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Bromhexine: in vitro and in vivo studies of release from mono- and bi-component preparations

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Summary

The in vitro dissolution characteristics of bromhexine and its hydrochloride salt have been investigated in the presence and absence of a cephalosporin antibiotic. Bioavailability of the salt and of the antibiotic from combination capsules have also been studied. In conditions typical of dissolution tests for formulated products, no significant differences were observed in the in vitro dissolution rates of the base and its salt in mono-component preparations. Some slowing of solution rate of the base was observed in bi-component preparations. In vivo, peak plasma levels of bromhexine hydrochloride and cefaclor were 18.1 ± 2.3 ng·ml^{-1} and 15.3 ± 0.9 μg·ml^{-1}, respectively, from the combination formulation and were similar to levels found with the mono-component preparations.

Introduction

Bromhexine hydrochloride B.P. is often formulated with antibiotics for the treatment of congestive respiratory disease. Its absorption profile has been studied using ^{14}C-labelled compound (Jauch and Hankwitz, 1975) and an electron-capture GLC method, suitable for specific plasma level determinations, has been described recently (De Leenheer and Vandecasteele-Thienpont, 1980). The use of the hydrochloride salt has been questioned by Miyazaki et al. (1980) who showed that, at high concentration in vitro, the solution rate of the base may be faster than that of the hydrochloride salt and may therefore be the more suitable dosage form. This paper

reports on the in vivo absorption of bromhexine hydrochloride in the presence of a cephalosporin antibiotic (cefaclor) and on further in vitro comparisons of bromhexine and its hydrochloride salt.

Materials and methods

Materials

Raw materials. Bromhexine hydrochloride B.P., particle size less than 150 μm, was obtained from K. Thomae GmbH, Biberach, F.R.G., and cefaclor was obtained from Eli Lilly, Indianapolis, U.S.A. Both compounds were used as supplied. Bromhexine was derived from the hydrochloride salt. The salt was suspended in hot ethanol–water, mixed with sodium bicarbonate solution and the base extracted with ethyl acetate. After evaporating to dryness, the base was identified by NMR, IR spectroscopy and melting point. Particle size was controlled by sieving to be less than 150 μm.

Capsule preparation. Capsule formulae are shown in Table 1. Drugs and excipients were blended for fixed periods in a Y-cone mixer and capsules were filled manually to stated weight ± 5%, being characterized by HPLC assay prior to use. For bioavailability and in vitro testing, capsule a represented a minimum weight control, capsules b, e and f typical size 2 weights, and capsules g and h were maximum fill-weight/density examples of the formulations. Excipient formulae were qualitatively similar throughout but varied in proportion according to the particular drug/capsule combination.

TABLE 1

FORMULAE FOR CAPSULES USED IN IN VITRO AND IN VIVO STUDIES OF THE RELEASE CHARACTERISTICS OF BROMHEXINE, BROMHEXINE HYDROCHLORIDE AND CEFACLOR

Capsule	Capsule size	Compound quantity (mg)			
		Bromhexine	Bromhexine hydrochloride	Cefaclor	Excipient to
a(○)	2	8	–	–	270
b(□)	2	8	–	250	283
c(▲)	2	–	8.78	–	271
d(△)	2	–	8.78	–	270
e(■)	2	–	8.78	250	285
f(◆)	2	–	–	250	288
g(●)	0	–	8.78	500	572
h(▼)	0	–	–	500	575

Methods

In vitro studies. Dissolution testing was carried out according to the 1980 B.P. in Hanson Corporation 6-pot dissolution apparatus. Samples were tested in 1 litre of 0.1 N and 0.001 N HCl, with a basket rotation rate of 50 rpm. Samples (10 ml) were withdrawn at 10, 20, 40 and 60 min using a microprocessor-controlled automated sampling device (Papworth and Bowtle, 1979). Drug release was measured spectrophotometrically (Bowtle et al., 1981). Cefaclor was determined at 275 nm (Cecil Instruments CE 272 spectrophotometer, 10 mm cell path length) and bromhexine and bromhexine hydrochloride at 422 nm after reaction with bromocresol purple and chloroform extraction of the ion-pair reaction product (Technicon Instruments Colorimeter, 15 mm cell path length).

Sample series were compared by *t*-tests applied at each time interval.

Bioavailability studies. A three-way cross-over study was conducted on 6 normal healthy subjects (2 males, 4 females, age range 22–37 years, body weight range 48.5–82.5 kg). At intervals of not less than one week and after overnight fasting, each subject received a single capsule (namely capsule c, g or h, see Table 1) with 150 ml water. Blood samples were taken 30 min before dosing and 0.5, 1.0, 1.5, 2.0, 3.0, 4.0 and 6.0 h after dosing. Plasma was obtained by centrifuging at $1000 \times g$ for 5 min, frozen immediately and stored at $-50°C$ prior to assay. No food or fluid was taken until after the 1.5-h blood sample, after which a light breakfast was taken and fluid intake not further restricted.

Plasma assays. Cefaclor in plasma was assayed as described previously (Glynne et al., 1978). Bromhexine hydrochloride plasma levels were determined using a modification of the method developed by De Leenheer and Vandecasteele-Thienpont (1980). The derivatization procedure was omitted and the hexane extracts (2 μl) injected directly into a Hewlett Packard 5710A gas chromatograph fitted with an electron-capture detector and a 2 m × 4 mm column containing 3% OV17 on H.P. Chromosorb W. The retention times at 220°C for bromhexine hydrochloride and the internal standard N-cyclohexyl-N-*n*-propyl-2-(2-amino-3,5-dibromo)benzyl ammonium chloride were 6.4 and 7.8 min, respectively. A specimen trace is shown in Fig. 1.

Results and Discussion

In vitro tests

Bromhexine and its hydrochloride salt are known to have low water solubilities and their dissolution rates may be controlling factors in gastrointestinal absorption. In vitro tests at high concentration have recently shown that the chloride common-ion effect can significantly reduce the solution rate of bromhexine hydrochloride relative to the base in simulated acid gastric conditions and that the solution rates of the compounds are pH-sensitive (Miyazaki et al., 1980). Dissolution profiles for for-

mulated capsules of bromhexine and bromhexine hydrochloride (capsules a and d), respectively, in 0.001 N HCl and 0.1 N HCl are shown in Fig. 2. At the first time interval (10 min), solution of the base but not the salt varied with degree of acidity ($P_{base} < 0.02$, $P_{salt} > 0.05$). At 20 and 40 min, solution of each compound was greater than 75% and 80%, respectively, with neither being affected by a change in acidity ($P > 0.05$ in each case). These results, obtained in conditions typical of pharmaceutical dissolution testing and considered compatible with in vivo pH and concentration conditions, indicate that, in 0.001 N HCl and 0.1 N HCl, there are no biologically significant differences between the dissolution rates of either compound from formulated capsules.

Fig. 3A and B shows dissolution profiles for mono- and bi-component capsules

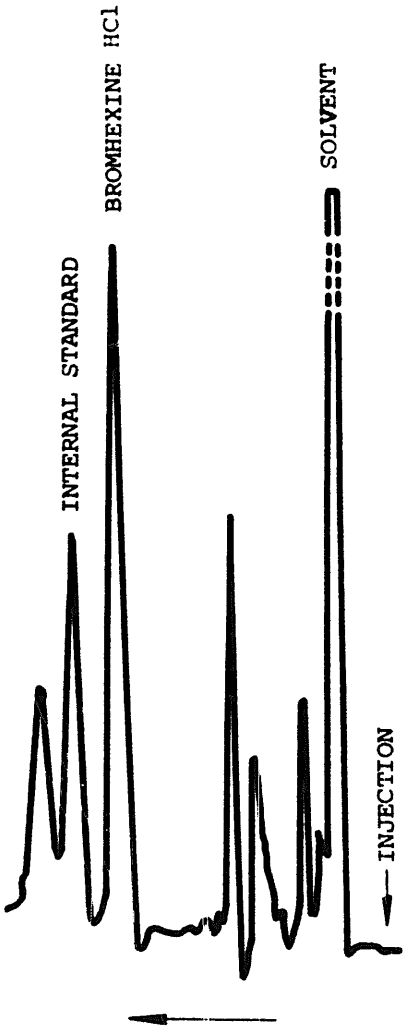

Fig. 1.

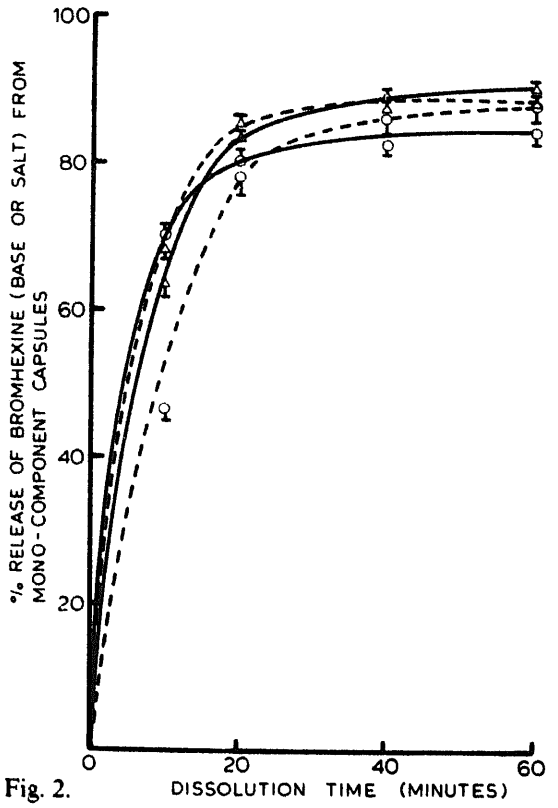

Fig. 2.

Fig. 1. Gas chromatograph trace for bromhexine hydrochloride extracted from plasma.

Fig. 2. Effect of degree of acidity on in vitro release of bromhexine (base or salt) from formulated capsules. Key: ○, bromhexine base (capsule a); △, bromhexine hydrochloride (capsule d); - - - - - -, in 0.001 N HCl; ————, in 0.1 N HCl. Results are averages of 6 determinations at each time interval.

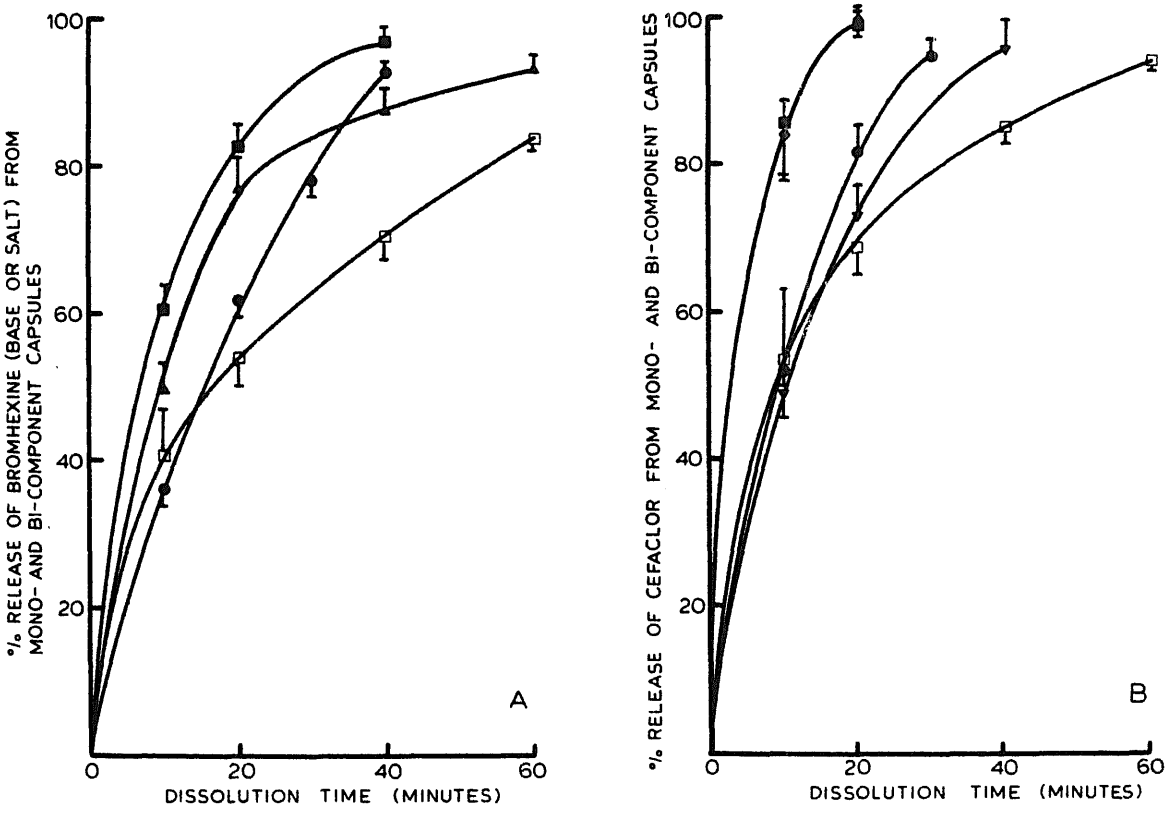

Fig. 3A and B: In vitro release of bromhexine (base or salt) and cefaclor from formulated capsules. Key: for capsule identity, see Table 1. Results are the averages of 6 determinations in 0.1 N HCl.

used for in vitro studies (capsules b, e and f) and in bioavailability testing (capsules c, g and h). In the more acidic condition (0.1 N HCl), dissolution of bi-component capsules is faster from those containing the hydrochloride form of bromhexine ($P < 0.02$ at 20, 40 and 60 min). The present in vitro tests, therefore, show that using the hydrochloride form, about 80% of bromhexine would be expected to be in solution at 40 min. The combination test using cefaclor shows that no advantage accrued from using bromhexine base. This combination was, therefore, not studied further.

The dissolution profile of neither bromhexine hydrochloride nor cefaclor was adversely affected by combination with the other component.

Newton and Rowley (1970) have shown that increasing the packing density of capsules may affect drug in vitro release rates. In a separate experiment, capsules containing 8.8 mg bromhexine hydrochloride and prepared to the maximum density used here (858 mg/ml) showed 75, 85 and 89% solution at 20, 40 and 60 min and, except at 10 min, compared closely with capsules prepared to the minimum density used in these studies (730 mg/ml). The minor differences between packing densities of the range of capsules used in this study are therefore considered to be unimportant in relation to release of the compound.

Absorption of bromhexine hydrochloride and cefaclor

Mean plasma levels of bromhexine hydrochloride and cefaclor are shown in Figs. 4 and 5 and statistical comparisons of the means in Table 2. Neither compound affected the time to or size of peak plasma levels of the other component. At each time interval, differences in plasma levels of each compound were statistically insignificant when the mono- and bi-component formulations were compared. Measurements of area-under-curve (0–6 h) showed no differences in bioavailability of bromhexine hydrochloride between preparations. Cefaclor bioavailability from the combination capsule was statistically lower ($P < 0.02$) than from the mono-component preparation but this difference was considered clinically inconsequential.

Cephalexin, a cephalosporin analogue of cefaclor with a similar pK_a profile, has been shown to be absorbed from the duodenum and jejunum in rats and dogs (Welles et al., 1968) and the absorption pattern for cefaclor may be considered similar. Bromhexine is a moderately strong base (pK_a 9.2, aqueous) and pH-partition theory predicts that it will be absorbed later than cefaclor since it will be less dissociated in the more neutral region of the small intestine. The current in vivo findings comply with this prediction since maximum absorption of bromhexine

Fig. 4.

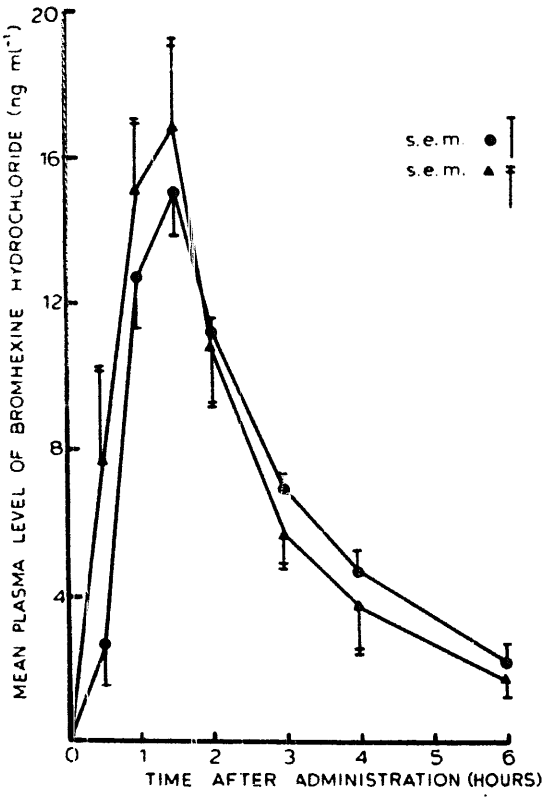

Fig. 5.

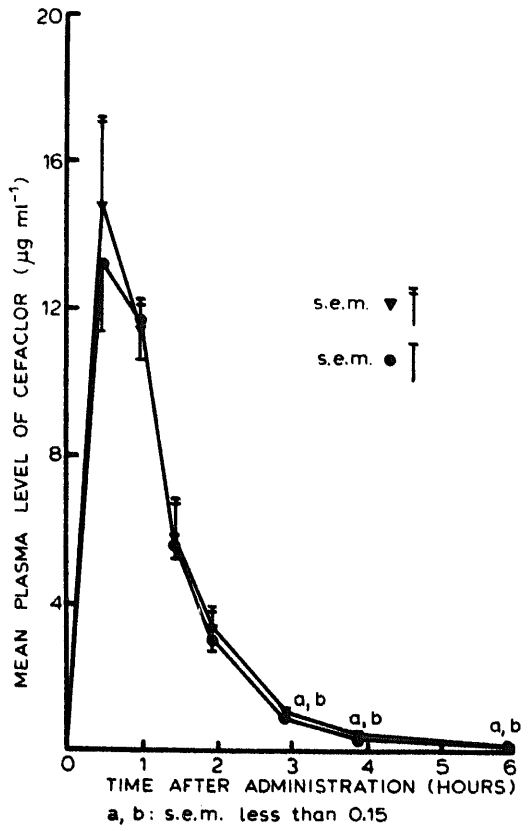

Fig. 4. Mean plasma levels of bromhexine hydrochloride after administration of 8.78 mg in a mono-component (capsule c, ▲) or bi-component (capsule g, ●) preparation.

Fig. 5. Mean plasma levels of cefaclor after administration of 500 mg in a mono-component (capsule h, ▼) or bi-component (capsule g, ●) preparation.

TABLE 2

BIOAVAILABILITY OF BROMHEXINE HYDROCHLORIDE AND CEFACLOR FROM CAPSULE FORMULATIONS

Mean plasma level parameter	Bromhexine hydrochloride		Cefaclor	
	Mono-component capsule	Bi-component capsule	Mono-component capsule	Bi-component capsule
Peak concentration [a,d]	15.1 ± 1.1	18.1 ± 2.3	16.0 ± 1.7	15.3 ± 0.9
Time to peak [b,d]	1.3 ± 0.1	1.3 ± 0.1	0.75 ± 0.17	0.67 ± 0.11
Area-under-curve (0–6 h) [c,d]	39.5 ± 1.8	40.5 ± 5.0	20.7 ± 0.7	18.9 ± 0.8
Paired *t*-value	0.17		3.853	
P	N.S.		< 0.02	

[a] $ng \cdot ml^{-1}$ (bromhexine hydrochloride) and $\mu g \cdot ml^{-1}$ (cefaclor).

[b] h.

[c] $ng \cdot ml^{-1} \cdot h^{-1}$ (bromhexine hydrochloride) and $\mu g \cdot ml^{-1} \cdot h$ (cefaclor).

[d] Figures show mean ± S.E.M. (n = 6)

Note: individual data on subject profiles and compound bioavailability parameters are too extensive to be presented here.

occurs 30–40 min after that for cefaclor, although their 80% dissolution times differ here by only 10 min.

The method used here to determine bromhexine hydrochloride is more specific for the parent compound and less likely to be influenced by the presence of metabolites than the ^{14}C-method of Jauch and Hankwitz (1975). Assuming 42 ml plasma per kg body weight and accounting for the average 62.4 kg body weight of subjects in this study, the mean peak plasma level of bromhexine hydrochloride corresponds to 0.6% of the administered dose.

This figure is 10 times less than that found previously (Jauch and Hankwitz, 1975) and is explained by the greater specificity of the electron-capture GLC method for the original compound. The ^{14}C-method determined bromhexine hydrochloride and its metabolites and hence overestimated unchanged compound in plasma.

Elimination of bromhexine hydrochloride and cefaclor from plasma

Rate constants and half-times for elimination of each compound from plasma after administration of mono- and bi-component capsules are shown in Table 3. These parameters were derived from the linear plots of log mean plasma level versus time at 3, 4 and 6 h after administration and showed that the presence of neither drug affected the elimination profile of the other compound.

In vitro–in vivo correlation

A previous study (Jauch and Hankwitz, 1975) has shown that, following oral administration of 4 mg of bromhexine hydrochloride in aqueous solution, maximum plasma levels occurred at one hour. In vitro tests used here indicated that, with doses of bromhexine hydrochloride and bromhexine hydrochloride plus cefaclor, solution of each component was 80% complete in 40 min and that neither component adversely affected the solution rate of its complement. The absorption profiles for the solid dosage forms found here comply closely with the present in vitro findings and with those found previously for the orally administered solution. It has been suggested that the hydrochloride form of bromhexine shows slower solution characteristics than the base and that the base may be the better dosage form (Miyazaki

TABLE 3

RATES AND HALF-TIMES FOR ELIMINATION OF BROMHEXINE HYDROCHLORIDE AND CEFACLOR FROM PLASMA

Parameter for elimination from plasma	Bromhexine hydrochloride		Cefaclor	
	Mono-component capsule	Bi-component capsule	Mono-component capsule	Bi-component capsule
Rate constant [a]	−0.383	−0.384	−0.976	−1.060
Half-time [b]	1.81	1.80	0.71	0.65

[a] h^{-1}.

[b] h.

et al., 1980). The current in vitro tests, conducted in theoretically poor conditions for solution of bromhexine hydrochloride, show, however, that no significant differences exist between the solution rates of either form at concentrations appropriate to testing of dosage forms. Further, since absorption of bromhexine hydrochloride has been shown to be rapid, any minor difference in the solution rates of bromhexine and its hydrochloride salt would be biologically insignificant, even if the base were not converted to the hydrochloride in the stomach.

The Miyazaki model is therefore considered less appropriate to in vitro dissolution evaluation of bromhexine than current standard methods.

Conclusion

In vitro tests show that in relation to release rates no advantage accrues in the use of bromhexine rather than its hydrochloride salt and that the Miyazaki model is not appropriate to release predictions of formulated bromhexine. In vivo, bioavailability of bromhexine hydrochloride is unaffected by the presence of the cephalosporin antibiotic, cefaclor.

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