±7.74[1,2,3]	1.67±0.67[1,2]
	Percentage of wound reduction (%)	30.9±11.36[1,2]	52.5±8.39[1,2,3]	88.9±2.29[1,2,3]	99.2±0.05[1,2]

Note: [1]-$p<0.05$ when compared series of Levomecol and MirNaCMC, and MirMetNaCMC with the control series (Dunnett test). [2]-$p<0.05$ when compared series of MirNaCMC and MirMetNaCMC with the series of Levomecol (Newman-Keuls test). [3]-$p<0.05$ when compared series of MirNaCMC with the series of MirMetNaCMC (Newman-Keuls test).

From the analysis of the data presented in Table 3 can be concluded that the initial experimental wounds of all animals were comparable in their area (252.4±4.85 mm^2). During the time there was a significant reduction of wound area and percentage of wound reduction area in all the series compared with the control series, the same for the series of MirNaCMC and MirMetNaCMC as compared with the series of Levomecol.

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MirNaCMC promotes the wound reduction area on the 15th day in 99.5%, which is 10.63% more than in the series of Levomecol. Moreover, the maximum difference is on 8th day, when the percentage of wound reduction area in the series of MirNaCMC is 1.4 times higher than in the series of Levomecol and 1.74 times higher than in the control series. The same situation was observed in the series of MirMetNaCMC in comparison with the control series and the series of Levomecol.

When we compared MirNaCMC and MirMetNaCMC on the 15th day, there was no significant differences.

The speed of wound healing of the series of MirNaCMC is higher in comparison with the control series from the 1st to the 10th day, and compared with the series of Levomecol – 1th-8th days. In addition, the speed of wound healing of the series of MirNaCMC is maximum on the 1th - 3rd days, which is 1.4 times higher than in the series of Levomecol and 2.9 times higher than in the control series. The speed of wound's healing of the series of MirMetNaCMC is stably high from the 1st till the 8th day, indicating that this combination has a high activity in the phase of the hydration and the dehydration of the wound process.

The analysis of the results of the microbiological examination of wounds is presented in Table 4.

Table 4: The dynamics of the determination of the wounds' bacterial load contamination of wounds (M±sigma)

Series	(CFU in 1 g of tissue of wound)				
	1th day	3rd day	5th day	8th day	10th day
	n=6 (in each examination)				
Control series	$14.7\pm3.06x10^7$	$8.8\pm1.12x10^7$	$5.0\pm0.31x10^7$	$4.2\pm0.66x10^6$	$3.9\pm0.42x10^6$
Levomecol	$14.7\pm1.09x10^7$	$19.2\pm7.55x10^{6(1)}$	$16.6\pm1.29x10^{5(1)}$	$1.5\pm0.38x10^{5(1)}$	$7.3\pm0.60x10^{4(1)}$
MirNaCMC	$14.8\pm2.44x10^7$	$15.5\pm1.92x10^{6(1)}$	$13.9\pm4.49x10^{5(1,2)}$	$1.0\pm0.22x10^{5(1,2)}$	$4.2\pm1.14x10^{4(1,2)}$
MirMetNaCMC	$14.6\pm1.95x10^7$	$13.4\pm2.84x10^{6(1)}$	$12.9\pm1.57x10^{5(1,2)}$	$0.9\pm0.21x10^{5(1,2)}$	$4.2\pm1.35x10^{4(1,2)}$

Note: [1]-$p<0.05$ when compared series of Levomecol and MirNaCMC, and MirMetNaCMC with the control series (Dunnett test). [2]-$p<0.05$ when compared series of MirNaCMC and MirMetNaCMC with the series of Levomecol (Newman-Keuls test). [3]-$p<0.05$ when compared series of MirNaCMC with the series of MirMetNaCMC (Newman-Keuls test).

The bacterial load of the wounds was in average $14.7\pm2.56x10^7$ CFU/g. on the 1st day in all series. The bacterial load in the control series remains at a high level at all stages of observations, the bacterial load of the series of Levomecol at all stages was significantly lower than in the control series, and was 1.5 ± 0.38 x10^5 CFU/g. on the 8th day, that in 28.0 times less than in the control series.

It was noted that the bacterial load of the wounds was lower when using MirNaCMC and MirMetNaCMC in comparison with the series of Levomecol, beginning from the fifth day of an observation, that the averaged difference was in 1.5 times.

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In order to identify the distinctive patterns of the process of reparative regeneration of compared experimental series we studied diametrical sections of experimental wounds with the surrounding skin tissues and muscles and performed morphometry, the results of which are presented in Tables 5-7.

Table 5: The dynamics of changes in the composition of the infiltrate wounds during the process of the treatment (M±sigma) in %

Days	3	5	8	10	15
The control series (1)					
fibrocytes	12.5±1.78	14.3±2.58	15.8±2.66	18.8±2.57	20.4±2.01
fibroblasts	14.6±2.17	15.2±1.55	18.4±2.27	21.1±2.02	25.4±2.12
macrophages	5.8±0.79	7.6±1.43	8.4±1.71	8.2±0.92	8.8±1.48
lymphocytes	8.0±1.56	7.9±1.85	8.0±1.49	8.6±1.26	11.5±0.97
granulocytes	57.9±1.79	53.3±1.25	44.7±3.86	41.1±2.02	35.1±3.48
Levomecol (2)					
fibrocytes	10.3±1.34	10.9±1.19	14.8±1.93	22.5±3.54	35.2±1.62
fibroblasts	11.6±1.17	13.0±0.94	18.5±1.96	22.9±4.07	26.1±1.97
macrophages	27.6±1.51	25.4±1.26	22.4±1.51	17.7±1.64	12.6±2.32
lymphocytes	20.5±1.27	24.1±2.13	21.5±2.42	18.4±1.58	16.2±2.57
granulocytes	34.1±1.91	29.9±1.66	25.4±2.01	20.4±0.97	13.8±2.04
MirNaCMC (3)					
fibrocytes	13.9±1.66	17.3±1.34	20.8±1.32	32.3±1.16	37.1±2.23
fibroblasts	8.9±1.19	14.6±1.43	27.4±1.35	26.9±2.08	30.4±1.51
macrophages	20.9±1.19	15.4±1.26	13.2±0.92	11.7±1.25	8.2±1.03
lymphocytes	28.9±2.28	26.7±2.36	23.6±3.03	17.0±3.49	13.4±3.03
granulocytes	26.7±1.06	26.0±1.15	15.0±0.94	12.1±1.10	10.9±0.74
MirMetNaCMC (4)					
fibrocytes	15.1±1.10	16.6±0.97	28.9±1.19	34.7±1.16	42.7±1.25
fibroblasts	11.9±0.88	18.7±1.25	25.4±1.07	30.4±1.07	39.0±1.15
macrophages	23.1±1.66	18.5±1.35	14.6±1.58	9.1±1.37	5.3±1.06
lymphocytes	24.8±2.25	24.8±2.89	15.0±3.37	12.3±2.26	8.2±1.55
granulocytes	24.9±2.74	21.4±1.26	16.1±1.45	13.5±1.27	4.8±0.92

According to the data shown in Tables 5-7 it can be concluded that during the treatment in all the series the quantity of fibrocytes and fibroblasts increased as compared with macrophages, lymphocytes and granulocytes. The predominance of fibroblasts and fibrocytes over other cellular elements is early noted in all series of MirNaCMC and MirMetNaCMC also the maximum values of fibrocytes and fibroblasts are observed on the 15th day in the series, where the treatment was carried out by immobilized forms of Miramistinum and Metronidazole on a sodium salt of carboxymethylcellulose, indicating a high wound healing activity of this combination.

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Table 6: The accuracy of differences (p) between the control (1) and others series (2, 3, 4) presented in Table 5 (Dunnett test)

Series	3rd day.	5th day	8th day	10th day	15th day
Fibrocytes					
2 and 1	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05
3 and 1	p>0.05	p<0.05	p<0.05	p<0.05	p<0.05
4 and 1	p<0.05	p>0.05	p<0.05	p<0.05	p<0.05
Fibroblasts					
2 and 1	p<0.05	p<0.05	p>0.05	p>0.05	p>0.05
3 and 1	p<0.05	p>0.05	p<0.05	p<0.05	p<0.05
4 and 1	p>0.05	p<0.05	p<0.05	p<0.05	p<0.05
Macrophages					
2 and 1	p<0.05	p<0.05	p<0.05	p<0.05	p>0.05
3 and 1	p<0.05	p>0.05	p>0.05	p<0.05	p>0.05
4 and 1	p<0.05	p<0.05	p<0.05	p>0.05	p<0.05
Lymphocytes					
2 and 1	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05
3 and 1	p<0.05	p<0.05	p<0.05	p<0.05	p>0.05
4 and 1	p<0.05	p<0.05	p>0.05	p>0.05	p>0.05
Granulocytes					
2 and 1	p<0.05	p>0.05	p>0.05	p<0.05	p>0.05
3 and 1	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05
4 and 1	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05

RESULTS

Thus, the analysis of the results, obtained by microbiological research, showed that our developed drugs MirNaCMC and MirMetNaCMC have a broad spectrum of an antimicrobial activity against both gam-positive and gram-negative microorganisms. Our conducted experimental studies on the model of a purulent wound confirmed the efficiency of our developed immobilized on the basis of a sodium salt of carboxymethylcellulose, an antiseptic Miramistinum and antimicrobial Metronidazole in the phase of hydration and dehydration of the wound process, due to the high sorption activity of a sodium salt of carboxymethylcellulose and protract antimicrobial effects of immobilized components of Miramistinum and Metronidazole.

CONCLUSION

It was found out that MirNaCMC and MirMetNaCMC possess a broad spectrum of an antimicrobial activity against test strains St. aureus ATCC 6538-P, Bac. cereus ATCC 10702, E. coli ATCC 25922, Proteus vulgaris, Candida albicans ATCC 885-653 and Pseudomonas aeruginosa ATCC 9027.

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Table 7: The accuracy of differences (p) between the series of Levomecol (2), MirNaCMC (3) and Mir-MetNaCMC (4) based on the data presented in Table 5 (Newman-Keuls test)

Series	3rd day.	5th day	8th day	10th day	15th day
Fibrocytes					
4 and 2	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05
4 and 3	p<0.05	p>0.05	p<0.05	p<0.05	p<0.05
3 and 2	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05
Fibroblasts					
4 and 2	p>0.05	p<0.05	p<0.05	p<0.05	p<0.05
4 and 3	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05
3 and 2	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05
Macrophages					
4 and 2	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05
4 and 3	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05
3 and 2	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05
Lymphocytes					
4 and 2	p<0.05	p>0.05	p<0.05	p<0.05	p<0.05
4 and 3	p<0.05	p>0.05	p<0.05	p<0.05	p<0.05
3 and 2	p<0.05	p>0.05	p>0.05	p<0.05	p<0.05
Granulocytes					
4 and 2	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05
4 and 3	p<0.05	p<0.05	p>0.05	p<0.05	p<0.05
3 and 2	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05

Their use significantly reduces the wound healing area in 1.4 times, and the bacterial load of the wounds in 1.5 times, compared with the use of the series of Levomecol. Our developed drugs for estimated parameters (the bacterial load of the wounds, the percentage of the wound reduction area) were not significantly different from each other. Statistically significant differences were observed only between the speed of the wound healing, which was higher in the series of MirMetNaCMC. The same data was obtained during the morphometric examination, where the number of fibroblasts and fibrocytes was significantly higher compared with the rest of the series.

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