EXPERIMENTAL EVALUATION OF THE CHEMOTHERAPEUTIC EFFICACY AND TOXICITY OF A NEW MEDICINAL FORM OF DIOXIDINE: LIDOXYCOL OINTMENT

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Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 42, No. 7, pp. 34 – 37, July, 2008.

Original article submitted June 4, 2007.

The antibacterial activity *in vitro* and *in vivo* and the toxicity of Lidoxycol ointment, which is a new medicinal form of dioxidine, have been studied in comparison to the properties of Dioxycol ointment. The results showed that Lidoxycol is identical to Dioxycol with respect to the spectrum of antimicrobial action, chemotherapeutic efficiency, and tolerance.

The preparation Dioxidine has been used for more than 25 years in Russian clinics to treat various forms of purulent infection. It is sold in forms for local application and for injection into cavities and veins [1 – 4].

Dioxidine is a preparation with bactericidal action that is based on disruption of DNA synthesis in microbial cells with severe structural damage to the nucleoid even at sub-inhibitory concentrations. A feature of dioxidine as an antimicrobial agent is the lack of a correlation of *in vitro* effects (determined under aerobic conditions) and those in an infected organism.

The high antibacterial activity of dioxidine, the broad spectrum of action that includes activity against anaerobic strains, and the effectiveness in the clinic for serious forms of purulent infections are combined with several undesirable toxicological properties [5].

Because of the toxicological properties, dioxidine is countraindicated for individual nontolerance of the preparation, insufficient adrenal functioning, pregnancy, and breastfeeding. Dioxidine is also prohibited for systemic use in pediatric practice, especially for infants and newborns and cannot be justified, considering the low therapeutic effect, the high risk of overdosing, and the harmful action on the adrenals.

Russian researchers have clearly defined indications for dioxidine: infection caused by gram-negative bacteria, ineffectiveness of prior antibacterial therapy, and treatment of infection after cardiac by-pass surgery and coronary stent implantation with artificial circulation. The preparation is used primarily for injection into cavities, endobronchially, or by inhalation as solutions and locally as ointments.

The low therapeutic effect of dioxidine requires strict adherence of dosing not only for i.v. administration but also locally, especially for injection into cavities, prolonged constant irrigation of wounds, and application of ointments to large burn areas.

Clinical use found that Dioxycol ointment, which contains dioxidine in combination with trimecaine and methyluracil, is highly effective. The dioxidine concentration in this ointment is reduced to 1%, which provides completely an antibacterial effect and simultaneously, upon application to large burns and wounded areas, reduces the risk of undesired reactions with possible absorption of dioxidine [6].

The new medicinal form of dioxidine, Lidoxycol ointment, which contains 1% dioxidine in combination with lidocaine and methyluracil in a polyethyleneoxide base, was developed at the Center for Drug Chemistry, All-Russia Research Institute of Pharmaceutical Chemistry [7].

The goal of our investigation was to study the antibacterial activity in *in vitro* and *in vivo* experiments and to compare the toxicity of Lidoxycol ointment with that of Dioxycol ointment sold by OSC NIZhFARM.

MATERIALS AND METHODS

The antimicrobial activity was studied in *in vitro* experiments against the following test strains of aerobic and anaerobic bacteria and fungi: *Staphylococcus aureus* ATCC 6538-P, *S. aureus* NCDC 25923; *S. Aureus* Gure, *S. aureus* Smith, *S. Aureus* 178, *Bacillus subtilis* ATCC 6633, *Esche-*

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TABLE 1. Antibacterial Activity of Lidoxycol Ointment Compared to Dioxycol Ointment *in vitro* for Aerobic Bacteria

Microorganism strain	MIC, %	
	Lidoxycol	Dioxycol
S. aureus ATCC 6533-P	0.12	0.12
S. aureus NCDC 25923	0.12	0.12
S. aureus 178	0.25	0.25
S. aureus Gyre	0.12	0.12
S. aureus Smith	0.12	0.12
B. subtilis ATCC 6633	0.06	0.06
E. coli NCDC 25922	0.03	0.03
E. coli M-17	0.015	0.015
P. vulgaris ATCC 6896	0.06	0.06
P. vulgaris NCDC 4636	0.12	0.12
P. aeruginosa NCDC 27853	0.12	0.12
P. aeruginosa 165	0.06	0.06
C. albicans 885 – 653	0.5	0.5

TABLE 2. Antibacterial Activity of Lidoxycol Ointment Compared to Dioxycol Ointment *in vitro* for Anaerobic Bacteria

Microorganism strain	MIC, %	
	Lidoxycol	Dioxycol
B. fragilis 323	0.015	0.015
B. melaninogenicus 9337	0.03	0.03
B. distasonis 255/82	0.03	0.03
B. asaccharolyticus 1092	0.03	0.03
B. asaccharolyticus 17	0.03	0.03
Peptostreptococcus anaerobius 891	0.06	0.06
Peptococcus asaccharolyticus 416	0.06	0.06
F. nucleatum 143	0.06	0.06
F. necroforum 22	0.06	0.06
Cl. perfringens t. A 88	0.12	0.12
Cl. septicum 286	0.06	0.06

richia coli NCDC 25922, *E. coli* M-17, *Pseudomonas aeruginosa* NCDC 27853, *P. aeruginosa* 165, *Proteus vulgaris* ATCC 6896, *P. vulgaris* NCDC 4636, *Candida albicans* 885 – 653, *Bacteroides fragilis* 323, *B. distasonis* 255/82, *B. melaninogenicus* 9337, *B. asaccharolyticus* 1092, *B. asaccharolyticus* 17, *Fusobacterium nucleatum* 143, *F. necroforum* 22, *Peptostreptococcus anaerobius* 891, *Peptococcus asaccharolyticus* 416, *Clostridium perfringens* t. A 88, and *C. septicum* 286. The strains were obtained from L. A. Tarasevich Institute of Standardization and Control of Medical and Biological Preparations.

The antibacterial activity against aerobic bacteria was determined by double serial dilutions in Hottinger broth [8, 9]. The activity of the ointments was studied at concentrations from 0.5 to 0.007%. Bacteria were prepared according to a turbidity standard in physiological saline and were innoculated into tubes with dilutions. The microbial loading was 1×10^6 CFU/mL. The bacteria were incubated at 37°C for 18 – 24 h. The activity of the preparations was calculated visually from the lack of visible growth. The minimal inhibiting concentrations (MIC, %) of the preparations were determined.

The activity of the preparations against anaerobic bacteria was studied by double serial dilutions in Schaedler anaerobe broth (Oxoid). The MIC values were determined. The microbial loading was 1×10^8 CFU/mL [10]. Bacteria were incubated at 35°C for 48 h. Anaerobic conditions were created by placing the samples in an anaerobic station (Jouan) filled with a mixture of CO_2 (10%), H_2 (10%), and N_2 (80%). The results were calculated after 48 h of incubation.

The chemotherapeutic activity in *in vivo* experiments was studied using a model localized purulent-necrosis process (s.c. infection) [10, 11]. We used strains *S. aureus* Gure and *S. aureus* 178 (strain resistant to methicillin) and gram-negative *P. aeruginosa* 165 and *E. coli* 25922, obtained from the L. A. Tarasevich Institute of Standardization and Control of Medical and Biological Preparations.

We used white mongrel mice of both sexes (mass 15 – 16 g). A one-day culture of pathogen grown on Hottinger agar was used to infect the animals. The culture was injected as a suspension in isotonic saline s.c. in the peritoneum (shaving fur beforehand). The infecting dose (ID) was a single LD_{100}, for *P. aeruginosa*, 0.5×10^7 CFU/mL; for *E. coli*, $(1 – 3) \times 10^8$ CFU/mL; for *S. aureus*, 8×10^8 CFU/mL. The infecting dose was injected in 0.05 – 0.1 mL. The purulent-necrosis process developed 24 – 48 h after infection at the site of injection. The nature and extent of the infections were designated arbitrarily as follows:

1. +, first stage of infection, pinpoint and small necroses up to 2 mm in diameter and seepages not associated with the necrotic process;
2. ++, second stage of infection, necroses from 3 to 5 mm in diameter;
3. +++, third stage of infection, necroses from 6 mm to more in diameter;
4. —, no infections.

Depending on the size and extent of the infection, purulent—necrotic sites healed 10 days after infection. The average index of infection per mouse in each group of animals was derived by summing the infection indices of each mouse during the whole observation period. An infection denoted + was taken as the unit. The effectiveness of the tested preparations considered 1) the number of animals for which the purulent—necrosis process did not develop and 2) a comparison of the extent of infections in groups of treated and control animals.

The observation of test animals was continued until complete liquidation of the process in the control group (average 12 – 15 days) for the chemotherapeutic test using these models.

Lidoxycol and Dioxycol ointments were applied daily to mice at infected sites for 10 days.

TABLE 3. Chemotherapeutic Activity of Ointments Lidoxycol and Dioxycol for a Model Purulent-Necrosis Process in Mice Induced by Various Strains

Infection – necrosis strain	Lidoxycol		Dioxycol		Control	
	Infection index, ball	TE, %	Infection index, ball	TE, %	Infection index, ball	TE, %
S. aureus Gyre	12.4	53	13.6	48	26.1	–
S. aureus 178	105	57	9.9	58	22.3	–
E. coli	11.6	52	12.1	49	23.8	–
P. aeruginosa	11.8	53	12.3	51	25.2	–

The toxicity of Lidoxycol ointment was studied relative to that of Dioxycol in experiments on rats. Ointment was applied to rats for one month on depilated wounded spinal skin at a dose of 4.0 g/kg. This dose was greater than the recommended therapeutic daily human dose by about 18 times. Hematological, biochemical, and pathological studies were carried out after the experiment was finished.

RESULTS AND DISCUSSION

The *in vitro* results found that Lidoxycol ointment, like Dioxycol, is active against aerobic bacteria. Both ointments were most active against gram-negative bacteria including the two strains of *E. coli* 25922 and M-17 (MIC was 0.015 and 0.03%, respectively) (Table 1). Strains *P. aeruginosa* NCDC 27853 and 165 were more resistant (MIC varied from 0.06 to 0.12%). The activity of both ointments against the two *P. vulgaris* strains was from 0.06 to 0.12%; *B. subtilis*, 0.06%. The activity of the ointments against gram-positive bacteria was found for five strains of *S. aureus* including ATCC 6533-P, NCDC 25923, Gure, and Smith at a concentration of 0.12%; for semi-resistant strain *S. aureus* 178, 0.25%. They were least active against *C. albicans* with a MIC of 0.5%.

TABLE 2 gives the results for the antibacterial activity of Lidoxycol and Dioxycol ointments against obligate anaerobic bacteria.

TABLE 2 shows that Lidoxycol and Dioxycol ointments are highly active against a broad spectrum of obligate anaerobes that do and do not form spores (nonclostridial). The ointments were most active against bacteroids. The MIC against *B. fragilis*, *B. melaninogenicos*, and *B. distasonis* and two strains of *B. asaccharolyticus* varied from 0.015 to 0.03%. Lidoxycol and Dioxycol inhibited growth of nonclostridial anaerobic bacteria *P. anaerobius* and *P. asaccharolyticus* and two strains of *Fusobacterium* at a concentration of 0.06%. The activities of the ointments against strains of clostridia were 0.06% (*C. septicum*) and 0.12% (*C. perfringens*).

The chemotherapeutic effectiveness of Lidoxycol and Dioxycol ointments was compared in *in vivo* tests using a localized purulent—necrosis process model in white mice that was induced by s.c. administration of *S. aureus* 178 and *S. aureus* Gure and gram-negative bacteria *E. coli* and *P. aeruginosa* on the background of a serious purulent—necrotic process. Treatment began on the third day after infection and was carried out daily for 10 days. Table 3 gives the results of the *in vivo* experimental study.

The process developed rapidly in animals infected with *S. aureus* Gure. The maximum size of third-stage infections reached up to 1 cm in diameter after 48 h. Table 3 shows that both ointments were highly effective. The effectiveness of Lidoxycol ointment was 53%; of Dioxycol, 48% for a serious purulent—necrotic process (infection index in the control, 26.1).

The effectiveness of Lidoxycol ointment increased up to 57%; of Dioxycol ointment, 58% for infection of animals with *S. aureus* 178 (semi-resistant strain, resistant to methicillin). The high activity of the ointments against a resistant strain of *S. aureus* provides a basis for recommending these preparations for resistance or intolerance to antibiotics.

Both ointments had a distinct chemotherapeutic effect on purulent processes caused by *P. aeruginosa* and *E. coli*. The therapeutic effectiveness coefficient was 49 – 52% for a purulent process caused by *E. coli*; 51 – 53%, for *P. aeruginosa* infection.

Thus, the investigations found that Lidoxycol and Dioxycol ointments typically have a broad antibacterial spectrum against gram-positive and gram-negative aerobic and anaerobic bacteria with the highest activity primarily against obligate anaerobes (forming and not forming spores). The spectrum of antibacterial activity of Lidoxycol ointment was identical to that of Dioxycol ointment.

A comparison of the chemotherapeutic activity of Lidoxycol and Dioxycol ointments showed that both medicinal forms had a distinct effect on purulent—necrotic process models caused by various pathogens. The therapeutic effectiveness coefficient (TE) reached 48 – 53%. The activity of one ointment or another reached 57 – 58% for a purulent—necrotic process model caused by *S. aureus* strain 178, which is resistant to methicillin.

A comparison of the general toxicity of Lidoxycol and Dioxycol ointments showed that application of both preparations for one month at a dose of 4.0 g/kg did not cause death of animals and did not affect their general condition and behavior. Clinical investigations carried out after the experiment was finished showed that the homeostatic condition of the test animals did not undergo noticeable changes com-

pared with the control. The hematological and biochemical blood indices were within physiological norms. A pathological investigation did not find traces of damage by the preparations on the structure of internal organs. Visual inspection of the skin of test animals also did not reveal any changes (reddening, exfoliation, loss of elasticity) that would indicate the presence of local irritation by the preparations. Only traces of wounding on control and test rats were observed at the site of action on the skin.

Histological studies of the skin of rats also were consistent with a lack of local irritating action of the compared ointments. Inflammatory infiltrations in the dermis and changes in the epidermis were not found. The preparations did not cause changes of the layer thickness and shifts in the degree of proliferative-desquamatory processes in the epidermis. The epidermis surface layer with signs of structural-functional activity was penetrated by gland excretions and fur fibers. Hair follicles and undamaged sweat and fat glands were located in the filamentary dermis.

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