Microflora of Acute Festering-Necrotic Processes of Soft Tissues under Local Application of Adsorbed Antibiotics

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Abstract

Background: Microbial contamination of chronic wounds plays an important role in prevention of healing and development of complications. Existing approaches for decontaminating of chronic wounds lack efficacy.

Objectives: The aim of the study is to study the effectiveness of local use of the combined form of adsorbed antibacterial drug in the surgical treatment of purulent necrotic processes of soft tissues.

Methods: The study included 45 patients with various chronic wounds and trophic ulcers in the phase of exudation (post-thrombophlebitis disease, peripheral arteries atherosclerosis and diabetic foot syndrome). Drug composition containing gentamicin sulfate, polymethylsiloxan and coordination compound of zinc with L-tryptophan was topically used in study group patients for local treatment once daily covering the whole wound area with thin layer of powder. Microbiological methods included determination of species composition of aerobes and anaerobic flora, cultivation and study of microorganisms' population levels in wound's biofilms.

Results: Microbial spectrum dominated by Gram-positive cocci (*S. aureus*, *S. epidermidis*, *Streptococcus* spp.). Pathogens were presented by members of other taxonomic groups, including the *Enterobacteriacea* family; in single cases, anaerobic *Bacteroides* spp and Peptostreptococci were found. The total number of species in both groups of patients at the time of hospitalization were similar – 28 strains of different taxonomic groups. Treatment in research group dramatically reduced microflora population levels: *E. coli* by 35,6%, *P. aeruginosa* by 39,5%, *S. aureus* by 45,1%, *S. epidermidis* by 24,3%. Population levels of these pathogens become significantly lower than in the control group ($P<0.001$-0.005).

Conclusion: Local application of sorbent and antibiotic compositions in the local treatment of purulent necrotic processes of soft tissue allows achievement of elimination or significant inhibition of pathogenic and conditionally pathogenic microorganisms from wound surfaces, thus creating conditions for faster healing.

Key words: Purulent-necrotic processes of soft tissues, chronic wound, topical treatment, microflora.

Introduction / Background

An important aspect concerning the disappointing results obtained from the treatment of chronic wounds and ulcers, as well as other slowly healing skin and other soft tissues defects which are defined by several authors as the group of festering-necrotic processes of soft tissue (FNPST), is a burdened state of patients due to regulatory, metabolic and vascular disorders (atherosclerosis, chronic venous insufficiency, hypertension, metabolic syndrome, etc.).[1-2] However, the change in the structure of pathogens, their virulence, and host resistance actualize efforts on improvement of methods and techniques of surgical treatment of purulent necrotic processes on the background of burdened general condition of the patient[3]. In addition, the majority of literature sources indicate that the most clinically significant pool of pathogenetic microflora is present not only in the exudate (fluid) of wounds but also in the bio-film that is formed on the surface of the wound or ulcer. This is a kind of wound microbiocenosis.[4] Use of vacuum assisted wound treatment or Negative Pressure Wound Therapy (NPWT) greatly improves the treatment results in this category of patients. However, NPWT has its own complications and limitations. Its benefits in treatment of diabetic ulcers of the feet are encouraging while clinical results for bedsores and other wounds remain conflicting.[5-6]

Generally, it is considered that the structure of chronic wound microflora is dominated by Gram-positive pathogens including cocci, but current studies show that FNPST microflora represents a significantly wider range of microorganisms, including anaerobes and *Enterobacteriaceae* family. Under such circumstances, the systemic etiotropic antibacterial therapy (SEAT) in these patients doomed to failure in the case of ignoring changes in the structure of microflora and its resistance to antibiotics.[4,7] Moreover, systemic antibacterial therapy has multiple limitations, which are caused by the burdened status of the patient, as well as insufficient drugs concentration is tissues and wound biofilm. It prompts clinicians and scientists to explore the possibility of using combined formulations of drugs, particularly for local action that will not only achieve adequate control of microorganisms' growth, but also to avoid toxic effects of systemic antibiotic therapy. Among comparatively recent improvements are the use of hydrogels and other water soluble ointments. Another promising technique is Negative-pressure wound therapy or vacuum assisted closure. However, it has several contraindications and limitations.[7] Currently, a new combined form of drug, antibiotics on absorbing carrier powder is developed to prolong and potentiate the local antimicrobial effect of antibiotics.

The aim of the study is to investigate the effectiveness of local use of the combined form of adsorbed antibacterial drug in the surgical treatment of purulent necrotic processes of soft tissues.

Methods

This study conforms to international bioethical standards (European Convention on Human Rights and Biomedicine, the Declaration of Helsinki of the World Medical Association on ethical principles of scientific medical research involving human subjects, GCP, EUC directive #609, etc.) and approved by Commission for Bioethics in Research (IRB) of the

Bukovinian State Medical University, Ukraine (Protocol #3, 15/11/2012). All patients signed written permissions and obtained full information about the study prior to participation.

The study included 45 patients with various FNPST. Main group consisted of 25 patients (mean age 64.15±12.79) with purulent wounds and trophic ulcers in the phase of exudation. Etiological factors were post-thrombophlebitis disease (syndrome) of the lower extremities – 5 patients (20.0%), atherosclerosis – 4 patients (16.0%) and diabetes mellitus (DM) – 16 patients (64.0%). Drug composition containing gentamicin sulfate (2.4%), polymethylsiloxan (95.2%) and coordination compound of zinc (1.0%) with L-tryptophan (1.4%) was topically used in study group patients for local treatment once daily covering the whole wound area with thin layer of powder.

Control group consisted of 20 patients (mean age 65.10±8.89), who were locally treated with antiseptic solutions (0.02% decasan or dimexide). Etiological factors were postthrombophlebitis syndrome in 2 patients (10.0%), atherosclerosis – 2 patients (10.0%) and DM – 16 patients (80.0%). Other group' characteristics, including general aspects were similar to research group.

Microbiological methods included determination of species composition of aerobes and anaerobic flora, cultivation and study of population levels of different taxonomic groups of microorganisms in wound's biofilms. Material was collected during procedures and prepared in accordance with existing recommendations. Frequency rate (ΔnC%) and coefficient of dominance (Pi) among species were selected to analyze taxonomical structure of the microorganisms' associations. Analysis of microflora's population levels was performed according to the recommendations of the literature[8] with the use of quantitative significance coefficient (QSC) and coefficient of quantitative dominance (CQD).

Distribution of variables was checked in the Kolmogorov-Smirnov test; t-test and exact Fischer test for samples less than 5 were calculated. Database created using MS® Excel 2010 and statistical processing performed in StatSoft® Statistica v7.0.

Results

To begin with, analysis of the species composition of investigated microflora obtained from patients of both research and control groups was performed. Figures depicting species composition of microflora in both groups is shown in Table 1. According to the table 1, the total number of species in both groups of patients at the time of hospitalization were similar – 28 strains of different taxonomic groups. This indicates that not only monomicrobial cultures were obtained, but also the associations of pathogens, where microbial spectrum dominated by Gram-positive cocci (*S. aureus*, *S. epidermidis*, and *Streptococcus* spp.). However, pathogens were presented by members of other taxonomic groups, including the *Enterobacteriacea* family. In single cases, anaerobic bacteria – *Bacteroides* spp and peptostreptococci were found.

As shown in Table 2, the highest population level was observed for *Pseudomonas aeruginosa*, *S. aureus* and S. *epidermidis*, *Streptococcus* spp and *E. coli* spp. Population levels of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and streptococci were significantly higher in patients of study group.

Repeated microbiological examination showed (Table 3) that the material obtained in patients of the main and control groups contains significantly decreased diversity of microorganisms. In particular, in patients of group isolated and identified only 9 strains of microorganisms' species. The research group samples gave growth of nine strains belonging to five species while control group – 16 and 7, respectively. In the research group all obligate anaerobes, *Klebsiella* spp, *Proteus* and *Citrobacter* spp. eliminated during the course of treatment.

Treatment dramatically reduced the most important microflora population level (Table 4): *E. coli* by 35,6%, *P. aeruginosa* by 39,5%, *S. aureus* by 45,1%, *S. epidermidis* by 24,3%. Population levels of these pathogens become significantly lower than in the control group ($P<0.005$).

Discussion

Treating chronic, slowly healing wounds remains a puzzle with many unknown. Background pathology, including changed blood vessels, metabolic disorders and impaired immunity potentiate local manifestation of the disease and aggravate its course, and outcome. While it is well known that without influencing general conditions local treatment of FNPST is doomed to failure, this causes another problem of drug and metabolic interference.[9]

Microbial contamination of chronic wounds plays important if not decisive role in their healing. However, different studies do not confirm this supporting the need for further research.[10] Although, Gram-positive cocci are generally accepted as main pathogens, more attention must be paid to representatives of other taxonomic groups. Among them *Pseudomonas aeruginosa*, *Escherichia coli* and several anaerobes attract major attention.[4]

This study supports recent publications in defining microflora as a major pathogenetic mechanism in FNPST development. In addition, the local use of combined antibiotic and sorbent in treatment of patients with FNPST has positive effect on wound surface decontamination, which is much more expressed when compared to use of antimicrobial drugs without sorbents.

Conclusion

Purulent-necrotic processes of soft tissues in patients with complicated common condition are caused by microorganisms of different taxonomic groups, which are dominated by Gram-positive cocci, *Pseudomonas aeruginosa* and *Escherichia strains*. Both monocultures and associations of microorganisms were found in wound biofilms. Local application of sorbent and antibiotic compositions in the local treatment of purulent necrotic processes of soft tissue allows achievement of elimination or significant inhibition of pathogenic and conditionally pathogenic microorganisms from wound surfaces, thus creating conditions for faster healing.

Conflict of Interest: None

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Table 1: Species composition of biofilm microflora in research and control groups patients on admission

Microorganisms	Research group (n=25)			Control (n=20)		
	Qty of strains	Δn C%	Pi	Qty of strains	Δn C%	Pi
Anaerobic bacteria						
Bacteroides spp	1	4.0	3.57	–	–	–
Peptostreptococci	–	–	–	1	5.0	3.57
Aerobic and facultative anaerobic bacteria						
E.coli	2	8.0	7.14	3	15.0	10.71
Klebsiella spp	1	4.0	3.57	–	–	–
P.aeruginosa	3	12.0	10.71	3	15.0	10.71
Proteus spp	1	4.0	3.57	–	–	–
S.aureus	5	20.0	17.86	7	35.0	25.0
S.epidermidis	7	28.0	25.0	5	25.0	17.86
Streptococcus spp	6	24.0	21.43	6	30.0	21.43
Enterococcus spp	1	4.0	3.57	2	10.0	7.14
Citrobacter spp	1	4.0	3.57	1	5.0	3.57
Total	28			28		

ΔnC% – frequency rate; Pi – coefficient of dominance.

Table 2: Population levels of biofilm microflora in research and control groups patients on admission

Microorganisms	Research group (n=25)			Control (n=20)			P value
	M±m, lg CFU / ml	QSC	CQD	M±m, lg CFU / ml	QSC	CQD	
Anaerobic bacteria							
Bacteroides spp	2.87	2.13	2.33	–	–	–	–
Peptostreptococci	–	–	–	3.13	2.27	2.54	–
Aerobic and facultative anaerobic bacteria							
E.coli	5.11±0.42	7.59	8.29	4.96±0.41	10.78	12.07	>0.05
Klebsiella spp	4.47	3.32	3.63	–	–	–	–
P.aeruginosa	6.89±0.25	15.34	14.97	3.49±0.38	7.58	8.50	<0.001
Proteus spp	5.71	4.75	4.63	–	–	–	–
S.aureus	6.41±0.08	23.81	26.0	5.31±0.12	26.93	30.16	<0.001
S.epidermidis	5.34±0.24	27.76	30.33	5.33±0.15	19.31	21.62	>0.05
Streptococcus spp	4.09±0.21	18.22	19.91	4.52±0.21	19.65	22.01	0.005
Enterococcus spp	4.32	3.20	3.51	4.14±0.17	6.0	6.72	–
Citrobacter spp	3.50	2.60	2.84	3.63	2.63	2.95	–

QSC – quantitative significance coefficient; CQD – coefficient of quantitative dominance.

Table 3: Species composition of biofilm microflora in research and control groups patients after 7-14 days of treatment

Microorganisms	Research group (n=25)			Control (n=20)		
	Qty of trains	Δn C%	Pi	Qty of strains	Δn C%	Pi
Anaerobic bacteria						
Bacteroides spp	–	–	–	1	5.0	6.25
Aerobic and facultative anaerobic bacteria						
E.coli	2	8.0	22.22	2	10.0	12.50
P.aeruginosa	1	4.0	11.11	3	15.0	18.75
Proteus spp	–	–	–	2	10.0	12.5
S.aureus	3	12.0	33.33	4	20.0	25.0
S.epidermidis	2	8.0	22.22	2	10.0	12.5
Streptococcus spp	1	4.0	11.11	2	10.0	12.5
Enterococcus spp	–	–	–	1	5.0	6.25
Total	9			16		

ΔnC% – frequency rate; Pi – coefficient of dominance.

Table 4: Population levels of biofilm microflora in research and control groups patients after 7-14 days of treatment

Microorganisms	Research group (n=25)			Control (n=20)			P value	P_1 value
	M±m, lg CFU / ml	QSC	CQD	M±m, lg CFU / ml	QSC	CQD		
Anaerobic bacteria								
Bacteroides spp	–	–	–	3.01	4.42	5.19	–	–
Aerobic and facultative anaerobic bacteria								
E.coli	3.29±0.27	19.75	7.11	4.78±0.25	14.03	11.22	0.029	0.036
P.aeruginosa	4.17	12.52	4.51	5.31±0.18	23.37	18.70	–	–
Proteus spp	–	–	–	3.75±0.22	11.0	8.80	–	–
S.aureus	3.52±0.11	31.69	11.41	4.93±0.30	28.93	23.15	<0.001	<0.001
S.epidermidis	4.04±0.26	24.25	8.73	3.73±0.34	10.95	8.76	>0.05	0.03
Streptococcus spp	3.49	10.47	3.77	3.53±0.25	10.36	8.29	–	–
Enterococcus spp	–	–	–	5.04	7.39	5.92	–	–

QSC – quantitative significance coefficient; CQD – coefficient of quantitative dominance; P – compared to control; P_1 – compared to previous period (Table 2, research group).