SEARCH FOR NEW DRUGS

SYNTHESIS AND ANTISEPTIC AND ANTIDOTE ACTIVITY OF POLYHEXAMETHYLENEGUANIDINE HYDROXYETHYLIDENEDIPHOSPHONATE SALT

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The synthesis and characterization of the new drug polyhexamethyleneguanidine hydroxyethylidenediphosphonate salt are presented. Its bacteriological activity is comparable to that of previously obtained polyhexamethyleneguanidine salts and higher than that of already known chlorhexidine digluconate while its toxic effect is lower. A specific feature is that aqueous solutions of this drug help to eliminate heavy metals from warm-blooded animals. Thus, polyhexamethyleneguanidinium hydroxyethylidenediphosphonate can be used as a cleansing, antiseptic, and sterilizing agent for treating pyoinflammatory processes, purifying water in water-cycling systems, and resolving antidote problems.

Key words: polyhexamethyleneguanidine, polyhexamethyleneguanidinium hydroxyethylidenediphosphonate, antibacterial activity, antidote properties

It has been shown that polyhexamethyleneguanidine (PHMG) salts are highly effective antimicrobial agents [1].

The present work addresses the synthesis and characterization of a new compound, PHMG hydroxyethylidenediphosphonate (PGHP), which is the salt of PHMG and hydroxyethylidenediphosphonic acid (HEDP) [2].

EXPERIMENTAL PART

Preparation of PGHP. PHMG hydrochloride (16.1 g, 0.02 mol) was treated with NaOEt or NaOH (0.1 mol) in alcohol (150 mL). The precipitated NaCl was filtered off. The filtrate was treated with HEDP (20.6 g, 0.1 mol) dissolved in alcohol (180 mL). The solid PGHP was separated, washed, and dried. Yield 32.7 g (91%) [2].

PGHP was prepared by polycondensation of guanidinium carbonate with hexamethylenediamine in boiling xylene. After the release of ammonia stopped, the resulting PHMG was treated with HEDP in alcohol [3].

IR spectra of PGHP were recorded on a Specord IR-10 instrument (KBr, ν_{max}, cm^{-1}): 1040 (P-OH), 1250 (P=O), 1650 (=NH), 2860, 2920 (–CH_2–), 3220 (–NH–).

The antimicrobial activity of PGHP was studied using the Autosceptor bacteriological complex. The controls were chlorhexidine digluconate and PHMG. The test drug was used at concentrations from 2 to 0.00136%. Standard suspensions of bacteria test cultures (10^{10} CFU/mL) were prepared for the experiment. Test strains were innoculated into PDA nutrient medium and treated with aqueous solutions of PHMG salts at the appropriate concentrations. Growth of bacteria colonies in dense nutrient media was assessed after incubation for 42 – 48 h at 37°C.

Toxicological studies were performed on a large contingent of animals (>11,000) of various species (rats, guinea pigs, rabbits) of both sexes and various ages including newborns, adults, and older subjects according to requirements of the Pharmacological Committee and the GLP system.

The application area for the study of local irritation was 16 cm_2 for white rats; 25, guinea pigs; and 56, rabbits. The application area was 12% of the animal body surface area for the study of resorptive general toxicity. The effect on skin was assessed openly on previously shaved areas dosed per unit area and body mass of experimental animals. Control

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TABLE 1. Minimal Bacteriostatic Concentrations of Polyhexamethyleneguanidinium Hydroxyethylidenediphosphonate

Microorganism type	MBC, μg/mL		
	PGHP	PHMG phosphate	Chlorhexidine digluconate
Staphylococcus aureus	0.5	0.3	2.0
Proteus aeruginosa	10.8	6.2	25.0
Proteus vulgaris	1.6	0.6	15.0
Escherichia coli	1.2	0.09	10.0
Salmonella tiphi murium	19.0	15.0	40.0
Candida albicans	4.2	8.0	15.0
Serratia marcenscens	8.6	10.0	30.0

TABLE 2. Main Toxicity Parameters of PGHP for Topical Administration to Rats

Toxicity parameter	PGHP	Chlorhexidine digluconate
Mean lethal dose, mg/kg	13700	> 2500
Topical-oral coefficient (LD_{50}, *per os*, mg/kg)	8	4.4
	2200	1800
Accumulation coefficient	not accumulated	4.0
Age-difference sensitivity coefficient	1.8	2.0
Threshold of chronic general toxicity, mg/kg	80.0	20.8
Rate of transepidermal resorption, $\mu g/cm^2 \cdot h$	1.5	10.3

animals were kept under identical experimental conditions. The corresponding solvents for the test compounds (water, sunflower oil) were applied to the corresponding skin areas. The mean lethal doses were calculated by the Litchfield-Wilcoxon [4] and Kerber [5] methods. The ability of an organism to accumulate the studied compounds was studied by administering them at doses 1/2, 1/5, 1/10, and 1/50 of the LD_{50} as before [5]. The age-difference sensitivity coefficient, threshold of chronic general toxicity, and rate of transepidermal resorption were estimated according to methodical instructions "Establishing limiting allowable levels of skin contamination by chemical compounds" (1987) and "Toxicological-hygienic studies of polymeric materials and items from them" (1988) [6].

The antidote properties of PGHP with respect to heavy metals were studied by acute experiments. Experimental animals (rats) were injected once into the stomach with $HgCl_2$ at a dose 1/10 of the maximally tolerated, which was 0.4 mg/kg calculated as metal. PGHP was injected into the stomach two weeks after the mercury injection at doses 1/5 and 1/20 of LD_{50} (400 and 100 mg per animal). The Hg content was determined in urine, liver, and kidneys. The Hg content in urine and organs was determined 2, 4, 12, and 24 h after injection of PGHP.

It was found that the antibacterial activity of PHMG phosphate was practically the same as that of PGHP, which indicates that the acid used to form the salt had little effect (Table 1). However, the minimal bacteriostatic concentration (MBC) of PGHP was much lower than that of the known foreign antiseptic chlorhexidine digluconate. The PGHP aqueous solution even at 20°C had antiseptic activity for vegetative forms of bacteria, lipophilic viruses, and pathogenic microflora. The activity for microflora increased if the temperature of the PGHP aqueous solution was raised to 50°C or the pH of the medium was increased to 10 – 11. However, the probability of hydrolysis of guanidine groups increased simultaneously, which could with time lead to decreased antibacterial activity of PHMG salts. The aqueous solution of PGHP salt was stable with time and had a pH of 7. Use of PGHP salts as vehicles for biologically active compounds, e.g., antibiotics, through the membrane into the cell is just as important [6]. This property is based on the ability of polyguanidines to suppress the activity of enzymes that inactivate antibiotics and to increase the permeability of the cell wall and cytoplasmic membrane. This intensifies the activity of antibiotics toward resistant forms of bacteria and viruses and creates conditions that assist the antibiotic in finding its target within the cell.

PGHP was selectively toxic, i.e., was poison for microorganisms and slightly toxic for warm-blooded animals (Table 2). The results showed that PGHP did not have species, sex, and age sensitivity. An aqueous solution of the drug placed on skin was absorbed through undamaged tissue. However, the rate of transdermal resorption was slow because of the small distribution coefficient. Absorption occurred mainly in the first 5 min of contact. The PGHP solu-

TABLE 3. Mercury Content in Rat Urine and Organs after Administration of PGHP for 12 d

Mercury content	Group I, receiving Hg	Group II, receiving Hg + 1/5 LD_{50} PGHP		Group III, receiving Hg + 1/5 LD_{50} PGHP	
	$X \pm S_x$	$X \pm S_x$	P	$X \pm S_x$	P
Urine	0.045 ± 0.003	0.023 ± 0.001	< 0.02	0.021 ± 0.001	< 0.02
Liver	0.058 ± 0.014	0.032 ± 0.006	> 0.05	0.034 ± 0.004	> 0.05
Kidneys	0.420 ± 0.150	0.350 ± 0.006	< 0.01	0.270 ± 0.008	< 0.01

tion dried on the surface and formed a polymeric film that prevented further resorption of the antiseptic through the skin and made it less toxic than chlorhexidine digluconate. Thus, it can be concluded based on the aforementioned facts that PGHP is a promising and safe antiseptic.

Additional experimental studies of PGHP were performed in order to use PGHP as an antidote for eliminating heavy metals (in particular, $HgCl_2$) from animals and humans. The Hg content in biological tissues of animals was determined after decapitation. Table 3 lists the results. The data indicate that injection of aqueous solutions of PGHP to rats for 2 – 12 d led to an increased Hg content in urine that depended little on dose and, therefore, to elimination of the metal from the organism. The level of metal content decreased significantly also in internal organs (liver, kidneys). The results indicate that PGHP can bind metal and eliminate it from warm-blooded animals. This suggests that PGHP has antidote properties.

Thus, polyhexamethyleneguanidinium hydroxyethylidenediphosphonate can be used as a disinfectant, antiseptic, and sterilizing agent for treating pyoinflammatory processes, for purifying water in water-cycling systems, and for resolving antidote problems.

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