ORIGINAL RESEARCH

In vitro antibacterial activity of some systemic and topical antihistaminic preparations

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Abstract

Purpose: *In vitro* antibacterial activity of topical and systemic antihistaminic preparations containing different active substrates against the standard strains of two bacteria was evaluated.

Methods: Four topical and 3 systemic preparations containing pheniramine maleate, chlorphenoxamine hydrochloride, and diphenhydramine hydrochloride were studied. The antibacterial activities of these preparations against strains of *S. aureus* (American Type Culture Collection, ATCC 29213) and *S. epidermidis* (ATCC 25212) were tested using the disc diffusion method. In addition, the Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of parenteral preparations for these two bacteria were determined.

Results: Pheniramine maleate-topical and pheniramine maleate-systemic had no activity against bacteria, but the others showed various rates of activity. Chlorphenoxamine hydrochloride-topical and chlorphenoxamine hydrochloride-systemic were the most effective ($P < 0.05$). Despite the same active substrate content, diphenhydramine hydrochloride-topical-1 and diphenhydramine hydrochloride-topical-2 yielded different results when they were compared with each other or with the other preparations. Diphenhydramine hydrochloride-topical-2 had a relatively higher rate of activity than diphenhydramine hydrochloride-topical-1. Inhibition zone diameters were 16.9 ± 1.5 mm 12.3 ± 0.5 mm for *S. aureus*, 17.4 ± 1.0 mm 0 mm for *S. epidermidis* respectively ($P < 0.05$).

MIC values of parenteral preparations were equal to or above 125 µg/ml.

Conclusion: MIC values of parenteral preparations were higher than their blood levels in clinical use. Thus, effects of parenteral preparations may not have been reflected in routine clinical practice. However, topical forms have antibacterial activity due to additive substrates and the use of high concentration levels at the site of application. Therefore, in selection of topical forms for appropriate cases, these effects should also be taken into consideration. The antibacterial activity of topical antihistaminic preparations may be useful in certain dermatological pathology.

Several pharmacological agents with various effects and indications were also found to have antibacterial activity.¹ Based on the findings of earlier studies, the number of these agents has increased, and they have been termed non-antibiotics.²⁻⁶ Some of these drugs can show their antibacterial effects at routine doses. However, sometimes these routine doses are lower than the antibacterial doses. When that is the case, their antibacterial activity cannot be seen in practical use, especially in systemic application when compared with their local and topical applications used for ophthalmic and dermatologic lesions. Another prob-

lem related to non-antibiotics is that antibacterial activity studies are performed with pure forms, but the pharmaceutical forms usually consist of additive substrates used as filling or protective agents. Non-antibiotics are manufactured considering their main effect instead of their antibacterial activity and, thus, the results obtained with their laboratory and commercial forms are not same. In addition, antibacterial activity of only the additive substrates in their certain pharmaceutical forms has been shown (e.g. ophthalmic pharmaceutical form).⁷ In light of this information, drugs must be investigated for their available pharmaceutical forms, but not for their pure active substrates alone to determine their antibacterial effects in routine practice.

Antihistaminic drugs were developed to prevent some pathophysiological events resulting from histamine. These drugs, in addition to their basic effects, have antiemetic, parasympatholytic anti-parkinson effects. Dastidar and coworkers demonstrated that antihistaminic preparations also had antibacterial activity.²⁻⁴ Antihistaminic preparations in topical and systemic forms are used in allergic reactions such as urticaria, sunburn, and bites. These reactions are usually reflected through pruritus, in which the dermal integrity is broken and microorganisms can enter easily via these entrances and lead to formation of infected lesions.

Four topical and three parenteral (systemic) formulations of three commercially available antihistaminic preparations were studied *in vitro* to determine whether they had any antibacterial activities against the standard strains of *S. aureus* and *S. epidermidis*, which were usually isolated from dermal infections.

Materials and Methods

Antibacterial activity of some commercially available drugs containing pheniramine maleate (T1: ointment, topical and P1: intravenous, parenteral), chlorphenoxamine hydrochloride (T2: ointment, topical and P2 intravenous, parenteral), and diphenhydramine hydro-

chloride (T3: ointment, topical-1; T4: ointment, topical-2 and P3 intravenous, parenteral) as active substrates was investigated *in vitro*. Although T3 and T4 have the same active substrate, their additive contents are different. While T3 contains zinc oxide, glycerine, sodium citrate, and alcohol, T4 contains zinc oxide, lidocaine, benzalkonium chloride, and alcohol. Mupirocin and ciprofloxacin were used in topical and parenteral preparations, respectively, to confirm the validity of the methodology. The antibacterial activities of these drugs against the strains of *S. aureus* (American Type Culture Collection, ATCC 29213) and *S. epidermidis* (ATCC 25212) were tested.

Sterile discs containing approximately 20-22 mg/disc amount of topical preparations and 10- μ l amount of parenteral preparations were prepared. Standard discs containing an active substrate (5 μ g/disc) was used for ciprofloxacin (Oxoid). The topical drugs were placed on one side of empty standard antibiogram discs using sensitive balance under aseptic conditions. For parenteral drugs, a sterile pipette and its tip were used. The bacteria were diluted to a density of 0.5 Mc Farland units (1.5×10^8 bacteria/ml) with sterile non-bacteriostatic 0.9% saline. Then, suspensions were adapted to Mueller Hinton agar (Oxoid). Seven discs containing each of the drugs were used for each bacterium. The discs with their surfaces down (the side containing the drug) were placed on the plates. The plates were incubated at 35°C, and after 16-24 hours, the inhibition zones were measured in millimeters⁸.

In addition, the Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of parenteral preparations for these two bacteria were determined based on CLSI M7-A6 protocol.⁹ Serial dilutions of P1, P2 and P3 (2000-31.25 μ g /ml) were prepared. Muller Hinton broth medium culture with cation was used. The first concentration at which growth inhibition was detected with visual assessment was considered MIC for these bacteria after incubation at 35 °C for 18 hours. Determination of the MBC

was defined as the concentration at which 99.9 % of growth inhibition was observed, and an aliquot from each well in the dilution series showing inhibition of visible bacterial growth was sub cultured onto sheep blood agar. Control cultures were performed for bacteria, broth, and drug dilutions.

Furthermore, the micro plates were measured at the wavelength of 450 nm using a micro plate reader (Micro Quant®, BioTek) before and after the incubation period during MIC determination. Each absorbance value obtained from the well was compared with the results of visual assessment.

The results were compared using Mann Whitney U test for statistical analysis. $p < 0.05$ was considered statistically significant. The data were presented as mean $\pm$ SD.

Results

Although each preparation contained different concentrations of the active substrate, the mean dosage of the topical drugs added to disc was approximately 20-22 mg and parenteral forms was 10 μ l (Table 1).

The inhibition zones of the drugs measured with disc diffusion method are shown in Table 2. The inhibition zones obtained with mupirocin and ciprofloxacin verified the methodology of the study, as expected.

Whereas T1 and P1 had no activity against the bacteria, the others showed activity at different rates, and T2 and P2 were found to be the most effective.

Several additives, especially lidocaine and benzalkonium chloride, the antibacterial activities of

TABLE 1. The amount of drugs added to discs (n:7; mean $\pm$ SD).

	<i>S. aureus</i>	<i>S. epidermidis</i>
Topical	Drug	Drug
T1	21.65 $\pm$ 1.48 mg	20.95 $\pm$ 1.52 mg
T2	20.19 $\pm$ 0.84 mg	20.36 $\pm$ 1.52 mg
T3	22.65 $\pm$ 1.24 mg	22.41 $\pm$ 1.82 mg
T4	21.54 $\pm$ 1.83 mg	22.96 $\pm$ 3.59 mg
Mupirocin	20.96 $\pm$ 2.37 mg	22.59 $\pm$ 3.00 mg
Parenteral		
P1	10 μ l	10 μ l
P2	10 μ l	10 μ l
P3	10 μ l	10 μ l

Pheniramine maleate (T1: topical, P1: parenteral form), chlorophenoxamine hydrochloride (T2, P2), diphenhydramine hydrochloride (T3, T4, P3)

which were shown earlier, changed the results, with the most significant difference between T3 and T4. Although T3 and T4 had the same active substrate, their topical preparations had different effects. The activity of T4 containing lidocaine and benzalkonium chloride was more prominent than the activity of T3 was. T3 showed less activity on *S. aureus* and no activity on *S. epidermidis*.

There are several additive substances in topical forms, but parenteral forms are relatively pure and thus, in this study, parenteral forms were used to determine the MIC and MBC values. The MIC value of P2 for *S. aureus* and *S. epidermidis* was 125 μ g/ml, and the MIC value of P3 for two bacteria was 1000 μ g/ml (Table 3). The MIC and MBC values of P2 and P3 were the same. Although the highest concentration used for P1 was 2000 μ g/ml, there was no visible in-

TABLE 2. The diameters of inhibition zones of topical and systemic antihistaminic preparations (n:7, mm, mean $\pm$ SD).

Topical	Mupirocin	T1	T2	T3	T4
<i>S. aureus</i>	36.5 $\pm$ 2.9	0	19.2 $\pm$ 2.1*	12.3 $\pm$ 0.5	16.9 $\pm$ 1.5**
<i>S. epidermidis</i>	41.5 $\pm$ 1.4	0	18.6 $\pm$ 1.8	0	17.4 $\pm$ 1.0
Parenteral	ciprofloxacin	P1	P2	P3	
<i>S. aureus</i>	25.0 $\pm$ 0	0	14.0 $\pm$ 0	8.0 $\pm$ 0	
<i>S. epidermidis</i>	26.0 $\pm$ 0	0	20.0 $\pm$ 0	8.0 $\pm$ 0	

Pheniramine maleate (T1: topical, P1: parenteral form), chlorophenoxamine hydrochloride (T2, P2), diphenhydramine hydrochloride (T3, T4, P3)

*Significantly higher than the values of T3 and T4 ($P < 0.05$)

**Significantly higher than the value of T3 ($P < 0.05$)

TABLE 3. Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) values of parenteral forms ($\mu\text{g/ml}$).

	P1		P2		P3	
	MIC	MBC	MIC	MBC	MIC	MBC
<i>S. aureus</i>	>2000	>2000	125	125	1000	1000
<i>S. epidermidis</i>	>2000	>2000	125	125	1000	1000

Pheniramine maleate (P1: parenteral form), chlorphenoxamine hydrochloride (P2), diphenhydramine hydrochloride (P3)

hibition. However, a decrease was observed in measured absorbance value of micro plates at 450 nm (Figure 1). This result suggests that P1 might have the MIC and MBC values above 2000 $\mu\text{g/ml}$. Besides, the absorbance values at the wavelength of 450 nm for each well were concordant with visual evaluation and MIC values.

Discussion

Antibacterial activity of drugs containing three different antihistamines, which are used topically and systemically, was investigated. It was found that T1 had no activity by disc diffusion method, and the MIC value of P1 was undetermined. T2 and P2 showed the

highest rate of activity with regard to inhibitions zones and MIC values (Table 2 and 3). T3 and T4 yielded different results from those of one another and the other preparations. The preparation containing benzalkonium chloride and lidocaine (T4) showed higher rate of activity, but T3 had lower rate of activity on *S. aureus* and no activity on *S. epidermidis* (Table 2). The MIC values of the parenteral forms were fairly high (Table 3). The MIC values obtained from parenteral forms were higher than the therapeutic blood values of routine usage. Thus, antibacterial activity was not expected with parenteral forms. However, it is possible to observe an antibacterial effect with topical forms in clinical practice. The diameters of the inhibition zones measured for T2, T3 and T4 indicate the antibacterial activities of these preparations *in vitro*. However, whether they will show antibacterial activities in clinical applications remains to be investigated by clinical studies.

The antibacterial activity of diphenhydramine hydrochloride has been shown. However, literature reveals no information on the antibacterial activities of the other two antihistamines we tested. In addition,

FIGURE 1. Absorbance values of bacteria at different drug concentrations after incubation Pheniramine maleate (P1: parenteral form), chlorphenoxamine hydrochloride (P2), diphenhydramine hydrochloride (P3)

there are no studies evaluating the antibacterial effects of commercially available forms of these drugs. In this study, the efficiency of some antihistaminic preparations, which are commercially available in Turkey, was investigated. In different countries, even if the preparations contain the same antihistaminics, the preparations may vary in concentrations as well as additives; thus, the antibacterial efficiency of available preparations should be tested individually in each country.

Several non-antibiotic pharmacologic agents have been shown to have antibacterial activity. Antibacterial activity of some antihistamines (e.g. methdilazine and trimeprazine) has also been shown.^{2,10,11} In clinical practice, with the use of pharmaceutical forms and original indications, the antibacterial activity of antihistamines may be altered by additives. In our study, this difference was observed prominently with T3 and T4. The preparation containing lidocaine and benzalkonium chloride had a higher rate of antibacterial activity than without such additives. Lidocaine has been reported to possess antibacterial activity, as in our study.¹²⁻¹⁸ Our aim was not to show the possible use of these preparations as antibiotics but, rather, to investigate whether they had any antibacterial effects and if so, to emphasize that these preparations may provide protective effects in pathology for which they are used as antihistamines. Topical and systemic antihistaminic preparations are used in allergic reactions such as urticaria, sunburn, and bites. These reactions are often accompanied by pruritus, in which the dermal integrity is broken and microorganisms can easily enter via these entrances. The lesions with pruritus become infected lesions. Antibacterial activity of topical antihistamines may be useful in these dermatologic pathologies by preventing development of such secondary infections.

Parenteral forms are not expected to have any antibacterial activity in routine use; however, topical forms may have shown an antibacterial activity at the site of application with the use of their high concentrations and with their additive substrates. Therefore,

in selection process of the preparations for treatment, awareness of the antibacterial activities of these preparations might be helpful.

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