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Antimicrobial resistance. New antimicrobial agents in the treatment of chronic trophic ulcers

Antimikrobinės atsparumas. Naujos antimikrobinės medžiagos lėtinėms trofinėms opoms gydyti

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Background / objective

The steady growing number of pathogenic microorganisms resistant not only to antibiotics but also to antiseptics complicates the cure of the wound process. The sensibilization of skin to the majority of locally applicable preparations creates certain difficulties in the treatment of chronic trophic ulcers. The new herbal remedy PhytoMP was developed based on two components (*Macleaya microcarpa* (Maxim.) Fedde, *Papaveraceae* family and *Plantago major* L., *Plantaginaceae* family). A new atraumatic combined dressing was developed based on polyester curtain nets coated with copper particles 0.2 mcm in size and a linen nonwoven sorbent containing silver nanoparticles stabilized by PhytoMP. The aim of the present study was to investigate the possibility of these developments to improve the effectiveness of the complex therapy of trophic ulcers of the lower limbs.

Methods

A clinical trial study was conducted in the Republic Center of Surgical Infection, Belarus. Patients (n = 42) with chronic trophic ulcers of the lower limbs participated in the study. The sensitivity of microorganisms to antibiotics, herbal remedy, and metallic fabrics was determined by the method to diffusions in agar. By the method of the dose- and time-dependent killing of pathogens, the minimal inhibitory concentration for PhytoMP and the duration of its antimicrobial effect was evaluated. The total treatment response was evaluated on the basis of changes in the clinical manifestations of the disease, according to the subjective feelings of patients, the degree of bacterial contamination, the terms of the appearance of granulation, epithelialization, healing, and the cytological picture. The parameters were evaluated before the treatment and every third day in the period until complete ulcer healing. The data were analyzed statistically.

Results

Bacteriological examination revealed that the microflora of trophic ulcers in 37.04% of cases was introduced by *S. aureus* which was resistant to the majority of antibiotics. A comparative analysis demonstrated the significant differences in terms of a complete cleansing of ulcers from pathogens: with use of PhytoMP on the 7.38 ± 0.65 day, while in the control group it was on 8.30 ± 0.48 day ($p < 0.01$). The average hospital stay of patients with trophic ulcers from the main group was 30.77 ± 2.01 bed-days and from the control group 35.50 ± 1.72 bed-days ($p < 0.01$). It was experimentally established that the use of PhytoMP infusion as a matrix for stabilizing silver nanoparticles allows to halve the amount of the metal in a linen nonwoven

sorbent, and to achieve a strong antimicrobial effect against *S. aureus* and *E. coli*. It was noted that, due to the strong antimicrobial effect, the physical properties of the developed combined dressings, their high anti-adhesive activity allowing carrying out the atraumatic change of bandage without moving a flap, successful results of skin plastic were observed.

Conclusions

This study suggests that the developed herbal remedy PhytoMP and atraumatic combined dressings are a promising alternative to be used in the treatment of chronic trophic ulcers.

Key words: chronic trophic ulcers, wound infection, PhytoMP, dressings, nanoparticles

Introduction

Venous trophic ulcer prevails in the overall structure of leg ulcers of various origin. They occur in 1–2% of the population of industrialized countries. The low efficiency of antibacterial agents, multiple adverse reactions dictate the need of searching for new highly effective remedies. So, researchers often pay attention to herbal substances as sources for biologically active compounds which have a modifying activity against the pathogenic and persistent potential of microbes [1–6].

We have developed the PhytoMP herbal remedy. When selecting components with the antimicrobial effect, leaves of *Macleaya microcarpa* were a choice as a source of the drug sanguirythrine widely used in the Russian Federation for the treatment of bacterial and fungal infections. Leaves of *Plantago major* have long been known and used in folk medicine because of their hemostatic properties and were included for providing a wound-healing effect.

A fundamentally new solution of the problem of treatment of purulent wounds and trophic ulcers that do not require the use of antibiotics and antiseptics is to create dressings based on nanotechnology. The study of metal nanoparticles, due to the specific properties of the modified materials, playes a significant role [7–11]. The atraumatic combined dressing developed by us was based on polyester curtain nets coated with copper particles 0.2 mcm in size and a linen nonwoven sorbent containing silver nanoparticles stabilized by PhytoMP.

The aim of the present study was to investigate the possibility of the herbal remedy PhytoMP and atraumatic combined dressing to improve the efficiency of the complex therapy of trophic ulcers and surgical wounds of the lower limbs.

Patients and methods

A clinical trial study was conducted at the Republic Center of Surgical Infection, Belarus. According to the Program of clinical testing of PhytoMP 22 patients aged 43 to 83 years (14 women and 8 men) with chronic trophic ulcers of the lower limbs participated in this study. Thrombophlebitis or diabetic angiopathies was the reason for the appearance of ulcers. At the time of admission to the hospital, three patients underwent phlebectomy. Eleven patients had signs of acute thrombophlebitis, and the erysipelas skin with the presence of vesicles with serous-purulent contents was diagnosed in three events. The beginning of gangrene of the toes was observed in eight events. Disease duration averaged to 8 years. Most patients (86.4%) had a single ulcer defect; only three men came to the clinic with two ulcers. As a rule, the localization of ulcers was on the front, inside or outside of the leg, 6 patients with diabetes had them on feet. Their size ranged from 0.5 x 0.5 cm to 5.3 x 3.7 cm. The ulcerous surface of 8 patients was covered with fibrinous masses, in 6 events they were fibropurulent and in 5 events pyonecrotic. All patients reported a severe pain. A control group was formed of 20 patients of similar age with the identical clinical picture.

The sensitivity of microorganisms isolated from wounds to antibiotics, the herbal remedy and metallic fabrics was determined by the method to diffusion in agar [12]. By the method of the dose- and time-dependent killing of pathogens, the minimal inhibitory concentration for PhytoMP and the duration of its antimicrobial effect were evaluated [13].

All patients from both groups were appointed the general and the local treatment, including aspirin 0.5–1 g once a day, infusion therapy (*Solutio Rheopolyglucinum pro infusionibus* 200 ml + trental 5 ml + *Acidum*

ascorbinicum 5 ml / drip one time a day for 5 days). Eleven patients with symptoms of acute thrombosis, in addition, were injected with heparin 5000 units under skin 4 times a day for 6 days. All patients underwent a toilet of the ulcer surface and the adjacent skin, while in phase I of wound healing the bandagings were made daily. In addition, all patients used an elastic compression in the form of bandaging in phase I of the wound healing process or compression stockings at the stage of epithelialization.

In the main group, for the topical treatment in the case of abundant exudation and a high degree of microbial contamination, the PhytoMP powder at the ratio of the components *Macleaya microcarpa* : *Plantago major* 2 : 1 was applied as a thin layer on the surface of the ulcer with the Rapoleks spray. On a surface with the moderate exudation of the ulcer, the sterile gauze, heavily soaked with the PhytoMP infusion with the same ratio of components was applied. In phases II and III of the wound healing process, PhytoMP was used at the ratio of the components *Macleaya microcarpa* : *Plantago major* 1 : 2 or 1 : 1. The dosage form was selected individually depending on the state of granulation. The infusion was prepared in accordance with the requirements of the State Pharmacopea in a pharmacy in sterile conditions from the PhytoMP powder immediately before use. A sterile gauze armband was superimposed over. After filling, the ulcer granulation application was performed one time in two days.

For the topical treatment in the control group, traditional antiseptics were used.

The total treatment response was evaluated on the basis of changes in the clinical manifestations of the disease, according to subjective feelings of patients, the degree of bacterial contamination, the terms of the appearance of granulation, epithelialization, healing, and the cytological picture. The parameters were evaluated before the treatment and every 3rd day in the period until complete ulcer healing.

The data were analyzed statistically by using the Statistical Package for Social Sciences for Windows (SPSS, version 21; SPSS Inc., Chicago, IL, USA) and MS Excel 2002 by the methods of descriptive statistics. The p value < 0.05 was considered to be statistically significant.

Results

The bacteriological examination revealed that the microflora of ulcers in 37.04% of cases was introduced by *S. aureus*. Strains of *Str. pyogenes* amounted to 14.82% of all isolates, at 3.7% were the levels of *S. cohni* and *B. subtilis*. *P. vulgaris* was received in 7.41% of cases from *Enterobacteriaceae* and from *E. coli* in 14.82%. Non-fermentative gram-negative bacilli *P. aeruginosa* were found in the wound contents in 11.11% of cases, *A. baumannii* – in 7.41%. *S. aureus* + *P. aeruginosa, A. baumannii* + *S. cohni, S. aureus* + *P. vulgaris, B. subtilis* + *P. vulgaris* were identified as associations in trophic ulcers.

Staphylococci isolates showed a 100% resistance to ampicillin, gentamicin, ceftazidime, cephalexin, and they were highly resistant to penicillin, tetracycline, kanamycin, oxacillin; 100% of the isolates coagulase negative *Staphylococci* were resistant to azithromycin and penicillin, and they were highly resistant to erythromycin, oxacillin, tetracycline, lincomycin, amoxicillin clavulanate, co-trimoxazole, chloramphenicol. Kanamycin, tetracycline, amoxicillin, amoxicillin clavulanate, cephalothin, chloramphenicol were effective not only against *Enterobacteriaceae* strains. *Pseudomonas* isolates were resistant to pefloxacin, co-trimoxazole, ceftazidime, ticarcillin, netromycin, gentamicin, tobramycin, ofloxacin, piperacillin.

Laboratory studies have shown the most denominated antimicrobial activity by PhytoMP in respect of gram-positive microflora. For example, by the method to diffusions in agar it was established that the diameter of the zone of growth inhibition of *S. aureus* (mean ± S.D.) has 17.75 ± 0.48 mm, *E. coli* – 11.09 ± 0.69 mm, *P. aeruginosa* – 7.80 ± 0.40 mm when we used the PhytoMP powder at the ratio of the components *Macleaya microcarpa* : *Plantago major* 1 : 1. Against *P. vulgaris* activity was absent. When the amount of *Macleaya microcarpa* was increased two times, the diameter of the zone of the growth inhibition of *S. aureus* was 22.07 ± 0.66 mm, *E. coli* – 13.19 ± 0.97 mm, *P. aeruginosa* – 9.05 ± 0.49 mm, *P. vulgaris* – 4.0 ± 3.59 mm, what realistically differs from the activity of the powder at the ratio of the components 1 : 1 ($p < 0.001$).

The minimal inhibiting concentration of PhytoMP to *S. aureus* strains was detected as 0.008 mg/ml and

the length of the antimicrobial effect was 36 h, what allowed to forecast the frequency of bandaging one time in 1–2 days. The minimal inhibition concentration of PhytoMP to *E. coli* strains was 0.016 mg/ml, and the length of the antimicrobial effect was 18 h, what allowed forecasting the frequency of the the bandaging one time per day.

As a result of clinical testing of PhytoMP its positive influence on the course of the reparative process was revealed. Already in the first days of treatment with PhytoMP at the ratio of the components *Macleaya microcarpa* : *Plantago major* 2 : 1, the amount of wound exudation and the severity of inflammatory reactions significantly reduced, and microbial contamination decreased intensively from 10^6–10^5 to 10^3 microorganisms per 1 g tissue. Comparative analysis demonstrated significant differences in terms of a complete cleansing of trophic ulcers. The treatment by PhytoMP had an effect on the 7.38 ± 0.65 day, while in the control group it was on 8.30 ± 0.48 day ($p < 0.01$). By the powder the wound surface was rapidly cleared from the fibrin and necrotic masses, which were taken with a napkin from the tissue without pain for the patient.

Decreased exudation, a cloudy discharge from the purulent serous turning into a transparent serous, the appearance of a bright red fine granulation evidenced the positive course of the wound healing process. The terms of the appearance of granulation by the treatment with PhytoMP amounted to 7.54 ± 0.66 days, while in the control group to 9.50 ± 0.53 days, which was significantly different ($p < 0.001$).

Since the granulation tissue filling of the entire surface of the ulcer, the application was performed only once in two days using the PhytoMP infusion at the ratio of 1 : 1 ÷ 1 : 2. It should be noted that the choice of the ratio of components is important at certain stages of the treatment, because, in addition to the pronounced antimicrobial activity, *Macleaya microcarpa* has a strong drying and cauterizing action which can be negative for the growth of the granulation tissue. Therefore, in the II and III phases of the wound healing process, the ratio of the components *Macleaya microcarpa* : *Plantago major* 1 : 1 was used to maintain the antimicrobial effect, and at the ratio of 1 : 2 it enhanced the reparative activity.

In parallel with changes in the clinical manifestations of the disease, a series of changes in the favorable direction were noted in smears. In cytology, in smears obtained from the laboratory before treatment, we noted the predominance of neutrophils with the signs of degenerative changes; cocci tissue detritus and fibrin were present in large quantities. In the main group, on the 5th day of treatment microflora was absent in smears, the number of neutrophils with degenerative changes, fibrin, and tissue detritus were significantly reduced. With cleansing ulcers, the increase in the number of polyblasts and macrophages was noted. An active phagocytosis was observed along with the maturation of the granulation tissue and epithelialization. In the control group, in wound prints on the 5th day neutrophils were found in the degeneration stage with the phenomena of monocyte phagocytosis. On the 7th day, there were fibroblasts and polyblasts, and the number of neutrophils was reduced. On the 9th day, there was a significant decrease in the number of polymorphonuclear leukocytes to the presence of pronounced phagocytosis. On the 11th day of treatment, there was a further reduction in the number of neutrophils in the presence of a plurality of fibroblasts and macrophages.

The starting date of epithelialization by the treatment of trophic ulcers with PhytoMP was significantly different from the control: 12.00 ± 1.63 days and 15.50 ± 0.53 days ($p < 0.001$).

The reparative process was protracted in the treatment of trophic ulcers in patients with diabetes mellitus. Therefore, the average hospital stay of patients with trophic ulcers from the control group was 35.50 ± 1.72 bed-days and from the main group 30.77 ± 2.01 bed-days ($p < 0.01$). The difference in the timing was 4.73 bed-days.

It should be noted that five patients in the main group received only the conservative treatment in which the complete ulcer healing was achieved. The patients that required surgical treatment began to prepare for autodermoplasty after a complete cleansing of ulcer from pus and necrotic masses and when granulations became pink.

During clinical testing, in patients treated with PhytoMP no serious adverse events were reported. The portability was satisfactory in 100% of cases. However, it was found that in the first two days immediately after the application, in patients with the predominance of

inflammatory changes in the ulcer, the use of PhytoMP somewhat increased the pain in it, but it was present for 10–20 minutes only. Moreover, 11 patients in this group did not need to use analgesic drugs. They also noted an improvement in sleep and general well-being thanks to the absence of itching.

We present clinical examples.

Patient N. O., born in 1954, entered the Republic Center of Surgical Infection with the diagnosis: varicose veins, post-tromboflebit syndrome, trophic ulcer of the right shin. The patient was admitted with complaints on pain in the right lower leg, worse when walking, and the presence of trophic ulcer on the anterior surface of the lower third of the right tibia. Varicose veins of the right shin appeared 20 years ago. A month before hospitalization, trophic ulcer had appeared. On the examination it was stated: the right lower extremity is swelling, the shin has a bluish color and is painful at palpation. The trophic ulcer on the front surface of the lower third of the leg has the size 2 x 2 cm, its edges are hyperemic, and the fibrinous discharge is in the crater. The prescribed treatment included heparin 5000 units under skin every 6 hours, aspirin 0.25 g once per day, troxevasin, solkoseril.

After 5 days of treatment, the patient's general condition deteriorated significantly; the body temperature was 39.5°C. The skin of the right leg was sharply hyperemic, hypertrophied, and painful. The erysipelas of the right lower limb was diagnosed. The prescribed treatment included cefazolin 1 g 2 times per day and biseptol 480 mg 3 times per day. After two days, bubbles with a serous fluid, the dermis areas of necrosis (necrotic-bullous form of erysipelas) were formed.

The dissection of the bubbles and the removal of necrotic tissue elements were made, but the positive effect of the previous therapy was absent; it was proposed to use PhytoMP for wound healing. Three days after the start of treatment with PhytoMP infusion the reduction of microbial contamination to 10^2 microorganisms per 1 g of tissue was observed, and on the fifth day an intensive growth of granulation tissue was noted. The subsequent application of this remedy led to the formation of a smooth connective tissue scar on the 10th day of treatment.

Patient J. N., born in 1933, entered the Republic Center of Surgical Infection with the diagnosis of diabetes. The diabetic angiopathy of lower limbs and incipient gangrene of the third toe of the right foot were present. The patient had been treated repeatedly in the surgical hospital for 20 years. Two weeks before admission to hospital the worsening of the disease began, accompanied by general weakness and pain in the lower limbs.

An incision 2 cm long was made, excision of necrotic tissue in the area of lysed nail bone and the removal of sequestration were performed, and the wound was superimposed with a sterile gauze with the PhytoMP infusion. Subsequently, the application was carried out on a daily basis, while removing the necrotic tissue. In the course of treatment, a rapid clearance of the wound surface and the formation of granulation tissue were noted. By the 14th day of treatment a scar formed from the connective tissue. Finger amputation was avoided.

Patient R. I., born 1940, entered the Republic Center of Surgical Infection with an ulcer on the plantar surface of the foot, size 5.3 x 3.7 cm, with the towering and callus edges, the uneven, soft bottom covered with a purulent discharge containing *S. aureus* and *E. coli*. Microbial contamination was 10^7 microorganisms per 1 g tissue. During the 4 years, the ulcer had been hardly amenable to treatment by conventional means. The therapy with the PhytoMP powder (in the ratio 2 : 1) on the 3rd day allowed beginning to clear from the pus, microbial colonization was down with 10^7 to 10^5 microorganisms per 1 g tissue. Complete cleansing of the ulcer surface occurred on the 6th day, and on the 8th there were isolated areas of the granulation tissue, and then the PhytoMP powder was used at the ratio 1 : 2. On the 10th day of treatment, there were signs of marginal epithelialization, and a plastic closure of the ulcer by the deepithelialized skin graft was performed. After 3 weeks, the patient was discharged and kept under observation for 12 months; during this period, there was no recurrence (Figure).

Discussion

The majority of studies have analyzed the microflora of ulcers, and they have pointed at the dominance of *S. aureus* and coagulase-negative staphylococcis, in particular *S. epidermidis* and *S. hominis*, too. According to many authors, the lysis of grafts at autodermoplasty on ready-to-plastic granulation is most often associ-

Figure. Ulcer surface type using the PhytoMP powder (a) on the 6th day of treatment; (b) on the 20th day of treatment

ated with the microbial colonization of wounds [1, 3, 14, 15]. Therefore, the current selection is not only a means effective against pathogenic microorganisms, but also a method of treating postoperative wounds after autodermoplasty.

Atraumatic combined dressing was developed by us for improving the result of skin plastic.

According to literary data, the use of silver as an antimicrobial agent is able to add the high efficiency of dressing in respect of even methicillin-resistant and vancomycin-resistant pathogens. Herewith prolonged antimicrobial effect can be reached under minimum toxicity of this metal [7, 8]. However, it is necessary to take into account that even the most denominated antimicrobial effect of the dressing can be levelled by its adhesion to the wound, and, as a consequence, not only bandaging becomes to be painful for the patient, but also the process of regeneration is slowed down. One of the ways of giving antiadhesive properties to dressing is covering the surfaces turned to a wound with a fine layer of ZnO, Al_2O_3, Ag or Al [16].

To the mechanism of the antimicrobial action of copper, the authors refer the inhibition and breach of the synthesis of bacteria's protein [10, 11].

We used a polyester curtain fabric coated with copper particles 0.2 mcm in size to give an atraumatic effect to developed dressings. On the Orsha Linen Mill industrial enterprise (Belarus), nonwoven linen sorbents were obtained on the basis of a short fiber. In G. A. Krestov Institute of Solution Chemistry of the Russian Academy of Science they were treated with silver nanoparticles stabilized by PhytoMP infusion to give them an antimicrobial effect.

According to scientific literary data, the possibility to immobilizate silver nanoparticles in different materials for achieving a pronounced antimicrobial effect is being intensively studied at present. However, there is a problem of the stabilization of nanoparticles. Uusing natural polymers for this purpose is promising, but it is not a well-developed direction. There are data on obtaining a reception of colloidal silver solution by means of a herbal extract [17, 18, 19].

In our earlier study, silver nanoparticles with an average size of 40 ± 2 nm were synthesized by a green method using the PhytoMP aqueous infusion containing polysaccharides as a reducing agent and an efficient stabilizer [20]. It was experimentally established that the use of PhytoMP infusion as a matrix for stabilizing silver nanoparticles allows to halve the amount of the metal in a linen nonwoven sorbent to achieve a strong antimicrobial effect against *S. aureus* and *E. coli*.

We had the first experience of the using this development in clinic. The use of atraumatic combined dressing of a polyester curtain fabric with the coating

particles of Cu 0.2 mcm and linen nonwoven sorbent impregnated with a silver nanoparticles stabilized by PhytoMP infusion allows cleaning the wound surface from the pathogen on 3.21 ± 0.41 days versus the control group (on 7.90 ± 0.57 days). The atraumatic combined dressing was applied directly in the operating room on a postoperative wound, packed with split-skin grafting for its strong fixation to the granulating wound. It was shown that the release properties due to the mesh fabric graft dressings shifted during the first dressing procedure and remained in the wound. The dressing prevents the accumulation of fluid through the sorbent and is removed without maceration, painlessly for the patient. It was noted that due to the strong antimicrobial effect, the physical properties of developed combined dressings, their high anti-adhesive activity allowing carrying out the atraumatic change of bandage without moving the flap, successful results of skin plastic were obtained.

Conclusions

The clinical testing has shown that the herbal remedy PhytoMP is one of the affordable and effective drugs for the topical treatment of ulcers. The use of PhytoMP allows to reduce the dose of antibiotics and eliminate the need for painkillers. The use of PhytoMP in phase I facilitates the rapid purification of wound surface from the pus, the formation of a bright, fine-grained granulation. In phases II and III, the wound healing under the influence of PhytoMP gives a faster repair of the ulcer surface to form a smooth scar.

The use of the polyester curtain fabric with sputtered particles of copper as an antimicrobial atraumatic mesh in combination with a silver-containing sorbent allows to clean the surface from the ulcer microbes twice faster in comparison with the control. The proposed combined atraumatic bandage can be used in the treatment of wounds for a solid graft fixation to the wound and protecting the wound from infection and trauma.

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