ORIGINAL ARTICLE

ORNIDAZOL-BASED APPLICATION SORBENT WITH NANO SILICA AND ITS ANTIMICROBIAL ACTIVITY

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ABSTRACT

The aim: To investigate the effect of application sorbent based on ornidazole with nanosilicon in experiment and clinic.

Materials and methods: In order to study the effectiveness of the Ornidasil application sorbent for the treatment of purulent wounds, we conducted an experimental study in rats. Also, we studied the effectiveness of the Ornidasil in the clinic for the treatment of patients with diabetic foot syndrome and to prevent the suppuration of postoperative wounds in patients with purulent peritonitis in toxic and terminal stages.

Results: The formation of active substance complexes with hydroxylated matrices is due to hydrogen bonds between the oxygen atom of the silanol group of the silica surface and the hydrogen atom of the alcohol group of the ornidazole molecule. This promotes the gradual release of ornidazole from the surface of such a matrix into the wound exudate. Thus, on day 13, 9 experimental rats of group I healed completely, 11 rats had a small wound surface, complete healing occurred on day 15. We also investigated the effectiveness Ornidasil in the clinic. In the comparison group, postoperative wound suppuration occurred in 6 patients (31.6%), and in the main group - in 3 patients (12.5%).

Conclusions: A study of the effectiveness Ornidasil in the complex treatment of Diabetic foot syndrome showed that in the experimental groups, wound healing occurred 1.6 -1.9 times faster. The use of polyurethane wound protector in combination with Ornidasil reduced the suppuration of postoperative wounds in patients of the main group by 2.5 times relative to patients in the comparison group.

KEY WORDS: ornidazole, nano silica, hybrid nanocomposites, quantum chemical calculations, in vivo therapeutic effect, clinical study

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INTRODUCTION

Combined studies using quantum chemical calculations and IR spectroscopy have shown that the interaction of ornidazole with hydroxylated nano-silica is due to the formation of hydrogen bonds between the oxygen atom of the silanol group of the silica surface and the hydrogen atom of the hydroxyl group of the molecule.

The highest adsorption energy of the ornidazole molecule is inherent in hydroxylated silica (-87.5 kJ / mol). The ornidazole OH group is the most active in forming a hydrogen bond with the silanol group of the silica surface.

Comparison of the IR spectra of nanocomposites obtained after the release of the active substance (ornidazole) within 24 h, showed that the residual concentration of ornidazole on the surface according to research and desorption kinetics is about 13%, indicating the possibility of prolonged action of the synthesized nanocomposite. Given the above, it can be assumed that the obtained nanocomposites can be further used as promising hybrid nanomaterials that prolong the action of traditional drugs.

This is confirmed by the comparison of the therapeutic effect of the nanocomposite with ornidazole immobilized on the surface of nanodispersed silica with the known long-acting drug Gentaxan in vivo, as well as comparative use in clinical studies.

The immobilisation of antimicrobial substances on the surface of inorganic carriers allows to design the modern nanohybrid materials of complex action, as this approach combines the therapeutic effect of the active ingredient with high adsorption capacity of the carrier,

such as silica [1]. One of the areas of improvement of these combined drugs is the creation of nanocomposite systems in which silica due to intermolecular interactions with the molecule of the active substance can affect the dynamics of its release. The interest in materials with programmed characteristics based on silica is due to the fact that they are non-toxic, biocompatible, biologically erosive and have a high adsorption capacity. That is why such materials allow to solve a number of topical interdisciplinary problems, primarily related to the development of modern forms of medicines and their delivery systems [1 - 3]. These properties also allow the active use of silica in medical and pharmaceutical practice and many other areas of human activity [4 - 6].

In particular, one of the promising areas of use of highly dispersed silica in modern pharmacology is its use as a carrier of drugs and disinfectants [7]. This is due to the fact that the immobilization of these compounds can improve their physicochemical properties, increase stability, reduce toxicity, change the bioavailability and kinetics of release of the active substance [8]. When creating hybrid nanocomposite systems, including on the basis of highly dispersed silica, it should be borne in mind that the specific properties of modified nanomaterials may be manifested as a result of their interaction with active centers occurring in the surface layer [9].

An important characteristic that plays a significant role in the use of silica in the biomedical field, as well as in the study of silica surface chemistry, are the large specific surface area of the carrier and the concentration of active groups [10,11]. In addition, in the case of hybrid nanocomposite materials for medical purposes, it should be borne in mind that the adsorption of antimicrobial compounds can be one of the effective ways to create long-acting drugs with controlled release of the active ingredient. In turn, all these features require detailed experimental studies, which should primarily relate to the interaction of silica (as a matrix) with specific biomolecules and / or drugs of different classes.

Due to the possibility of changes in certain physicochemical properties of the active ingredient when combined with a carrier matrix, as an active ingredient studied the known drug ornidazole (1-(2-hydroxy-3-chloropropyl)-2-methyl-5-nitroimidazole). It is a derivative of 5-nitroimidazole, has a heterocyclic structure, consists of a nitroimidazole nucleus and 2-hydroxy-3-chloropropyl group in position 1 and methyl group in position 2, soluble in water, ether, ethanol and chloroform [12]. The drug easily penetrates the blood-brain barrier [13], has antimicrobial action, and reduced bacterial resistance [14], low toxicity and optimal bioavailability [15].

The need to study in detail the intermolecular interaction of active drug components with nanomaterials used as carriers is extremely important for managing pharmacokinetic processes, elucidation of pharmacological properties, creation of appropriate design of combined drug delivery nanosystems, etc. To this end, the authors in many studies are actively using not only experimental methods, but also the apparatus of quantum chemistry, in particular the method of DFT (Density Functional Theory) [16 - 18].

The use of molecular modeling methods revealed that the ability to penetrate target cells of the macroorganism, the peculiarities of drug transport during delivery *in vivo*, the kinetics of release of the active ingredient, etc., significantly affect not only the properties of the active substance but also the carrier characteristics [17]. Therefore, the combination of theoretical modeling and experimental studies in vivo, as well as clinical studies allows to rationally designing the composition and structure of nanoparticles of carriers while creating effective systems of controlled drugs delivery to target cells [16].

Promising today is the direction of designing modern nanocomposite drugs for the treatment of purulent wounds by immobilizing drugs on the matrices of sorbents that have the property of being a carrier of drugs. Immobilization of the drug reduces the side effects of the drug and promotes the prolongation of the therapeutic effect.

THE AIM

In view of the above, the aim of the study was to investigate the interaction of ornidazole with nanosilica using quantum chemical calculations, IR spectroscopy and to test the effectiveness and prolonged antimicrobial action of potential nanocomposite drugs in vivo and in clinical trials.

MATERIALS AND METHODS

Pyrogenic silica (A-300 «Sillard P») produced by the Kalush Research and Experimental Plant of the ISTC «Surface Chemistry» with a specific surface area of 300 m^2/g (by low-temperature nitrogen adsorption by the BET method) was used as the initial matrix (S).

Ornidazole (1-(3-chloro-2-hydroxypropyl)-2-methyl-5-nitroimidazole) manufactured by Aarti Drugs Limited, India was used as the active substance. Immobilization of ornidazole was performed under constant stirring (500 rpm) for 20 min at a temperature of 20°C. Nanocomposite materials were obtained by immobilization of ornidazole on the surface of the hydroxylated matrix, so that the final content of active ingredient in dry nanocomposites was 0.184 mmol/g SiO_2, which is

much less than the number of adsorbable silanol groups on the surface of dispersed silica [19].

To confirm the presence of functional groups on the surface of matrices and the presence of ornidazole on the surface of nanocomposites synthesised on their basis after immobilization and after release of the active substance the method of IR spectroscopy with Fourier transform spectrometer Thermo Nicolet Nexus FT-IR (Nicolet, USA) in frequency range 500 - 4000 cm^{-1} was used. All samples were mixed with freshly calcined KBr at a mass ratio of 0.015: 0.3 [19].

The release of the active substance (ornidazole) from the surface of nanocomposites was carried out under static conditions in distilled water (V = 100 ml) at a temperature of 37 ° C, the weight of the nanocomposite was 0.1 g. The duration of release was 24 hours.

To determine the most active centers for immobilization of the ornidazole molecule on hydroxylated and functionalized silica, the method of density functional theory (DFT) with functional B3LYP [12, 13] and basic set 6-31G (d, p) was used. GAMESS (US) program was used for calculations [14]. To take into account the dispersion effects of binding, which determine the formation of non-covalent intermolecular complexes, the energy variance of intermolecular interaction took into account the Grimme D3 variance correction [15, 16]. Given that the experiment was performed in aqueous solution, the effect of the solvent was described within the continuum approximation of the PCM (polarizable continuum model) [17]. The energy effect of complexation (adsorption) (ΔE) according to the reaction A + B $\rightarrow$ A…B was calculated by the formula: $\Delta E = E_{tot}(A\ldots B) - (E_{tot}(A) + E_{tot}(B))$, where $E_{tot}(A)$ - the total energy of the ornidazole molecule taking into account the aqueous medium, $E_{tot}(B)$ is the total energy of the cluster that reproduces the surface, $E_{tot}(A\ldots B)$ is the total energy of the intermolecular complex of ornidazole with the cluster.

Study of the prolongation of the therapeutic effect of nanocomposite drugs *in vivo*. In order to study the effectiveness of nanocomposite based on silica and immobilized on its surface ornidazole (Ornidasil) for the treatment of purulent wounds, we conducted an experimental study in rats. Thirty adult white male Vistar rats were used in the study. All experimental animals were in the same conditions, painful manipulations were performed under local infiltration anesthesia with 0.5% novocaine solution, animals were removed from the experiment by sodium thiopental overdose, in accordance with generally accepted ethical standards and recommendations.

In planning the presented study, they were guided by generally accepted domestic and international laws in accordance with the «General Ethical Principles of Animal Experiments» (Ukraine, 2001), Order of the Ministry of Health of Ukraine № 281 of 01.11.2000, Law of Ukraine № 3447-IV «On Animal Protection from cruel treatment «of 21.02.2006, as well as in compliance with the basic provisions of the» Rules for the use of experimental animals «(1977), GCP 1996, the Council of Europe Convention for the protection of vertebrate animals used in experiments and other scientific purposes from 18.03.1986, EEC Directives № 609 from 24.11.1986, Order of the Ministry of Health of Ukraine № 373 from 22.07. 2005, Order № 95 of 16.02.2009, Order № 944 of 14.12.2009, Council of Europe Convention on Human Rights and Biomedicine (04.04.1997), Helsinki Declaration of the World Medical Association on the Ethical Principles of Medical Practice human research (1964–2000).

Study of the action of Ornidasil for the treatment of purulent wounds, compared with a known drug of prolonged action. In all experimental animals, a purulent wound was simulated according to the method [20]. The animals were divided into two groups. In the main group (group I) the study was performed on 20 rats, for them Ornidasil was used [21-22]. The comparison group (group II) consisted of 10 rats, for which was used the known application sorbent «Gentaxan», which includes: gentamicin sulfate, polymethylsiloxane, L-tryptophan and zinc sulfate [23].

Also, we studied the effectiveness of the «Ornidasil» application sorbent in the clinic for the treatment of patients with diabetic foot syndrome and to prevent the suppuration of postoperative wounds in patients with purulent peritonitis in toxic and terminal stages.

RESULTS

In the next stage of the study, nanocomposites based on hydroxylated silica matrix with immobilized ornidazole were created. The method of immobilization of ornidazole on the silica matrix used in this work is primarily simple. Another of its features is the weak fixation of the modifier on the matrix, which leads to the gradual release of the active ingredient from the surface of the carrier into the wet environment. The concentration of ornidazole chosen for immobilization was considered optimal on the grounds that the content of the active ingredient, according to previous studies [19] was sufficient for its antimicrobial action, and on the surface of nanosilica there were still enough free active centers may be involved in the adsorption of toxic wound destructs of polypeptide nature [24]. After immobilization of ornidazole, the presence of the active substance on the hydroxylated silica matrix was also identified by IR spectroscopy.

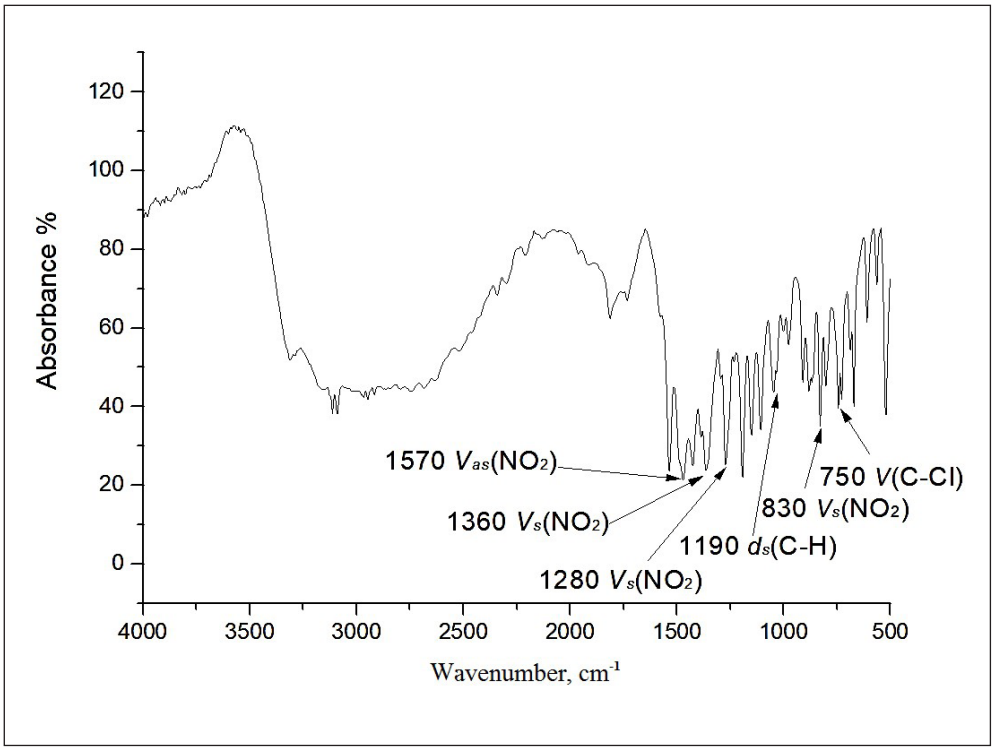

Fig. 1. IR-spectra of ornidazole.

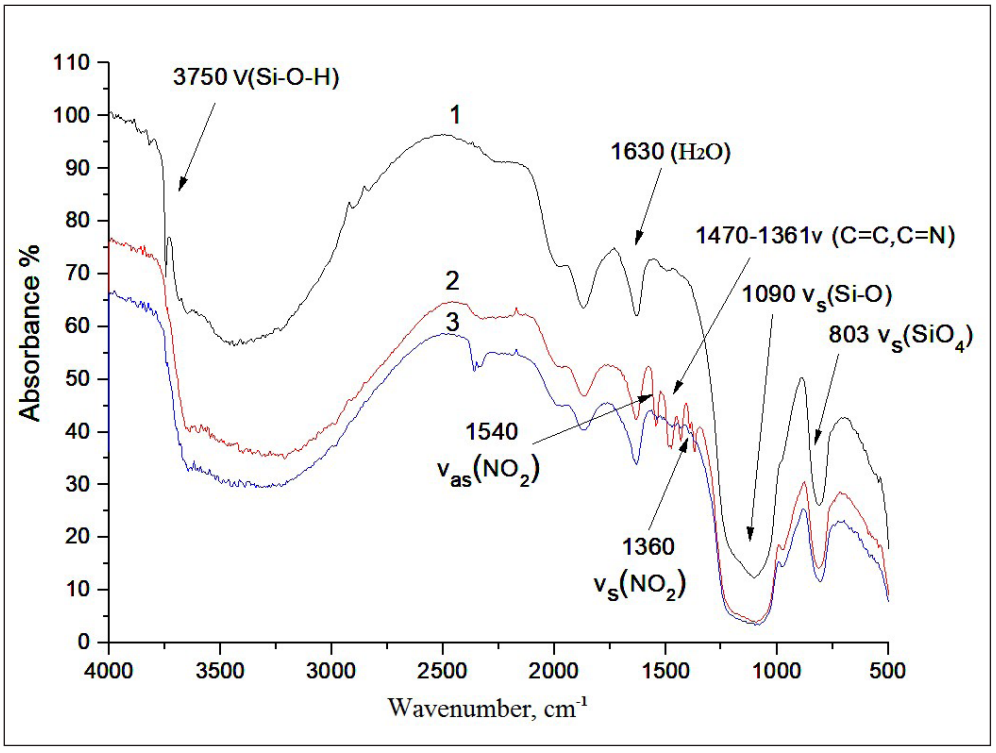

Fig. 2. IR-spectra of hydroxylated silica surfase (1), nanocomposite based on silica and ornidazole immobilized on (2) and nanocomposite after ornidazole desorption by water during 24 h (3).

The spectrum of ornidazole contains a number of characteristic bands, the assignment of which is shown in Fig 1.

The hydroxylated silica in the IR spectra (Fig. 2, curve 1) has a wide absorption band in the range 3200-3700 cm^{-1} due to the superposition of –OH streching and adsorbed water molecules. Peak 3750 cm^{-1} corresponds to the free silanol groups. The peak at 1870 cm^{-1} belongs to the oscillations of the SiO_2 framework, present in all spectra. The band at 1630 cm^{-1} refers to the deformation frequency of water molecules, the band 803 cm^{-1} corresponds to the symmetric vibration of the tetrahedron SiO_4. The band at 970 cm^{-1} belongs to the streching Si–O bond in ºSi-OH groups.

In the spectrum of the nanocomposite (Fig. 2, curve 2), there were absorption bands at 1540 cm^{-1}, and 1360 cm^{-1}, corresponding to asymmetric and symmetric stretching of NO_2 group of ornidazole (Fig. 1). The series

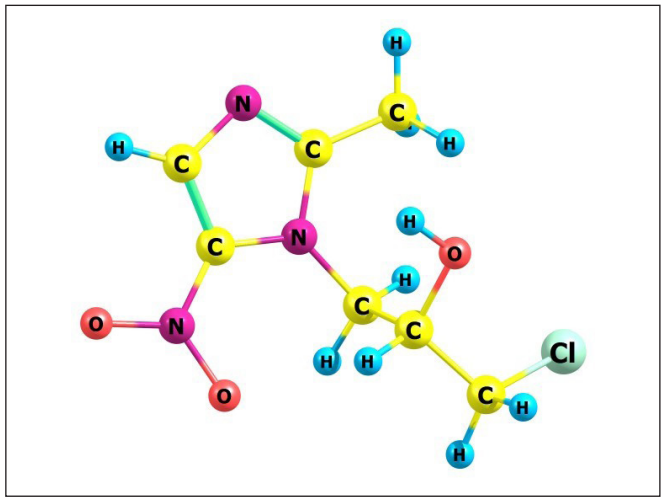

Fig. 3. Optimized structure of ornidazole conformere and its full energy.

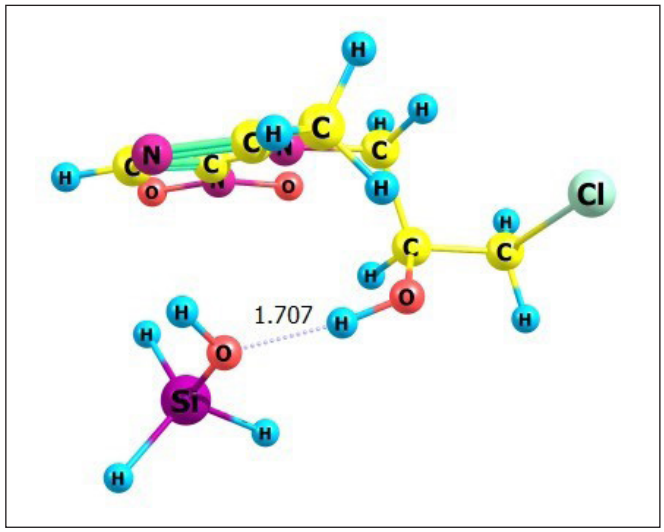

Fig. 4. Equilibrium structure of silanol and ornidazole intermolecular complex with lowest energy obtained in B3LYP-D3/6-31G(d,p) approximation. Here and then bond length is in Å [28].

of bands in the region 1470-1361 cm^{-1} belongs to the stretching of C=C and C=N bonds. After the water leaching process, the intensity of the characteristic peaks of ornidazole decreased significantly (Fig. 2, curve 3), which indicates a noticeable but not complete release of the active substance into solution. Confirmation of this fact is the study of desorption of ornidazole from the nanocomposite, which showed that after 24 h of leaching on the surface of the nanocomposite remains about 13% of the active substance (to be published).

The analysis of adsorption data raises the question of the structure of the sorption layer of the silica surface. It is also important to determine the most active adsorption centers of the ornidazole molecule relative to hydroxylated silica, the structure of ornidazole adsorption complexes on the surface, and to compare the energy of hydrogen bonds of ornidazole with water and

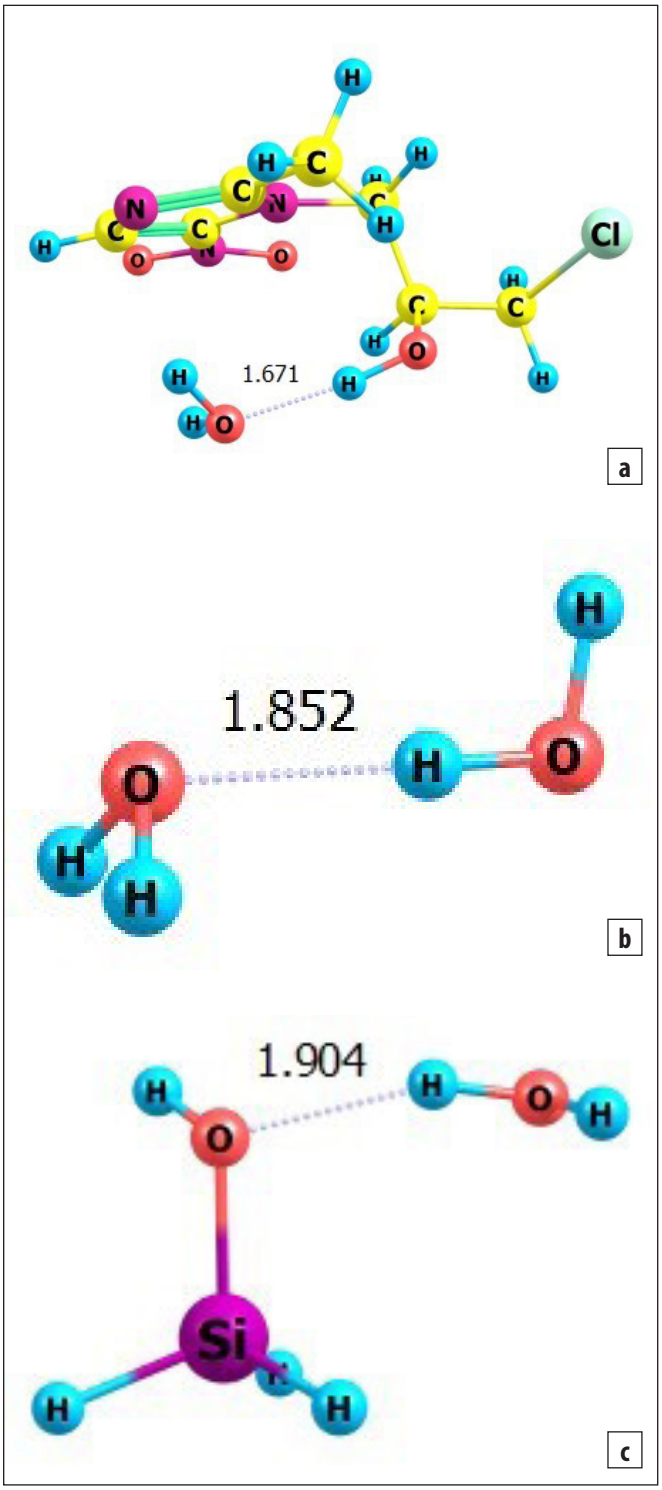

Fig. 5. Equilibrium structures of molecular complexes and their formation energies: a – water and ornidazole molecules, b – water dimer, c – water and silanol molecules [28].

silica molecules. Detailing of elementary processes at the level of intermolecular interactions allows a deeper interpretation of experimental data.

For quantum chemical modeling of physical sorption, the structure of the most probable conformer of the ornidazole molecule, which exists in aqueous solution,

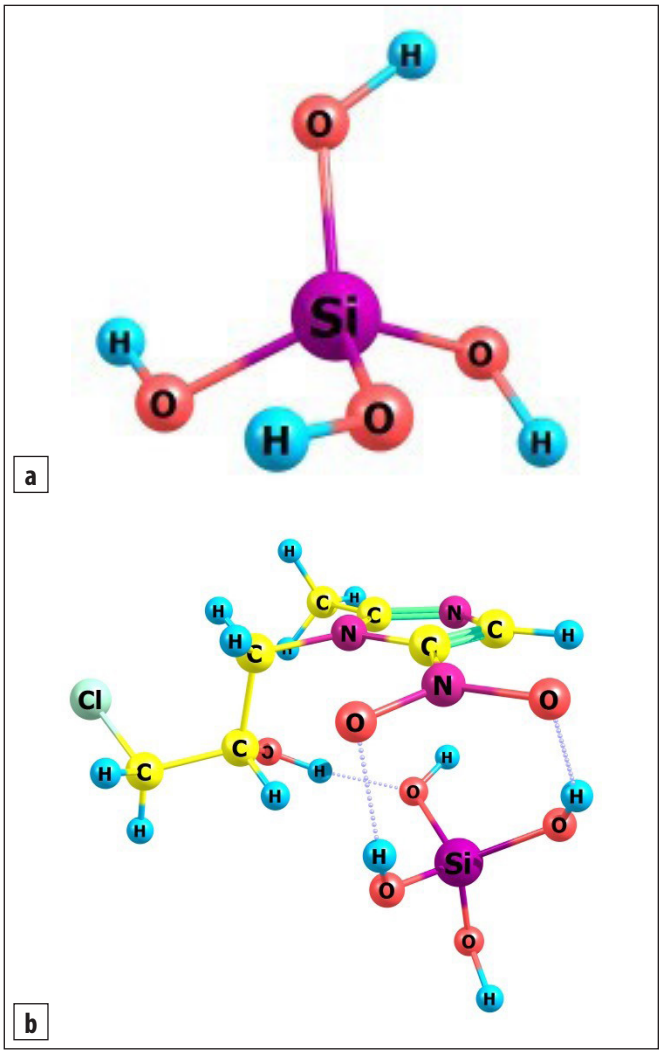

Fig. 6. Used for hydroxylated silica surface modeling orthosilicate acid (a) and most energetically efficient equivilibrium structure of ornidazole and orthosilicate acid molecular complex (b).

was first established, because, according to the literature, several options are possible [25 - 27]. In view of this, the structure of the most probable conformers of the ornidazole molecule was optimized and the values of their total energies were compared, and the most thermodynamically stable of them was determined [28]. This was the structure (Fig. 3), which has the lowest value of total energy. This conformer was involved in further calculations.

Taking into account that the ornidazole molecule has several functional groups, different in their chemical nature, which may participate in the adsorption interaction with the silica surface, to establish the most active of them was simulated interaction of the ornidazole molecule with the smallest possible silica surface model - silanol molecule [24]. There is only one silanol group in this molecule, and three hydrogen atoms are attached to the silicon atom, which are weakly polarized and do not participate in the formation of hydrogen bonds

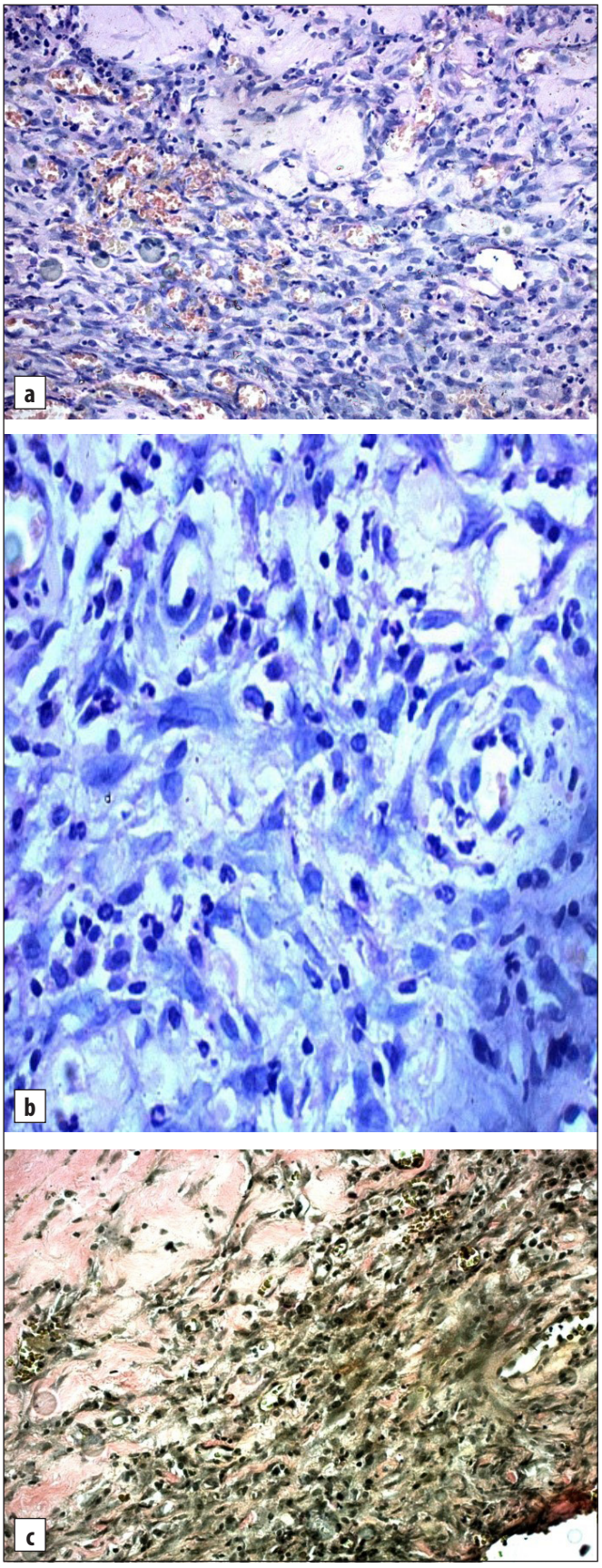

Fig. 7. Main group I, 14 days of the study. Intercellular infiltration extends to the entire depth of granulation tissue (a, b), endothelial cell proliferation (c). Staining: a, b - hematoxylin and eosin, c - van Gizon. Magnification: a, c x 100, b x 400.

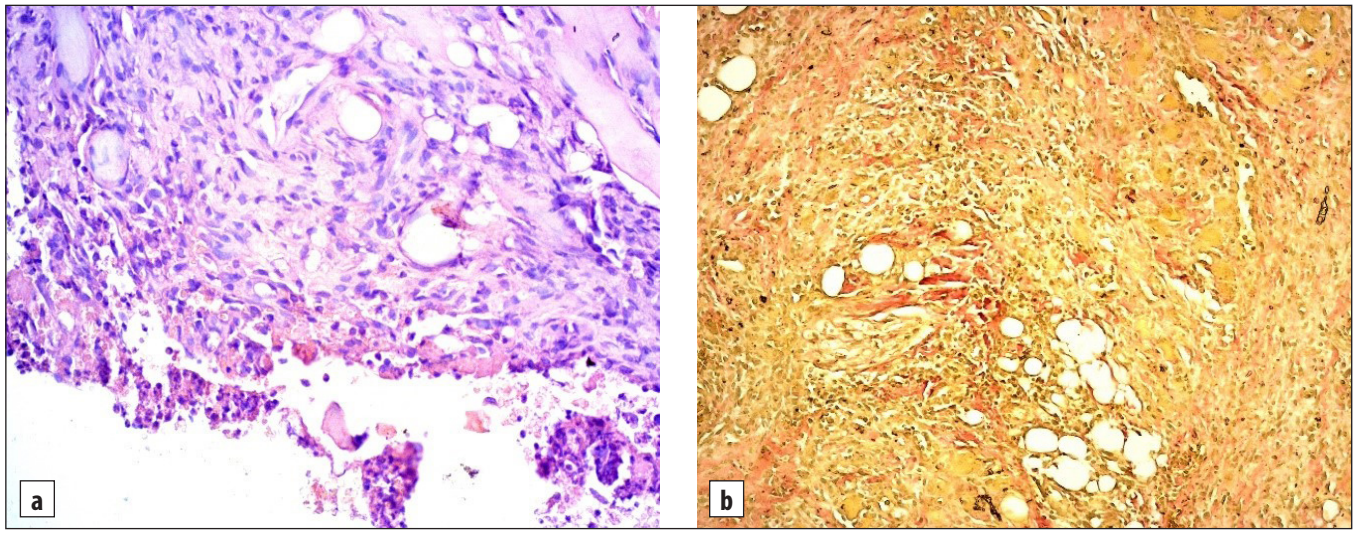

Fig. 8. Comparison group II, 14 days of the study. Fibrinous-purulent overlays (a(↑)), intercellular oedema and infiltration of granulation tissue by neutrophils and lymphocytes (b(↑)) are noted. Staining: a - hematoxylin and eosin, b - van Gizon. Magnification: x 200.

Table I. Dynamics of the wound process in clinical study groups

Group of patients	Clinical indicators				
	Cupping pain syndrome (day 24h)	Normalization of body temperature (day 24h)	Wound cleansing (day 24h)	The appearance of granulation (day 24h)	Marginal epithelialization (day 24h)
I	2,7±0,18	4,5±0,18	4,3±0,15	4,6±0,14	6,1±0,25
II	6,4±0,27	7,3±0,23	8,4±0,21	8,7±0,25	10,4±0,26
	p < 0,05	p < 0,05	p < 0,05	p < 0,05	p < 0,05

- this model is effective for preliminary estimation of adsorption energy.

When ornidazole and silanol molecules interact, the latter's silanol group may form intermolecular binding to ornidazole functional groups. In one of the adsorption complexes, the chlorine atom of the ornidazole molecule is located at a distance of 2,837 Å from the oxygen atom of the silanol group, which indicates a weak interaction, as this distance O···Cl is less than the sum of van der Waals atomic radii of oxygen and chlorine atoms (r_w(Cl) + r_w(O) = 3.27 Å). The interaction energy in this case is -10.4 kJ/mol [28]. Significantly higher complexation energy (-42.4 kJ / mol) when the nitrogen atom of the ornidazole molecule interacts with the hydrogen atom of the silanol molecule. The distance between these atoms (1,716 Å) is much smaller than the distance O···Cl in the above-mentioned complex, which indicates the possible formation of a hydrogen bond.

The hydroxyl group of the ornidazole molecule can form two types of hydrogen bonds with the silanol molecule: due to the oxygen atom of the alcohol group of the ornidazole molecule with a bond length of 1,711 Å or with the participation of its hydrogen atom with a hydrogen bond length slightly less than 1,707 Å (Fig. 4). The values of the interaction energy for these complexes are -41 and -53.0 kJ/mol, respectively.

In addition, the ornidazole molecule contains the polar group NO_2, which can also form hydrogen bonds due to the presence of negatively polarised oxygen atoms. In this case, the energy of complexation with the participation of the oxygen atom of the nitro group, which is located closer to the «tail» of the ornidazole molecule, is slightly higher (-45.4 kJ/mol) energy of a similar bond with another oxygen atom of this group (-41,1 kJ/mol), which correlates with their lengths.

Thus, based on the analysis of the results of calculations, it was found that the lowest energy obtained for intermolecular interaction of the silanol group of the silanol molecule with the chlorine atom of the ornidazole molecule, and the highest - with the OH group, whose hydrogen atom forms a hydrogen bond with the oxygen atom of the silanol group . In addition, the nominal nitrogen atom and the oxygen atoms of the nitro group may also be involved in the intermolecular interaction.

Due to the fact that the adsorption of ornidazole occurs from aqueous solution, it is desirable to consider the interaction of the water molecule with the OH group of the ornidazole molecule. It was found that the length

of the hydrogen bond between the oxygen atom of the water molecule and the hydrogen atom of the OH group of the ornidazole molecule is smaller (Fig. 5, a) and is 1.67 Å, compared with that formed by the interaction of the silanol group with the same group (Fig. 4, d) and is 1.707 Å, respectively. This means that the energy of this bond is higher and is 56 kJ / mol, which is 3 kJ / mol more than the absolute value of the energy of interaction with silanol (-53 kJ / mol). Comparing these values with the energy effect of hydrogen bonding between two water molecules (Fig. 5, b) (-31 kJ / mol), it can be argued that ornidazole will be washed away by water, passing into the contact solution, because the absolute value of the interaction energy between water molecules and ornidazole are higher compared to the binding energy between water molecules in the dimer (Fig. 5, b) and in the complex of water and silanol molecules, which is -28.4 kJ / mol (Fig. 5, c) .

To determine the influence of the chemical nature of the functional groups of the silica surface on its adsorption capacity relative to the ornidazole molecule, adsorption complexes were considered, in which the orthosilicic acid molecule ruled over the silica surface (Fig. 6).

The equilibrium structure of the adsorption complex of the ornidazole molecule with orthosilicate acid is presented in Fig. 6, b. It is obvious that the largest number of hydrogen bonds (namely - three) is formed by interaction with the original silica (Fig. 6, b), which is confirmed by the values of adsorption energy calculated by formula (1), which is -87.5 kJ / mol.

Therefore, complex studies using quantum chemical calculations and IR spectroscopy have shown that the interaction of ornidazole with hydroxylated silica is slightly different. The formation of active substance complexes with hydroxylated matrices is due to hydrogen bonds between the oxygen atom of the silanol group of the silica surface and the hydrogen atom of the alcohol group of the ornidazole molecule. This promotes the gradual release of ornidazole from the surface of such a matrix into the wound exudate.

The results of the study of the therapeutic effect of "Ornidasil" *in vivo* show that in the main group I already on the seventh day in comparison with group II was granulation tissue with a large number of newly formed vessels of the microcirculatory tract and pronounced proliferation of fibroblasts, indicating acceleration. In the comparison group, the regeneration process was slower (Fig. 8). Thus, on the 7th day of the study, the best results of healing purulent wounds are observed in the first group (main) of experimental animals.

Thus, on day 13, 9 experimental rats of group I healed completely, 11 rats had a small wound surface, complete healing occurred on day 15. In the group comparing rats on day 21 of the experiment, complete wound healing was not observed. On the 14th day of the experiment in 11 animals of the basic group from data of histological research, there was the formed granulation fabric that consists of young collagen fibers and plenty of vascular loops. There were areas where a wide strip of granulation tissue with the formation of thin multidirectional collagen fibres, between which many cells were seen: macrophages, lymphocytes, fibroblasts cells of varying maturity and large poorly differentiated cells (Fig. 7).

We also investigated the effectiveness of the aplication sorbent Ornidasil in the clinic. The main group of IA included 15 patients who received NO-therapy in combination with the application of sorbent Ornidasil in the complex treatment of DFS (Diabetic foot syndrome). The IB group included 23 patients who received the application sorbent Ornidasil for local treatment of DFS. The comparison group IIA included 27 patients who received the application sorbent Gentaxan in complex treatment. The IIB comparison group included 19 patients who received 10% NaCl solution for local treatment of DFS with subsequent transition to Levomecol ointment (Table I). The method of the application sorbent in patients with DFS of the main group was as follows: after treatment of the wound with 0.9%, NaCl solution on the wound surface for 24 hours applied application sorbent «Ornidasil» layer 1-3 mm with subsequent overlay by a sterile gauze bandage. Bandages are applied daily with this symptom until the wound is completely healed.

One of the important manifestations of diabetic foot syndrome (DFS) is the presence of pain. Cupping pain syndrome occurred in the IA group (main) compared to the IIA group (comparison) 2.1 times faster, with the IIB group (comparison) - respectively 2.4 times faster. In the IB group (main) compared with the IIA group (comparison) cupping pain syndrome occurred 1.2 times faster, with the IIB group (comparison) - respectively 1.4 times faster.

To determine the effectiveness of treatment, an important indicator is the purification of the wound from necrotic tissue, which in the IA group in comparison with the IIA group was 1.8 times faster, and with the IIB group - respectively 1.95 times faster. In the IB group, compared with the IIA group, wound cleansing was 1.4 times faster, and in the IIB group - respectively 1.5 times faster.

Wound cleansing begins with the appearance of granulation tissue, which is an objective criterion for the effectiveness of treatment. In the IA group, the appearance of granulation tissue occurred on 4.6 ± 0.14 days, while in the comparison groups (IIA, IIB) the appearance of granulation was observed 1.8 and 1.9

times later, respectively. In the IV group, the appearance of granulation tissue occurred at 5.3 ± 0.15 days, while in the comparison groups (IIA, IIB) the appearance of granulation was observed 1.6 times later.

Also, an important indicator of the wound process is the appearance of marginal epithelialization, which indicates the beginning of wound cleansing. In the IA group, the appearance of epithelialization was observed at 6.1 ± 0.25 days, which is 1.6 times faster than in the IIA group, and 1.7 times faster in the IIB group. In the IV group, the appearance of epithelialization was observed at 7.3 ± 0.15 days, which is 1.4 times faster than in the IIA group, and 1.7 times faster in the IIB group.

The wounding process in all patients ran with all the signs of each phase, but with their different duration. The most significant was the phase of hydration, which lasted much longer in patients with massive purulent processes.

One of the serious problems of abdominal surgery is the problem of peritonitis. Frequent complications of peritonitis are wound complications - seromas, hematomas and suppuration of wounds. The most dangerous complication is suppuration of postoperative wounds, which in diffuse and general peritonitis, according to various authors, occurs in 11 - 72% [29]. These complications prolong the length of stay of patients in the hospital, increase the duration of disability, the cost of treatment and worsen its results.

The critical number of microorganisms for the occurrence of suppuration is 105 microbial bodies per 1 g of tissue and in hypoxia enough 100 microbial bodies per 1 g of tissue. Therefore, in order to study the pathogenesis of suppuration of postoperative wounds and to develop mechanisms for its prevention, we studied the pollution by microorganisms of the surgical wound at the end of the operation. The study was performed in 43 patients with diffuse and general purulent peritonitis in the toxic and terminal stages. At the end of the operation, pollution of wounds with microorganisms above the critical level of suppuration was observed in 35 patients, which amounted to 81.4%.

We have proven the effectiveness of the application of sorbent «Ornidasil» for the prevention of suppuration of postoperative wounds in patients with purulent peritonitis in the toxic and terminal stages. The comparison group consisted of 19 patients who for the prevention of suppuration of postoperative wounds used a polyurethane wound expander (wound protector) with variable height. The main group included 24 patients in whom polyurethane wound protector was used in combination with the application sorbent «Ornidasil». The groups were comparable in age, sex, prevalence and nature of peritonitis. The method of protecting the wound from infection in patients of the main group was as follows: when performing surgical access to the abdominal organs (middle laparotomy) cut the skin, subcutaneous fat and aponeurosis to the parietal peritoneum; at the edge of the aponeurosis, clamps were applied and traction was performed upwards, after which the parietal peritoneum was dissected; a suction cup was introduced into the abdominal cavity with the help of which the exudate was evacuated; then the lower ring of the polyurethane wound protector was wound into the abdominal cavity and the clamps were removed from the aponeurosis, then the upward traction was performed behind the upper ring of the wound protector; between the wall of the wound protector and the surgical wound was applied a thin layer of application sorbent «Ornidasil», and then wound on the upper ring wall of the wound protector, carrying out its final installation and fixation in the surgical wound; at the end of the surgery, the wound protector was removed, and the remnants of the sorbent on the surgical wound were washed away with 0.9% NaCl solution. In the comparison group, postoperative wound suppuration occurred in 6 patients (31.6%), and in the main group - in 3 patients (12.5%).

DISCUSSION

On the basis of studies of the biological activity of the nanocomposition "Ornidasil" in *in vivo* and in clinical studies it's obvious advantages over traditional methods of treatment have been established. As shown by IR spectral data and data on the study of adsorption and desorption of ornidazole, as well as the results of quantum chemical calculations, the molecule of the active substance binds to the surface of the carrier with the formation of adsorption complexes.

The material thus obtained is a composite consisting of silica nanoparticles [10], the surface of which is covered with ornidazole adsorbed due to hydrogen bonds, mainly connected to the active silanol groups by the hydroxyl of adsorbate molecule. Moreover, the interaction of other polar groups of ornidazole with the active centers of the silica surface is not excluded. In particular, judging by the calculated interaction energies, a hydrogen bond with the oxygens of the nitro group is very likely [19].

The energy of interaction of ornidazole with water molecules, that is, the energy of solvation, is relatively large and close to the energy of interaction of the adsorbate with the surface due to one hydrogen bond. The solvation energy determines the ability of the adsorbate to be hydrated and dissolve in water.

Therefore, after the nanocomposite enters the wound solution, ornidazole, bound by one hydrogen bond,

begins to desorb and pass into the solution due to a shift in the equilibrium because of the predominance of concentrations of water molecules and destroys bacterial microflora [25]. The surface of silica freed in this way begins to adsorb pus and other contamination of the wound, primarily of oligo- and polypeptide nature [7]. Those ornidazole molecules that are tightly bound to the silica surface by multiple hydrogen bonds are retained by the surface and gradually releasing from the surface over longer periods of time [11]. Thanks to this multifunctional ability, the wound is cleaned of microorganisms, their waste products and debris [8].

At the same time, there is a synergism of the action of the adsorbent and the antimicrobial agent. Our studies of the effects of ornidazole and nanosilica separately on the most common microorganisms of wound infection, such as S. aureus and Pseudomonas aeruginosa [24], showed either their weak inhibitory effect or even the absence of activity (silica). While together they showed a high inhibitory capacity for all tested bacterial strains, and the main thing is the prolongation of the action of the composite. Such a prolonged effect can be explained precisely by the easy release of the active substance into the solution, as well as the presence of residual adsorbed molecules of ornidazole. Apparently, such molecules are tightly bound to the surface due to the simultaneous binding by several hydrogen bonds of different polar groups of ornidazole, which slows down the desorption of the active substance and determines the prolongation of the drug's effect [25].

The synergism or enhancement of the antimicrobial activity of ornidazole in the presence of silica can be explained by the adsorption of silica nanoparticles to the surface of bacteria due to the active centers of the surface of silica and the phospholipid membrane [4] of bacterial cells and the weakening of bacteria for their effective destruction by the antibacterial agent ornidazole.

Thus, the mechanism of action of the drug Ornidasil consists in the joint action of nanosilica and ornidazole, which is released in portions into the wound solution at first quickly and then slowly, creating a prolonged antimicrobial and simultaneously cleansing effect.

CONCLUSIONS

Nanocomposites obtained by immobilization of ornidazole on the surface of the hydroxylated silica matrix were studied using quantum chemical calculations and IR spectroscopy.

Comparing the IR spectra of nanocomposites, before and after the release of the active substance (ornidazole) after 24 h, it was found that this process occurs gradually and even after 24 h the active ingredient still remains in the sorbent.

Quantum chemical modelling of the interaction of ornidazole with surface functional groups showed that the lowest energy is inherent in the intermolecular interaction of the silanol group of the silanol molecule with the chlorine atom of the ornidazole molecule, and the highest with the OH group whose hydrogen atom forms a hydrogen bond. The nominal Nitrogen atom and the Oxygen atoms of the nitro group may also be involved in the intermolecular interaction. Also, quantum chemistry methods have shown that regardless of the size of the silica model, the highest adsorption energy of ornidazole is inherent in hydroxylated silica.

The results of the study in rats show that in the main group, which was treated with nanocomposite "Ornidasil" on the seventh day compared with the comparison group was granulation tissue with a large number of newly formed vessels of the microcirculatory tract and pronounced proliferation of fibroblasts, indicating acceleration. In the comparison group, which was treated with Gentaxan sorbent, the regeneration process was slower. In the main group of experimental animals, wound healing was observed in 9 rats on day 13, in 11 rats on day 15, while in the comparison group, even on day 21, complete wound healing did not occur.

A study of the effectiveness of allocating sorbet based on nanosilicon and ornidazole (Ornidasil) in the complex treatment of Diabetic foot syndrome showed that in the experimental groups, wound healing occurred 1.6 -1.9 times faster than in the control group.

The use of polyurethane wound protector in combination with the application of sorbent Ornidasil to prevent suppuration of postoperative wounds in patients with diffuse and general peritonitis in toxic and terminal stages reduced the suppuration of postoperative wounds in patients of the main group by 2.5 times relative to patients in the comparison group.

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