ELIMINATION OF R-FACTOR AND INHIBITION OF EPISOMAL DRUG RESISTANCE IN ENTEROBACTERIACEAE BY ANTIHISTAMINES

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Experiments in vitro showed that chlorpromazine, promethazine diphenhydramine, and chlorpyramine can irreversibly eliminate and inhibit episomal drug resistance in Enterobacteriaceae carrying the R-factor. The eliminating and inhibitory effects of the antihistamines depend on individual properties of the strains, the type of determinants incorporated into the R-factor, the drug itself, and the duration of contact of the bacteria with it. The presence of 2,4-dinitrophenol, an inhibitor of oxidative phosphorylation, in the medium increases the sensitivity of the tetracycline-resistant strain of E. coli to this antibiotic.

Recently another method of dealing with drug-resistant microorganisms has been discovered with the introduction into medical practice of substances preventing the transmission of extrachromosomal determinants of resistance from drug-resistant strains to sensitive during genetic exchange, and also of substances capable of eliminating and inhibiting episomal resistance in those microorganisms which already carry it.

The object of the present investigation was to study, in vitro, the possibility of eliminating R-factor and of inhibiting the expression of the R-determinants incorporated into it in Enterobacteriaceae under the influence of antihistamine drugs.

EXPERIMENTAL METHOD

Seven strains of Enterobacteriacea were used in the investigation (Escherichia coli M-17, E. coli K-12, Shigella flexneri 170, 2a, Sh. sonnei 8062, Salmonella typhi 4446, and S. typhimurium 79, obtained from the L. A. Tarasevich State Control Institute, and S. london 6968, obtained from the Saratov Regional Bactiological Laboratory). In preliminary experiments on these strains the identical R_1-factor, determining resistance to tetracycline and chloramphenicol, was transferred to them by the conjugation method. Besides the above strains, M-17 to which episomal resistance to monomycin, kanamycin, and ampicillin (R_2) was transferred in analogous experiments, also was used.

To abolish drug resistance, 24-h cultures of the microorganisms grown in the presence of maximal concentrations of antibiotics permitting growth of the bacteria were diluted with physiological saline and added (2×10^5 bacterial cells per ml medium) to tubes with Hottinger's broth (pH 7.4) containing serial dilutions of the antihistamine drugs. After incubation for 24 and 72 h at 37°C, seedings were taken from the tubes containing subbacteriostatic concentrations of these preparations and from control tubes (without the preparations) on agar plates in order to obtain isolated colonies. To detect bacteria which had lost their resistance to antibiotics, the growing colonies were transferred by the impression method to Endo's medium containing tetracycline (30 μg/ml), chloramphenicol (25 μg/ml), monomycin (50 units/ml), kanamycin (50 μg/ml), or ampicillin (50 μg/ml). The results were read 24 h later. Altogether 100 colonies of each strain, selected arbitrarily, were studied.

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TABLE 1. Effect of Chlorpromazine and Chlorpyramine on Level of Episomal Resistance of Enterobacteriaceae to Antibiotics

Strain of Enterobacteriaceae	Antibiotic	Level of resistance to antibiotics (in μg/ml)			Strain of Enterobacteriaceae	Antibiotic	Level of resistance to antibiotics (in μg/ml)		
		in absence of antihistamines	in presence of				in absence of antihistamines	in presence of	
			chlorpromazine	chlorpyramine				chlorpromazine	chlorpyramine
M-17R_1	Tc	100	25	50	170R_1	Cm	150	31	150
	Cm	200	100	100		Tc	250	31	50
	Mo*	5 000	1 250	1 250	8062R_1	Cm	150	150	31
M-17R_2	Ka	12 500	6 300	6 300		Tc	125	7,5	50
	Amp	1 250	4	4	4446R_1	Cm	150	15	31
	Tc	125	7,5	7,5		Tc	250	125	50
K-12R_1	Cm	150	15	75	79R_1	Cm	300	150	75
	Tc	125	3,7	63		Tc	250	31	15
					6968R_1	Cm	300	150	31

Note: Antihistamines were used in half the minimal bacteriostatic concentrations.

*Tc) tetracyclines; Cm) chloramphenicol; Mo) monomycin (concentration given in units/ml); Ka) kanamycin; Amp) ampicillin.

The possibility of specific inhibition of episomal resistance was investigated by comparing sensitivity of the bacteria to antibiotics during growth (seeding dose 10^5 bacterial cells per ml) in nutrient medium (in Hottinger's broth for 24 h, in synthetic medium for 48 h) containing or not containing the antihistamines.

The synthetic medium contained the following components (in g): glucose 10.0, K_2HPO_4 7.0, KH_2PO_4 3.0, $MgSO_4 \cdot 7H_2O$ 0.1, $(NH_4)_2SO_4$ 1.0, Na citrate 0.5, and water 1000.0. Amino acids (valine, leucine, tryptophan, threonine, lysine, histidine, arginine, methionine, phenylalanine) were added to the medium in doses of 200 μg/ml, and aspartic acid in a dose of 1000 μg/ml.

Standard preparations of histamine and its antagonists: diphenhydramine [2-(benzohydryloxy)-N,N-dimethylethylamine hydrochloride], chlorpyramine (N-dimethylaminoethyl-N-p-chlorobenzyl-α-aminopyridine hydrochloride), and two phenothiazine derivatives: promethazine [N-(2-dimethylaminopropyl)-phenothiazine hydrochloride] and chlorpromazine [N-(3-dimethylaminopropyl)-2-chlorophenothiazine] were used in the investigation.

EXPERIMENTAL RESULTS

The experiments showed that chlorpromazine, promethazine, chlorpyramine, and diphenhydramine possess marked antibacterial activity against the Enterobacteriaceae; the minimal bacteriostatic concentrations of the drugs were 50-300, 75-150, 125-250, and 125-500 μg/ml respectively. The species of Shigella and S. typhi were most sensitive to the antihistamines. The antibacterial action of the antihistamines was exhibited both in Hottinger's broth and also, to a still greater degree, in synthetic medium, but it was reduced when certain amino acids and histamine were added. Histidine, leucine, and threonine showed the strongest protective properties under these conditions.

Cultivation of the Enterobacteriaceae in the presence of subbacteriostatic concentrations of the antihistamines led to the elimination of drug resistance from the microorganisms studied. The percentage of colonies which consistently lost the R-factor or certain determinants of resistance varied within wide limits (from 0 to 40) depending on individual properties of the strains, the type of determinants incorporated into the R-factor, the drug used, and the duration of contact of the bacteria with it. Loss of resistance to antibiotics was observed most frequently under the influence of chloropyramine, followed by promethazine and chlorpromazine; the eliminating effect of diphenhydramine was least marked. Reversion to sensitivity was observed most frequently with S. typhi and Sh. flexneri. In strain M-17 R_1^+, resistant to monomycin and ampicillin, the frequency of appearance of cells resistant to antibiotics was higher than in M-17 R_2^+, resistant to tetracycline and chloramphenicol. Among bacteria of the strain M-17 R_2^+ which had lost their resistance to antibiotics, segregants sensitive to ampicillin were most frequently found, and those sensitive to monomycin and kanamycin less frequently, although some colonies were found which were sensitive to all three antibiotics at the same time. Prolongation of contact between the Enterobacteriaceae and anti-

histamines from 24 to 72 h led to an increase in the number of colonies with stable loss of their resistance to antibiotics. Cultivation of the Enterobacteriaceae in the presence of histamine (500 μg/ml) affected neither the rate of survival of the microorganisms nor the stability of the R-factor. In control experiments in which no antihistamines were added to the nutrient media, no mutant colonies with loss of resistance to antibiotics were found.

In the experiments to study inhibition the concentrations of antihistamines used did not themselves have any significant effect on growth and reproduction of the antibiotic-resistant bacteria, but if they were added to a medium containing antibiotics to which the tested strains were resistant, they increased the sensitivity of the microorganisms to a certain extent to these antibiotics (Table 1). The inhibitory effect of the antihistamines on the level of resistance depended on the individual properties of the strains and the type of determinants controlling episomal resistance to the antibiotics. The strongest inhibitory action was exhibited against resistance to tetracycline and, in particular, to ampicillin. Inhibition of resistance to tetracycline and ampicillin by chlorpyramine in E. coli was increased in the synthetic medium and reduced (by 15-20 times) relative to ampicillin by the addition of aspartic acid and histidine to the medium. Inhibition of resistance to the antibiotics was reversible in character, for after the bacteria had been washed to remove the antihistamines they completely retained their original resistance to the antibiotics.

The diverse action of antihistamines on living organisms is linked with their ability to inhibit carbohydrate metabolism and, in particular, activity of apodiastases and dehydrogenases [1, 2]. For this reason the effect of 2,4-dinitrophenol (an inhibitor of oxidative phosphorylation) on the episomal resistance of E. coli M-17 R_1^+ was studied. The presence of this inhibitor in the medium (1000 μg/ml) was found to increase the sensitivity of this strain to tetracycline by 16 times. In no case were mutants found which permanently lost their R-factor under the influence of 2,4-dinitrophenol. These results suggest that the action of the antihistamines was not entirely due to their inhibition of oxidation.

Further research is required in order to finally solve the problem of the mechanism of elimination and inhibition of episomal resistance in bacteria under the influence of antihistamines.

LITERATURE CITED

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