ANTIMICROBIAL AND ANTIVIRAL ACTIVITY OF THREE-COMPONENT COMPLEX OF CHLORHEXIDINE-EDTA-ZINC

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Chlorhexidine bigluconate (CHX) is widely used as a disinfectant, but it is not effective against spore-forming microorganisms, as well as viruses. In this work, a method has been found to increase the biocidal activity of chlorhexidine by using it as part of a complex including ethylenediaminetetraacetic acid (EDTA) and zinc chloride. The structure of the three-component complex CHX-EDTA-zinc is proved by the MALDI-MS method. The biocidal activity of the chlorhexidine complex has been studied in vitro and *in vivo* experiments. It is shown that the complex is significantly superior to chlorhexidine alone, both in terms of activity level and in the breadth of biocidal action. In relation to the studied bacterial and fungal strains, the CHX-EDTA-Zn complex was 4–5 times more active than chlorhexidine bigluconate. In concentrations from 1.0 mg/ml to 0.008 mg/ml (depending on the type of micro-organism), in vitro the complex showed both bacteriostatic and bactericidal effects against the main pathogens of bacterial diseases of birds. In clinical conditions, the complex has shown high efficiency in the treatment of dermatitis in small domestic and farm animals. Also, in vitro and *in vivo*, the complex showed unexpectedly high antitubercular activity comparable to that of monofloxacin, including on drug-resistant strains of mycobacteria. *in vitro* experiments involving polio virus and adenovirus have shown that the CHX-EDTA-Zn complex possesses virulent action.

Keywords: chlorhexidine, EDTA, zinc, complex, MALDI, antiseptic, anti-tuberculosis, antiviral activity

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Compliance with ethical standards: all procedures with animal models were carried out in accordance with the Directive 2010/63/EU of the European Parliament and of the Council of the European Union (2010) on protection of animals used for scientific purposes.

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АНТИМИКРОБНАЯ И ВИРУЛИЦИДНАЯ АКТИВНОСТЬ ТРЕХКОМПОНЕНТНОГО КОМПЛЕКСА ХЛОРГЕКСИДИН-ЭДТА-ЦИНК

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Хлоргексидина биглюконат (ХГ) широко используют в качестве дезинфицирующего средства, однако он мало эффективен в отношении спорообразующих микроорганизмов, а также вирусов. Целью работы было повысить биоцидную активность хлоргексидина путем его использования в составе комплекса, включающего этилендиаминтетрауксусную кислоту (ЭДТА) и хлорид цинка. Структура трехкомпонентного комплекса ХГ-ЭДТА-цинк доказана методом МАЛДИ-МС, биоцидная активность изучена в экспериментах *in vitro* и *in vivo*. Показано, что комплекс значительно превосходит индивидуальный хлоргексидин, как по уровню активности, так и по широте биоцидного действия. В отношении изученных бактериальных и грибных штаммов комплекс ХГ-ЭДТА-Zn был в 4–5 раз активнее, чем биглюконат хлоргексидина. В концентрации 1,0–0,008 мг/мл (в зависимости от вида микроорганизма) *in vitro* комплекс проявлял как бактериостатическое, так и бактерицидное действие в отношении основных возбудителей бактериальных болезней птиц. В клинических условиях показана его высокая эффективность при лечении дерматитов у мелких домашних и сельскохозяйственных животных; *in vitro* и *in vivo* выявлена высокая противотуберкулезная активность, сопоставимая с препаратом монофлоксацином, в том числе на лекарственно устойчивых штаммах микобактерий. В экспериментах *in vitro* на примере вируса полиомиелита и аденовируса доказано наличие у комплекса ХГ-ЭДТА-Zn вирулицидного действия.

Ключевые слова: хлоргексидин, ЭДТА, цинк, комплекс, МАЛДИ, антисептическая, противотуберкулезная, антивирусная активность

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Disinfectants are widely used in medical practice, everyday life and in many other areas of human activity. Disinfection measures are especially important in the context of epidemics or the risk of spread of dangerous infections, which makes provision of medical institutions and general population with effective antiseptic agents a task of national importance. Generally, such agents are expected to effectively suppress all types of pathogenic microflora, including causative pathogens of microbial, fungal and viral infections, as well as to be safe for humans and the environment. The currently existing agents meet these requirements only partially, so the matter of development of new ones remains urgent.

Chlorhexidine (1.6-di-[bis-(4-chlorophenyl)biguanide]hexane bigluconate) is one of the most famous antiseptics. It has been used medical, veterinary and household environments for over 60 years now; to this day, it remains one of the most popular antiseptic agents [1]. It is popular because of its strong bactericidal effect against a wide range of gram-positive and gram-negative microorganisms, including fungi, as well as because of the ability to remain highly active in contact with various biological substrates (blood, saliva, pus, etc.) [2].

A chlorhexidine molecule consists of two symmetrical chlorophenyl-substituted biguanide groups connected by a hydrophobic hexamethylene chain. At physiological pH, it exists as a bication [3]. Like most cationic antiseptics, chlorhexidine has its antimicrobial action realized at the level of the cell membrane [4].

Chlorhexidine is the primary active ingredient in various topical disinfectants; it is used to treat wounds, burns, to sterilize the surgical field, skin. Chlorhexidine is a component of anti-cold solutions and rinses [5]. It is indispensable in dentistry, where it became acknowledged as a "golden standard" and once served as a reference agent for the new antiseptics development efforts [6].

However, chlorhexidine has a number of significant drawbacks. In particular, at room temperature, it has practically no effect on bacterial spores, which makes it of little use against spore-forming pathogens [5]. Acid-resistant microorganisms [7], including pathogens of tuberculosis and leprosy, also exhibit high resistance to chlorhexidine. Another serious drawback of chlorhexidine is lack of pronounced virucidal activity. Although chlorhexidine has some effect on HIV, herpes 1 and 2, influenza A [2, 5], it is all but ineffective against most viruses, including, according to the recent clinical studies, such as SARS-CoV-2 [8].

Last but not least, the efficacy of all such commonly used agents diminishes with emergence of resistant microbial strains. For a long time it was believed that microorganisms cannot become resistant to chlorhexidine [2], but recent data refute this opinion. For example, it was established that *K. pneumoniae* can adapt to chlorhexidine, which leads to the appearance of pathogenic strains that are practically insensitive to the recommended antiseptic concentrations [9].

As our studies show, antiseptic potency of chlorhexidine can be increased by combining it with other chemicals. In this connection, it seems interesting to study the specific activity of chlorhexidine in the presence of excipients it creates a complex with, such as Trilon B (ethylenediaminetetraacetic acid disodium salt, EDTA) and zinc chloride. Zinc salts and complexes are moderately active against microbes, fungi and viruses [10], while EDTA, although it is not antiseptic individually, can form stable complexes with most metal cations [11] and improve permeability of cell membranes for other substances, including zinc and, possibly, chlorhexidine. The purpose of this work was to study the molecular structure of the new chlorhexidine complex and to thoroughly investigate its biocidal properties.

METHODS

The complex compound 0.2% chlorhexidine bigluconate water solution, 1% ethylenediaminetetraacetic acid disodium salt (EDTA) and 0.5% zinc chloride was provided by OOO Rosbio (St. Petersburg).

Mass spectrometry

The technique selected for the analysis was MALDI-MS, matrix-assisted laser desorption/ionization mass spectrometry. The original complex solution was diluted with water 100 times. 0.5 µl of the resulting solution were applied to the MALDI target cell, followed by 0.5 µl of the matrix solution (5 mg/ml). The mixture was then dried. The matrix was 2,5-dihydroxybenzoic acid dissolved in 70% acetonitrile with the addition of 0.1% trifluoroacetic acid (TFA). The samples were left to dry at room temperature and then examined with an ultrafleXtreme MALDI-TOF/TOF mass spectrometer (Bruker Daltonics; Germany) at the Science Park of St. Petersburg State University.

The mass spectra were recorded in the range m/z 600–1500 in the "reflectron" mode with detection of positive ions. For one spectrum, 15,000 instances of sample irradiation with a Nd:YAG 355 nm laser were summed up. The mass spectrometer was calibrated with a Peptide Calibration Standard II calibration mixture (Bruker Daltonics; Germany).

The elemental composition of the chlorhexidine-EDTA-Zn complex was determined by the standard method (CHN analysis), and the quantitative content of zinc was established with the help of inductively coupled plasma atomic emission spectroscopy enabled by the Optima 2100DV spectrometer (Perkin Elmer; USA). To isolate the individual complex, the initial solution was concentrated by evaporation in a vacuum to 1/10 of the primary volume, then the precipitated crystalline product was separated, washed with water and dried in air at 40 °C to constant weight. The resulting complex was a colorless fine crystalline powder, partially soluble in water. The initial solution contained 0.34% of this complex, which is 0.2% in terms of pure chlorhexidine. Elemental analysis data: found C — 44.50%, H — 5.21%, N — 19.44%, Zn — 7.57%. $C_{32}H_{44}Cl_2N_{12}O_8Zn$ — MW 858.21; calculated C — 44.64%, H — 5.15%, N — 19.52%, Zn — 7.60%. MALDI-MS mass spectrum data: m/z 859.27.

In vitro and *in vivo* microbiological studies

The *in vitro* experiments and clinical trials of antiseptic properties of the chlorhexidine-EDTA-Zn complex on animals were carried out at the Vitebsk State Academy of Veterinary Medicine (Belarus) and Agrokombinat Yubileyny (Belarus). The preparation was serially diluted in Petri dishes; each microorganism was tested in two series of experiments that made use of cultures grown on two different nutrient media [12].

The in vivo experiments included treatment of dermatitis in domestic and farm animals (dogs, cats, rabbits and sheep). The antiseptic was applied once a day for 5–14 days.

Investigation of antitubercular activity

This part of the study was carried out at Saint-Petersburg State Research Institute of Phthisiopulmonology of the Ministry of Healthcare of the Russian Federation. *In vitro*, the antitubercular activity of the complex was tested on *Mycobacterium tuberculosis* H37Rv, a sensitive reference strain (Institute of Hygiene and Epidemiology, Prague, 1976), and a clinical isolate

Table 1. Antimicrobial activity of the CHX-EDTA-Zn complex in comparison with chlorhexidine bigluconate (CHX)

Antiseptic	Culture					
	Bac. subtilis	*E. coli*	*Bac. mycoides*	*Sac. cerevisiae*	*P. breri-compactum*	*Asp. niger*
	Area of the no-growth zone, cm^2					
CHX-EDTA-Zn complex	16.8	13.7	15.2	16.8	19.4	18.5
CHX	3.6	4.1	4.1	4.0	4.4	3.6
CHX/Complex activity index	4.7	3.3	3.7	4.2	4.4	5.1

of *M. tuberculosis* 5582 with multidrug resistance (collection of MBT strains of Saint-Petersburg State Research Institute of Phthisiopulmonology). The minimum inhibitory concentration (MIC) was determined with the help of the REMA method [13] on cultures of *M. tuberculosis* H37Rv and clinical isolate 5582 with resistance to isoniazid, rifampicin, streptomycin, and pyrazinamide. The MIC value was taken as the minimum drug concentration at which the average fluorescence level did not significantly exceed 1% of the level registered for the control MBT culture that grew without inhibition. Bacterial growth was registered both visually, by the change in the color of resazurin from blue to pink, and using a FLUOstar Optima plate fluorimeter (Germany) at an excitation wavelength of 520 nm and emission wavelength of 590 nm.

In vivo, the antitubercular activity of the complex was studied in male C57black/6 mice weighing 16–18 g (Andreevka nursery, Scientific Center for Biomedical Technologies of the Federal Medical Biological Agency; Russia) [14, 15], with two models of tuberculosis, first involving infection with the standard test strain *M. tuberculosis H37Rv*, second — with the drug-resistant strain 5582. In both series of experiments animals received medications from the fourth day on after the infection. All preparations were administered intragastrically, daily, with the exception of Saturdays and Sundays, until the end of the experiment. The CHX-EDTA-Zn complex was administered in two doses, 7 mg/kg and 14 mg/kg. Moxifloxacin was the drug the efficacy of the complex was compared to. It was administered at an average therapeutic dose of 7 mg/kg. The total duration of the experiment was 40 days.

Investigation of virucidal activity

The virucidal activity of the complex was studied at the Ivanovsky Institute of Virology in accordance with the Guidelines [16]. Vaccine strain of poliomyelitis virus (type 1, virus titer 6.5 $lgTCID_{50}$; M.P. Chumakov Research Institute of Poliomyelitis and Viral Encephalitis, Russia) and human adenovirus (type 5, virus titer 5.5 lg $TCID_{50}$; State Collection of Viruses of the Ivanovsky Institute of Virology, Russia) were employed. For the poliomyelitis virus, we used a transplantable Vero green monkey kidney cell culture, for the adenovirus — transplantable HEp2 cell line.

Statistical data processing

Statistica 7.0 software package (StatSoft; USA) enabled statistical processing. The metric indicators were presented as mean and error of mean (M ± *m*). Student's *t*-test allowed establishing the significance of differences in metric indicators.

RESULTS

Biocidal activity assessment

The activity of 0.05% CHX-EDTA-Zn was compared in vitro with that of chlorhexidine bigluconate at the same concentration. For this comparison, we used a series of cultures of standard microorganisms. Table 1 shows the results of identification of the cultureless (no-growth) zones on Petri dishes.

Table 2. CHX-EDTA-Zn complex activity against avian pathogens

Type of culture	Nutrient medium	Sample number and active substance concentration, mg/ml									
		1	2	3	4	5	6	7	8	9	10
		1.0	0.5	0.25	0.125	0.06	0.03	0.015	0.008	0.004	0.002
E. coli-1	Plain broth	–	–	–	–	–	–	–	–	+	+
	Endo medium	–	–	–	–	–	–	–	±	+	+
E. coli-2	Plain broth	–	–	–	–	–	–	–	–	+	+
	Endo medium	–	–	–	–	–	–	–	–	+	+
S. enteritidis	Plain broth	–	–	–	–	+	+	+	+	+	+
	Endo medium	±	±	±	±	+	+	+	+	+	+
S. gallinarum	Plain broth	–	–	–	–	–	–	+	+	+	+
	Endo medium	–	–	–	±	+	+	+	+	+	+
S. typhimurium	Plain broth	–	–	–	–	–	+	+	+	+	+
	Endo medium	–	–	±	±	±	+	+	+	+	+
P. vulgaris	Plain broth	–	–	–	+	+	+	+	+	+	+
	Plain agar	–	+	+	+	+	+	+	+	+	+
St. aureus	Plain broth	–	–	–	–	–	–	–	–	+	+
	Plain agar	–	–	–	+	+	+	+	+	+	+
St. epidermidis	Plain broth	–	–	–	–	–	–	–	–	–	+
	Plain agar	–	–	–	–	–	–	+	+	+	+

Note: «–» — complete absence of growth; "+" — growth of single colonies; "+" — normal growth.

Table 3. CHX-EDTA-Zn complex efficacy indicators in treatment of dermatitis in animals

Indicators	Animals	Serous catarrhal dermatitis	Purulent catarrhal dermatitis	Dermatitis of parasitic etiology (in complex therapy)
Number of experimental animals	Dogs	14	10	6
	Cats	7	2	–
	Rabbits	–	–	12
	Sheep	–	–	7
Duration of treatment, days	Dogs	5	7–10	6–14
	Cats	5	10–12	–
	Rabbits	–	–	6–9
	Sheep	–	–	6–12
Animals recovered	Dogs	11	9	5
	Cats	7	2	–
	Rabbits	–	–	10
	Sheep	–	–	7

Table 2 presents the results of *in vitro* assessment of antimicrobial activity against the main pathogens of bacterial diseases in birds.

At a concentration of 1.0–0.008 mg/ml (depending on the type of pathogen), the CHX-EDTA-Zn complex has both bacteriostatic and bactericidal effects against all studied pathogenic cultures.

Table 3 shows the results of *in vivo* studies in clinical conditions that involved treatment of dermatitis in domestic and farm animals.

Application of the antiseptic for 5–14 days let the animals recover completely. There were no allergic reactions or other negative side effects registered that could be attributed to the treatment.

The results of *in vitro* investigation of the antitubercular activity of the complex (against the *M. tuberculosis* 5582 strain) are shown in Fig. 1.

According to the results of in vitro testing on the *M. tuberculosis H37Rv* model, the CHX-EDTA-Zn complex has a pronounced antitubercular activity with the MIC value at 6.2 μg/ml.

Results of the *in vivo* investigation of antitubercular activity of the complex. Table 4 shows the results of investigation of therapeutic efficacy of the CHX-EDTA-Zn complex in comparison with moxifloxacin; Table 5 presents the data on inoculation of MBT from the lungs.

By the end of the experiment, on the 40th day from infection mortality in the control group (no treatment) was 70% among *M. tuberculosis H37Rv* cases and 40% among the strain 5582 cases. Moreover, both infection models have shown a sharp increase in the mass ratios of lungs and spleen, overall affectation of the lung tissue with areas of necrosis. In the cultures of lung homogenates the MBT were recorded to grow continuously.

Therapeutic effect of the CHX-EDTA-Zn complex was confirmed in comparison with 7 mg/kg of moxifloxacin (average dose), a well-known antituberculous drug, in both models of tuberculosis, i.e. in mice infected with the standard test strain *M. tuberculosis H37Rv* and the drug-resistant strain 5582. The complex showed protective action and effectively prevented death of mice regardless of the model of tuberculosis (see

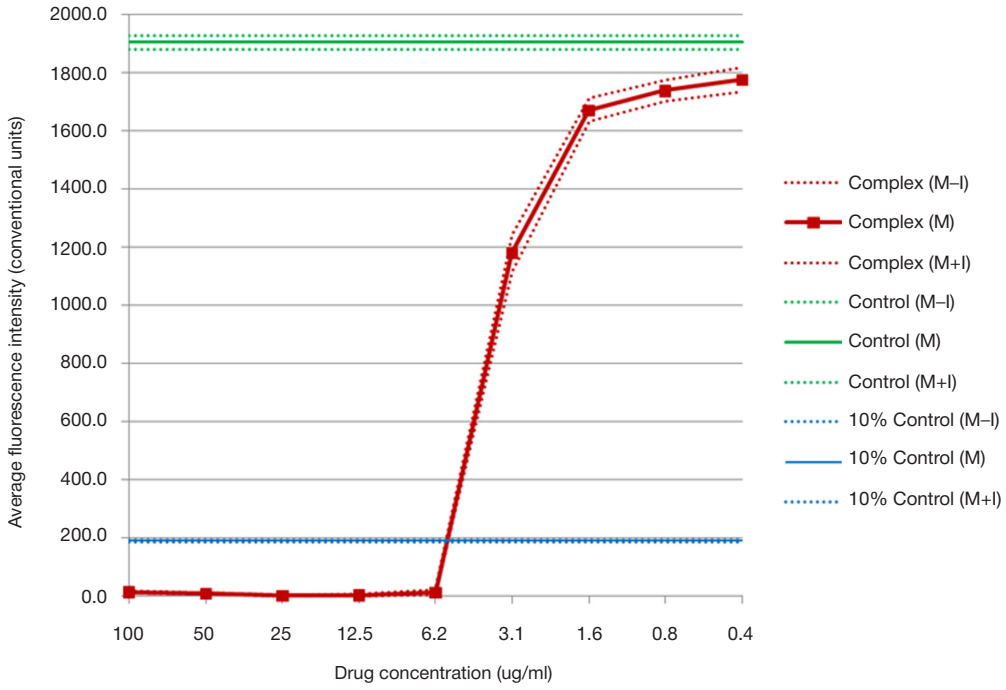

Fig. 1. Changes in the intensity of growth of the *M. tuberculosis H37Rv* test strain under the influence of the CHX-EDTA-Zn complex. M is the mean value, (M-I) and (M+I) are the lower and upper limits of the confidence interval (at α = 0.05)

Table 4. Tuberculosis infection severity in mice infected with *M. tuberculosis H37Rv* and infected with drug-resistant strain 5582 (after 5 weeks from the start of the treatment)

Group №	Drug, dose *per os*	Lethality, %	Mass coefficients, c.u.		Lung injury index, c.u.
			lungs	spleen	
M. tuberculosis H37Rv infection model					
1	Infection control (untreated)	70	2.13 ± 0.18	1.89 ± 0.19	3.25 ± 0.11
2	Moxifloxacin 7.0 mg/kg	0	1.12 ± 0.06 $p_{1-2} < 0.001$	0.83 ± 0.03 $p_{1-2} < 0.002$	2.45 ± 0.03 $p_{1-2} < 0.001$
3	CHX-EDTA-Zn 7.0 mg/kg	0	1.28 ± 0.08 $p_{1-3} < 0.002$	1.05 ± 0.07 $p_{1-3} < 0.002$	2.55 ± 0.08 $p_{1-3} < 0.001$
4	CHX-EDTA-Zn 14 mg/kg	0	1.24 ± 0.09 $p_{1-4} < 0.002$	1.01 ± 0.09 $p_{1-4} < 0.002$	2.52 ± 0.05 $p_{1-4} < 0.001$
Multidrug-resistant 5582 strain infection model					
5	Infection control (untreated)	40	1.13 ± 0.03	1.05 ± 0.04	2.92 ± 0.04
6	Moxifloxacin 7.0 mg/kg	0	0.98 ± 0.04 $p_{1-2} < 0.01$	0.69 ± 0.05 $p_{1-2} < 0.001$	2.30 ± 0.08 $p_{1-2} < 0.001$
7	CHX-EDTA-Zn 14.0 mg/kg	0	1.01 ± 0.03 $p_{1-3} < 0.05$	0.91 ± 0.05 $p_{1-3} < 0.05$. $p_{2-3} < 0.01$	2.48 ± 0.05 $p_{1-3} < 0.001$ $p_{2-3} < 0.$ 05

Table 4). In the cases with drug-susceptible *M. tuberculosis H37Rv*, the CHX-EDTA-Zn complex significantly reduced the values of all registered parameters compared to the control group. The effect from administration of the 14 mg/kg dose was significantly more pronounced than that produced by the dose of 7.0 mg/kg, i.e. it was dose-dependent. In terms of reducing lung damage, 14 mg/kg of the CHX-EDTA-Zn complex in both models of tuberculosis were almost as effective as 7 mg/kg of moxifloxacin, although the former was slightly inferior to the latter in terms of spleen parameters.

Results of investigation of virucidal activity Table 6 presents the results of assessing the potency of inhibition of viral reproduction expressed in $TCID_{50}$ units (50% tissue cytopathic infectious dose).

In both the suspension (mixing) and surface treatment tests, the solution of complex that contain of chlorhexidine 0.2%, inactivated the contaminating polio and human adenovirus in the course of 1–5 min.

DISCUSSION

It is known that EDTA forms a stable complex with the zinc cation (the $[Zn\ EDTA]^{2+}$ K_n instability constant is 3.2×10^{-17}) [11]. Preparation of a complex compound of chlorhexidine with EDTA has also been described [17]. However, more involved, three-component complexes including EDTA, zinc and chlorhexidine, have not been studied. As we assumed, in an aqueous medium, chlorhexidine bigluconate, EDTA disodium salt and zinc chloride can form a complex compound with the structure shown in Fig. 2. Indeed, MALDI-MS study revealed a signal with m/z 859.27 that corresponds to the protonated form (MH+) of the predicted structure. Fragmentation of this ion leads to the appearance of corresponding signals: m/z 567.2 (ion of protonated adduct of chlorhexidine and zinc), m/z 505.3 (ion of protonated chlorhexidine) and m/z 353.3 (ion of protonated EDTA-Zn complex), which unambiguously proved its structure. Apparently, the structure of this complex includes a central four-coordinated doubly charged zinc cation, a doubly charged chlorhexidinium cation and a four-charged anion of a completely deprotonated EDTA molecule. The rather high stability of this ternary complex should also be noted: it persists under the harsh conditions of laser-induced evaporation.

The stability of the ternary complex of chlorhexidine, EDTA and zinc can be explained by intramolecular salt formation, which makes cationic and anionic fragments in this system stoichiometrically balance each other, ultimately forming an electrically neutral molecule.

The inclusion of chlorhexidine in the strong ternary complex with EDTA and zinc translates into improvement of biocidal activity (see Table 1). The CHX-EDTA-Zn complex is 4–5 times

Table 5. Inoculation of MBT from the lungs of mice infected with *M. tuberculosis H37R* and strain 5582 after 5 weeks of treatment with the CHX-EDTA-Zn complex

Group №	Drug, dose per os	Number of MBT colonies in the lungs, CFU × 10^3	Number of viable mycobacteria in the lungs, lg
M. tuberculosis H37Rv infection			
1	Infection control (untreated)	129.6 ± 4.3	5.11 ± 0.01
2	Moxifloxacin 7.0 mg/kg	24.6 ± 4.6 $p_{1-2} < 0.001$	4.36 ± 0.09 $p_{1-2} < 0.001$
3	CHX-EDTA-Zn 7.0 mg/kg	41.99 ± 5.54 $p_{1-3} < 0.001$. $p_{2-3} < 0.05$	4.61 ± 0.05 $p_{1-3} < 0.001$. $p_{2-3} < 0.05$
4	CHX-EDTA-Zn 14 mg/kg	35.40 ± 3.19 $p_{1-4} < 0.001$	4.54 ± 0.05 $p_{1-4} < 0.001$
Multidrug-resistant 5582 strain infection			
6	Infection control (untreated)	18.39 ± 1.60	4.26 ± 0.04
7	Moxifloxacin 7.0 mg/kg	9.04 ± 1.57 $p_{1-2} < 0.01$	3.93 ± 0.07 $p_{1-2} < 0.01$
8	CHX-EDTA-Zn 14.0 mg/kg	11.48 ± 1.58 $p_{1-3} < 0.02$	4.04 ± 0.06 $p_{1-3} < 0.02$

Table 6. Virucidal activity of the CHX-EDTA-Zn complex

Object	Treatment method	Treatment time, min	Virus reproduction rate drop, lg $TCID_{50}$	
			Polio	Adenovirus
Viral suspension	1 : 9 blend	5	3.5	4.0
Faux leather	Swab	1	4.0	4.0
		2 × 1.5	5.0	4.5
Latex	Swab	3	4.0	4.0
Metal	Swab	5	4.7	4.5
Glass	Swab	5	5.0	4.3
Plastic	Swab	5	4.3	4.3

more active against the studied bacterial and fungal strains than chlorhexidine bigluconate.

In vitro, the chlorhexidine complex also demonstrates high efficiency against the main avian pathogens (see Table 2), and in vivo it proved effective against dermatitis in domestic and farm animals — dogs, cats, rabbits and sheep (see Table 3). It can be concluded that the CHX-EDTA-Zn complex can be an effective medicine for treatment of bacterial and fungal skin diseases of various etiologies, healing of scratches, cracks, burns, infected wounds and pyoderma.

As our experiments show, the CHX-EDTA-Zn complex exhibits a significant anti-tuberculosis activity, which is quite unexpected, since chlorhexidine alone is very weak against *Mycobacterium tuberculosis* (MBT). In *in vitro* experiments, the activity (MIC) of the complex compared applied to the standard *M. tuberculosis H37Rv* strain (see Figure 1) is 6.2 μg/ml, and against the drug-resistant strain 5582 the activity is 2 times higher (MIC 3.1 μg/ml). In other words, the complex is twice as effective against the drug-resistant strain than the standard tuberculosis strain.

Even more surprising is the antituberculous activity of the CHX-EDTA-Zn complex established *in vivo*, when it was administered orally to mice, although it is known that chlorhexidine alone is practically not absorbed from the gastrointestinal tract. The reliability of the therapeutic effect of the drug is confirmed in two models of tuberculosis, based on animal survival data, on the results of assessment of the physiological parameters (see Table 4) and data on the MBT inoculation from the lungs (see Table 5). The complex is inferior to the moxifloxacin when used against the drug-sensitive strain *M. tuberculosis H37Rv* and almost as potent against the drug-resistant strain.

The results obtained allow considering the CHX-EDTA-Zn complex not only as a disinfectant, but also as a potential anti-tuberculosis drug that can be used as an adjuvant in the treatment of drug-resistant forms of tuberculosis.

The biocidal properties of the CHX-EDTA-Zn complex cover not only bacteria but also viruses (see Table 6). The results obtained indicate that the complex can be used to disinfect objects contaminated with these viruses.

CONCLUSIONS

The three-component complex of chlorhexidine, EDTA and zinc has a pronounced biocidal effect against pathogenic bacteria, fungi, *Mycobacterium tuberculosis* and viruses. It significantly exceeds chlorhexidine in terms of level of biocidal activity and the breadth of range of action. Overall, the chlorhexidine-EDTA-Zn complex appears to be a promising disinfectant that can be used to combat the spread of dangerous bacterial and viral infections. On models of experimental tuberculosis, when administered systemically, the CHX-EDTA-Zn complex exhibits a therapeutic effect comparable to that of moxifloxacin. This allows recommending this complex for further study as a possible treatment for drug-resistant forms of tuberculosis.

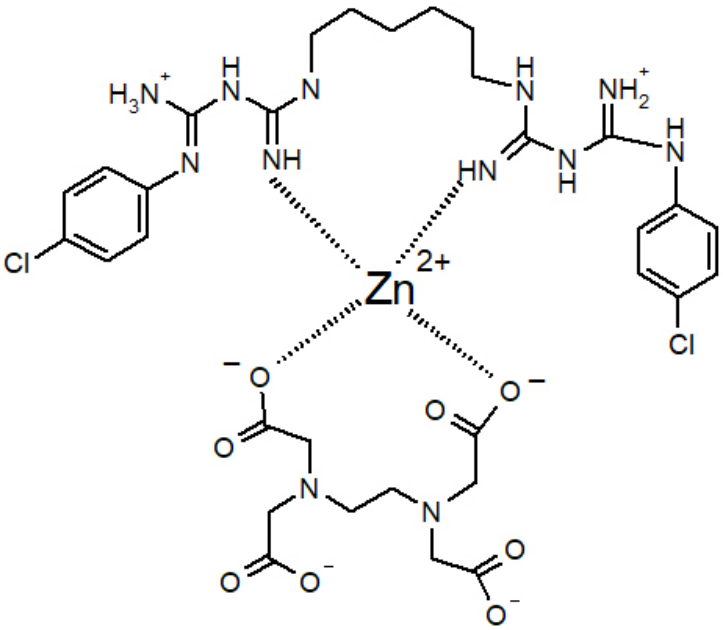

Fig. 2. Structural formula of the chlorhexidine-EDTA-zinc complex

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