International Journal of Pharmaceutics, 43 (1988) 77–81
Elsevier

IJP 01447

Potentiation of oxytetracycline antimicrobial activity in vivo by concurrent administration of bromhexine

Jaspal S. Malhi, Anita-Jane High, Geoffrey W. Hanlon, Paul R. Gard and Christopher Marriott

Pharmaceutical Sciences Research Group, Department of Pharmacy, Brighton Polytechnic, Brighton (U.K.)

(Received 9 October 1987)
(Accepted 23 October 1987)

Key words: Oxytetracycline; Bromhexine; Antibiotic; Mucolytic; Vaginal microflora

Summary

Female virgin guinea pigs were used to assess the effects of oxytetracycline hydrochloride (OTC) alone and in combination with bromhexine hydrochloride (BHC) on vaginal microflora. No synergistic activity exists between the two compounds but the number of surviving organisms was significantly lower in the group receiving OTC + BHC than in the group receiving OTC alone (t-test, $P < 0.005$). It is known that OTC induces greater mucus structure by binding mucus glycoproteins and it is suggested that BHC is able to reverse this effect and thus allow enhanced OTC penetration into genital tract secretions.

Introduction

The tetracyclines are a group of broad spectrum, bacteriostatic antibiotics which are employed clinically for the treatment of respiratory, genito-urinary and streptococcal or staphylococcal infections due to penicillin-resistant microorganisms. In many cases microorganisms are embedded within mucus secretions which provide a diffusional barrier to the antibiotic (Kearney and Marriott, 1987). Tetracyclines have been shown to exert a post-secretory mucospissic effect on bronchial mucus by binding to mucus glycoproteins (Marriott and Kellaway, 1975) and this acts to decrease the diffusion rate of the antibiotic through mucus gels.

Several authors have examined the penetration of tetracyclines into sputum following oral administration and the mean sputum concentration was normally 20% of that obtained in the serum. Following a 1 g dose of oxytetracycline (OTC) this would result in a concentration high enough to kill most sensitive pathogens such as pneumococcus and *Haemophilus influenzae* but any bacteria with a higher resistance would survive.

Mucolytic drugs, which are agents that destroy or lessen the tenacity of mucus, are a diverse range of compounds whose actions are still poorly understood. Bromhexine hydrochloride (BHC) has been reported to have a direct effect on the mucus-producing glands lining the respiratory tract leading to the production of mucus with reduced viscosity (Burgi and Makin, 1971).

Correspondence: J.S. Malhi, Pharmaceutical Sciences Research Group, Department of Pharmacy, Brighton Polytechnic, Moulsecoomb, Brighton BN2 4GJ, U.K.

A limited number of studies have shown that the concurrent administration of mucolytic and tetracycline has resulted in an increase in the concentration of antibiotic within the mucus secretions of the respiratory tract. In patients suffering from chronic bronchitis it was observed that BHC increased the OTC concentration in sputum by approximately 280% after oral administration (Bach and Leary, 1972). Bronchial secretions removed from piglets treated with BHC and OTC concurrently contained up to 65% more antibiotic than those obtained from control animals treated with OTC alone (Abdou and Kotzian, 1975).

However, very little is known about the action of mucolytic agents at sites of the body other than the respiratory tract. This is primarily due to difficulties encountered in obtaining sufficient quantities of mucus on which to perform rheological analysis. The first evidence to suggest that mucolytics do exert an effect on cervical mucus was reported by Malhi et al. (1986), who used the guinea pig as a mammalian model to show that the administration of mucolytic drugs compromised the integrity of the cervical mucus plug and allowed vaginal bacteria to invade the otherwise sterile uterus.

In this study we have used the female guinea pig to examine the effect of concurrent administration of BHC and OTC on vaginal microflora. The vagina is colonised by a wide variety of organisms constituting a micro-ecosystem associated with vaginal secretions and the rate at which they are killed following oral administration of an antibiotic will be determined by the concentration attained within these mucus secretions. This technique can be used indirectly to examine the effect of compounds such as mucolytics which may modify the concentrations of antibiotics achieved in the genital tract and its secretions.

Materials and Methods

Animals

Female Dunkin–Hartley guinea pigs (300–500 g) housed on wire-bottomed cages (North Kent Plastic, Dartford, Kent) were used for all experiments.

Quantitative estimation of vaginal microflora

Vaginal lavage was performed regularly using 2 ml of pre-warmed sterile, quarter-strength Ringer solution for each animal by repeatedly aspirating with 0.5 ml aliquots. The resultant suspension was diluted appropriately and 0.2 ml aliquots were plated onto nutrient agar and incubated aerobically at 37 °C for up to 48 h. After incubation the number of colony-forming units (cfu) per millilitre of the original wash solution was determined and the predominant microorganisms were isolated and identified using standard techniques (Cowan, 1974). At least 48 h were left between subsequent sampling of each animal in order to allow the microbial population to return to normal.

Sensitivity of isolated microflora to OTC

0.2 ml sterile double-strength broth was added to each well of a microtitre plate as well as 0.1 ml of a suspension of test organisms of a standardised optical density. To this mixture 0.1 ml of the appropriate dilution of a serially diluted OTC solution were added and the whole procedure was carried out in duplicate. Control determinations in the absence of any antimicrobial agents were also performed. The microtitre plate was then incubated at 37 °C for 24 h. At the end of the incubation period, each well was examined for bacterial growth.

Drug treatment

Twenty-eight animals were divided into two groups. The first (group A) received BHC for 7 days (phase I) followed by 7 days treatment with BHC concurrently with OTC (phase II). The second (group B) received vehicle control for 7 days (phase I) followed by 7 days treatment with OTC alone (phase II). Both groups were then given a 7-day drug-free period (phase III). The vaginal microflora was assessed regularly throughout the 21-day period, vaginal lavage being performed twice during each period. Drugs were administered orally via the diet, both BHC and OTC were administered at an approximate dose of 30 mg/kg/day.

Interaction between OTC and BHC

1 ml of an overnight culture of *E. coli* NCIB 8916 was mixed in 250 ml sterile molten DST agar and poured into a 23 × 23 cm assay plate. When set, 36 wells were cut using an 8 mm flamed and cooled cork borer. The wells were filled in a Latin square pattern with the following solutions buffered to pH 7.0:

(1) Oxytetracycline (OTC, Sigma, Poole)
(2) Bromhexine hydrochloride (BHC, Sigma, Poole)
(3) OTC and BHC combined
(4) Distilled water

The plate was incubated at 37 °C for 24 h when the zone diameters were recorded and assessed for evidence of interaction between OTC and BHC.

Analysis of data

Viable aerobic counts obtained throughout the study were expressed as percent survival. This was calculated by expressing all values as a percentage of the mean viable aerobic count obtained for each group during phase I. Comparison of group means was by use of Student's independent *t*-test.

Results

Table 1 shows the different species of bacteria isolated from the guinea pig vagina. The predominant species was *E. coli* with smaller quantities of *Proteus spp., Klebsiella spp., Lactobacillus spp.* and *Pasteurella spp.*, although the proportions given are approximate.

TABLE 1

Microorganisms isolated from the guinea pig vagina prior to and following drug treatment

Organism	Proportion of total isolated population	
	Before treatment	After treatment
Escherichia coli	40%	30%
Proteus spp.	20%	30%
Klebsiella spp.	20%	30%
Lactobacillus spp.	< 5%	Not present
Pasteurella spp.	< 5%	1–2% (gp B)
		Not present in group A

TABLE 2

Minimum inhibitory concentrations (μg/ml) of oxytetracycline hydrochloride against test organisms in nutrient broth at 37 °C (denotes resistant organisms)*

Organism	Minimum inhibitory concentration (MIC, μg/ml)
E. coli	25
Proteus spp. *	100
Klebsiella spp. *	100
Lactobacillus spp.	12.5
Pasteurella spp.	12.5

Testing of the microbial sensitivity to oxytetracycline showed that those organisms marked with an asterisk (Table 2) were classified as being resistant.

The results shown in Table 3 show that there were no statistically significant differences in the zone diameters observed for the OTC + BHC combination compared with OTC alone, indicating that there was no in vitro potentiation of OTC activity by BHC.

During phase I there was no statistically significant difference in the aerobic viable counts between the drug treament groups. Fig. 1 shows the log percent survival data obtained for each of the drug-treated groups throughout the study. The log percent survival for phase II was significantly lower in the group receiving OTC + BHC combination (4.55 ± 1.46%) than in the group receiving OTC alone (16.26 ± 3.80%), $P < 0.005$. During phase III, which was drug-free, the percent survival counts increased in both groups; however, again the percent survival for the group receiving the OTC alone (95.78 ± 17.95%) was significantly

TABLE 3

Inhibition of growth of E. coli NCIB 8916 by mixtures of OTC, BHC, OTC + BHC and distilled water buffered to pH 7.0

Solution	Mean zone diameter ± S.D. (mm)
Distilled water	No inhibition ($n = 9$)
BHC	No inhibition ($n = 9$)
OTC	24.9 ± 0.6 ($n = 9$)
OTC + BHC	25.0 ± 0.6 ($n = 9$)

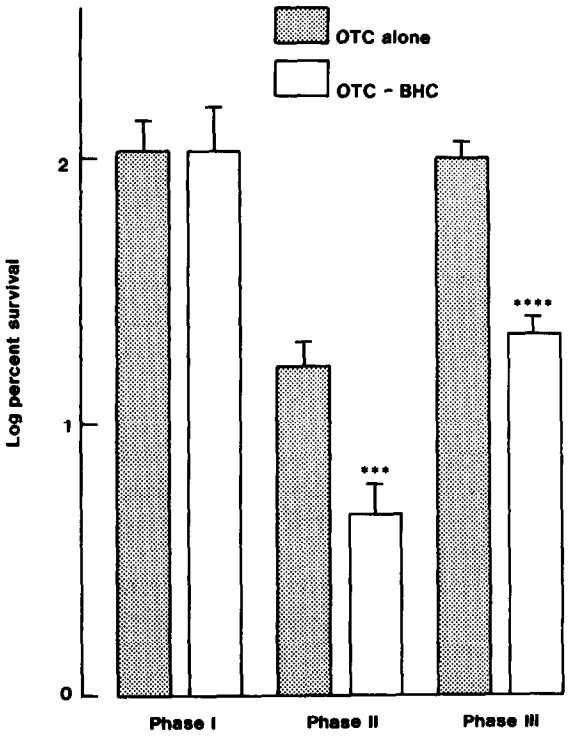

Fig. 1. Log percent survival rates for vaginal bacteria isolated from guinea pigs following treatment with oxytetracycline hydrochloride (OTC) or a combination of oxytetracycline hydrochloride and bromhexine hydrochloride (OTC + BHC). ***, $P < 0.005$; ****, $P < 0.001$.

higher than for the group receiving OTC + BHC combination (21.14 ± 3.85%), $P < 0.001$.

Discussion

Those microorganisms isolated from the vagina were assessed for their sensitivity to tetracycline. Organisms shown in Table 2 and marked with an asterisk were classified as resistant, hence the survival of any other organisms following oxytetracycline treatment in vivo can only be attributed to insufficient concentration of the antibiotic within the vaginal secretion rather than to any inherent resistance of the organisms to the antibiotic. It was also shown that BHC does not have any intrinsic antibacterial activity in vitro. Previous studies have shown that the same is true when the drug is used in vivo (Malhi et al., 1986), hence any enhanced kill observed by the combination of BHC with OTC is not due to any direct interaction between the compounds.

Vaginal microorganisms are embedded within mucus secretions and their survival on administration of OTC is a function of the concentration of antibiotic achieved within these secretions. Following oral administration the antibiotic concentration would normally be quite low and may only be sufficient to kill very sensitive organisms.

From the results presented it has been shown that when OTC and BHC are co-administered, the number of surviving vaginal organisms is significantly lower than when OTC is administered alone. Since there is no synergy between the two compounds in vitro this phenomenon can only be explained by an increase in the concentration of OTC within the vaginal secretions. This finding agrees with other workers (Bach and Leary, 1972; Kotzian et al., 1976) who have reported increased OTC levels in bronchial mucus with concurrent administration of mucolytic agents although this is the first report of an enhanced effect at other sites of mucus secretion.

The method employed in this study did not elucidate the mechanism underlying the increase in OTC concentration with concurrent BHC treatment. It is known, however, that tetracyclines are mucospissic and bind strongly to mucus glycoproteins, under acidic conditions such as those which exist in the vagina (Brown et al., 1983; Kearney and Marriott, 1987). This could significantly affect the amount of drug available to diffuse through the mucus (Turner et al., 1985). It has been shown in vitro that OTC-glycoprotein interactions lead to an increase in mucus viscosity (Marriott and Kellaway, 1975) and OTC is therefore termed a "structure-inducing" tetracycline. A mucus gel structure is more effective in reducing tetracycline transfer, than if the mucus were present in the form of a dispersed solution (Marriott and Kellaway, 1975).

It is therefore suggested that the thickened network of the vaginal mucus, brought about by OTC can be reversed by BHC to produce a gel which exhibits reduced diffusional resistance. The results therefore reinforce the observation of Malhi et al. (1986) that BHC can reduce the viscoelasticity of

cervical mucus and that this can result in the increased concentrations of OTC within these secretions. It might be expected that the increased concentration of OTC in the mucus may lead to increased viscoelasticity due to its mucospissic effect; however, this effect probably does not occur because the mucolytic action of BHC is more potent than the mucospissic effect of OTC.

References

Abdou, M.A.-F. and Kotzian, J., Observations on the increase in the oxytetracycline level in the bronchial mucosa of pigs when using a simultaneous dosage with bromhexine. *Tierarztl. Umschau*, 30 (1975) 565–566.

Bach, P.H. and Leary, W.P., The effect of bromhexine on the penetrance of oxytetracycline into sputum, *S. Afr. Med. J.*, 46 (1972) 1512–1514.

Brown, D.T., Marriott, C. and Beeson, M.F., Antibiotic binding to purified mucus glycoproteins. *J. Pharm. Pharmacol.*, 35 (1983) 80P.

Burgi, H. and Makin, E.J.B., Changes in sputum fibre systems and viscosity during treatment with bromhexine. *Clin. Trials J.*, 8 (1971) 37–40.

Cowan, S.T., Characters and characteristics. In Cowan, S.T. and Steele, K.J. (Eds.), *Cowan and Steele's Manual for the Identification of Medical Bacteria*, 2nd edn., Cambridge University Press, Cambridge, 1974, pp. 42–122.

Kearney, P. and Marriott, C., The effects of mucus glycoprotein on the availability of tetracycline. II. Binding. *Int. J. Pharm.*, 35 (1987) 211–217.

Kotzian, J., Abdou, M.A.-F. and Salamon, E., Increase in oxytetracycline level in the bronchial mucus following simultaneous administration of Bisolvon. *Tierarztl. Umschau*, 32Jg No. 3 (1976) 132–134.

Malhi, J.S., Gard, P.R., Hanlon, G.W. and Marriott, C., The effect of oral mucolytic agents on the microflora of guinea pig uteri, *J. Pharm. Pharmacol.*, 38 (1986) 38P.

Marriott, C. and Kellaway, I.W., The effect of tetracyclines on the viscoelastic properties of bronchial mucus. *Biorheology*, 12 (1975) 391–395.

Turner, N.C., Martin, G.P. and Marriott, C., The influence of native porcine gastric mucus gel on hydrogen ion diffusion: *J. Pharm. Pharmacol.*, 37 (1985) 776–780.