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Ketobemidone prodrugs for buccal delivery: Prediction of the extent of saliva-catalyzed hydrolysis of various ester prodrugs under simulated in vivo conditions

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Summary

As part of studies aiming at developing a ketobemidone prodrug suitable for buccal or sublingual administration, the potential impact of saliva enzyme-catalyzed hydrolysis of various ester prodrugs was assessed. The hydrolysis of three ketobemidone esters in human whole saliva, obtained under conditions ensuring maximal esterase activity, was studied as a function of ester concentration at 37°C The kinetics of hydrolysis could be accounted for in terms of the Michaelis-Menten equation and the rate parameters K_m and V_{max} were determined. Due to the occurrence of zero-order kinetics at pharmacologically relevant prodrug concentrations, degradation of the esters by saliva enzymes was predicted to occur to only a minor extent (1–6%) under conditions similar to those prevailing in vivo after administration of buccal or sublingual tablets of the esters. The mode of administration of tablets for use in the mouth and their rate of disintegration were shown to have some influence on the rate of saliva secretion and hence on saliva esterase activity but not to an extent compromising the efficient buccal or sublingual delivery of the ketobemidone prodrugs.

Introduction

Ketobemidone, 1-[4-(3-hydroxyphenyl)-1-methyl-4-piperidyl]-1-propanone (**I**), is a strong narcotic analgesic equipotent with morphine (Eddy et al., 1957; Anderson et al., 1986) which has been used clinically for more than 40 years, especially in Scandinavia and in some other European countries. The drug is usually administered perorally or rectally but its bioavailability is incomplete and variable due to pronounced first-pass metabolism (Bondesson et al., 1980; Anderson et al., 1981, 1986).

Studies have been initiated in our laboratories to develop a ketobemidone preparation suitable for buccal or sublingual administration in order to improve its bioavailability (through avoidance of the first-pass effect) and achieve a more rapid onset of action relative to the peroral or rectal preparations. Initial studies showed that ketobemidone did not penetrate the oral mucosa, presumably due to its poor lipophilicity, and therefore, we have prepared a number of carboxylate

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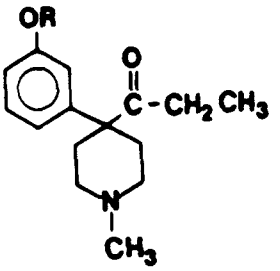
or carbonate ester prodrugs at the phenolic hydroxyl group in the drug (Hansen et al., 1991). These derivatives were found to be more lipophilic than the parent drug and to be rapidly and quantitatively hydrolyzed to ketobemidone in the presence of human plasma. However, most of the esters were also rapidly hydrolyzed when incubated in whole human saliva at 37°C (Hansen et al., 1991). Human saliva contains a variety of esterases (Chauncey et al., 1954, 1957; Burstone, 1956; Chauncey, 1961; Lindqvist and Augustinsson, 1975; Tan, 1976; Lindqvist et al., 1977) and the last finding thus makes it important to consider the possible impact of saliva enzyme-catalyzed degradation of the ester prodrugs in the development of buccal or sublingual preparations.

In the accompanying paper (Hansen et al., 1992), various factors influencing the esterase activity of human saliva have been studied. A most important factor was shown to be the salivation flow rate in that the esterase activity increased greatly with increasing salivary flow up to a rate of about 0.9 ml min^{-1}. Different stimuli such as paraffin chewing and sucking calcium phosphate tablets, acid drops or sour lemon candy result in increased salivary flow (Kerr, 1961; Zickert, 1974; Minaguchi et al., 1988). It may therefore be imagined that the type of device used for a buccal or sublingual preparation can effect the salivary flow and hence the salivary esterase activity.

The primary objective of the present study was to provide information on the stability of various ketobemidone ester prodrugs (**II–IV**, Scheme 1) in human saliva at various prodrug concentrations including clinically relevant concentrations. In addition, the effect of slowly and rapidly disintegrating buccal or sublingual tablets with and without taste on the salivary flow was examined.



I R = -H

II R = $-\overset{\overset{O}{\|}}{C}-CH_2C(CH_3)_3$

III R = $-\overset{\overset{O}{\|}}{C}-O-C_2H_5$

IV R = $-\overset{\overset{O}{\|}}{C}-O-CH(CH_3)_2$

Scheme 1

Materials and Methods

Chemicals

Ketobemidone hydrochloride was obtained from H. Lundbeck A/S, Copenhagen, Denmark. The ketobemidone esters **II–IV** were prepared as previously described (Hansen et al., 1991). The ester **II** was used as the fumarate salt whereas the other two esters were in the form of hydrochloric acid salts.

Influence of buccal / sublingual tablet composition and application site on salivary flow

Buccal/sublingual tablets (120–140 mg) having either fast or slow disintegration rates were used in the study. Each type of tablets was made with or without taste (0.5 mg of peppermint oil per tablet). The rapidly disintegrating sublingual tablets were placed under the tongue whereas the slowly disintegrating tablets were placed either sublingually or buccally (between the upper lip and gum). Control experiments without tablet administration were also performed. Two female subjects (age 29 and 36 years) participated in the

study. After application of the buccal/sublingual tablet, whole saliva was collected by expectoration into centrifuge tubes for every 2 min over a period of 20 min; whereupon any residues of the tablet were removed. Saliva collection was continued for 10 min. Seven experiments were performed per day with each subject, interrupted by 30 min intervals in which water but not food was allowed. The experiments were carried out with three replications.

Collection of saliva for stability experiments

Whole saliva, stimulated by normal frequent paraffin chewing giving a salivary flow of 1.0–1.2 ml min^{-1}, was collected from subject 1 before breakfast and following a fasting period of 10 h. When chewing the paraffin was moved from one side of the mouth to the other in order to activate all salivary glands (Kerr, 1961). Brushing of teeth was only allowed with toothpaste without fluoride and performed at least 1 h prior to saliva collection, since it has been found that the esterases in human whole saliva can be inhibited by several of the commonly used dental materials (Lindquist et al., 1980).

Hydrolysis studies in human saliva

The hydrolysis of the ketobemidone esters **II–IV** was studied in fresh whole human saliva obtained as described above. The initial concentrations of the esters were in the range 1.8×10^{-4}–1.5×10^{-2} M and the initial rate method was used to determine the kinetics of hydrolysis. The reactions were initiated by adding 25–500 μl of a stock solution of the esters in water or water-acetonitrile to 5.00 ml of whole human saliva. The solutions were kept in a water-bath at 37.0 ± 0.2°C, and at appropriate intervals, 250 μl samples were withdrawn and added to 500 μl of a 2% solution of zinc sulphate in acetonitrile-water (1:1 v/v) in order to deproteinize the saliva. After mixing and centrifugation for 3 min at 13000 rpm, 20 μl of the clear supernatant was analyzed for ketobemidone by a reversed-phase HPLC procedure described previously (Hansen et al., 1991). The detection limit for ketobemidone was 0.1 μg ml^{-1}, corresponding to 4×10^{-7} M. The initial rate of ester hydrolysis was determined from the slopes of linear plots of amount of ketobemidone formed against time. In all experiments, less than 5% of the ester was hydrolyzed, implying an almost constant substrate concentration during the runs.

Results and Discussion

Variation in salivary flow with composition of buccal / sublingual tablets

The influence of slowly and rapidly disintegrating tablets with or without 0.5 mg of peppermint oil as flavouring agent on the salivary flow was tested in two subjects after buccal or sublingual administration. The tablets with a fast disintegration rate were only administered sublingually. The results are shown in Fig. 1.

Significant differences ($p < 0.001$) in the salivary flow were found between the two subjects at all sampling times. A marked interindividual variation in paraffin chewing-stimulated salivation rates has previously been observed (Bertram, 1967; Heintze et al., 1983; Barenthin and Johnson, 1986). No significant effect of peppermint oil was observed and therefore, the data in Fig. 1 for each application site include both tablets with and without a content of this flavouring agent. Significant differences were found between the application sites at 2–28 min for subject 1 and at 2–6 min for subject 2 ($p < 0.05$), the highest salivary flow occurring after administration of rapidly disintegrating sublingual tablets. The disintegration time of these tablets in vivo was 1.5–3.5 min.

The weight loss of the buccal/sublingual tablets determined 20 min after their administration is listed in Table 1. Despite equal salivary flow rates after administration of slowly disintegrating buccal and sublingual tablets, the buccal tablets showed a smaller loss. Administration af slowly disintegrating or dissolving buccal tablets may be preferred over that of slowly disintegrating or dissolving sublingual tablets because of the prolonged retention time in the oral cavity and also because the presence of a buccal tablet is not noticeable to the patients after a short time (Livingstone and Livingstone, 1989).

Stability of ketobemidone prodrugs in human saliva

The ketobemidone esters **II–IV** have previously been found to undergo an enzyme-catalyzed hydrolysis to yield ketobemidone in quantitative amounts when incubated in whole human saliva at 37°C (Hansen et al., 1991). At an initial concentration of 10^{-4} M the hydrolysis proceeded according to first-order kinetics with half-lives of 295 min (**II**), 5.1 min (**III**) and 4.9 min (**IV**), respectively. The corresponding half-lives for the hydrolysis in 80% human plasma were 1.8 min (**II**), 0.03 min (**III**) and 0.04 min (**IV**) whereas those in buffer solutions of pH 7.4 and at 37°C exceeded 50–100 h (Hansen at al., 1991).

At higher initial ester concentrations the rate of hydrolysis of the esters in saliva changed to follow zero-order kinetics which is typical for enzyme-catalyzed reactions following Michaelis-Menten kinetics in which the initial substrate concentration is higher than the Michaelis constant K_m (Segel, 1975). The initial rates of formation of ketobemidone (V_0) from the esters were determined at different initial ester concentrations (Figs 2 and 3) and shown to conform to the Michaelis-Menten equation:

$$V_0 = \frac{V_{max} S_0}{K_m + S_0} \quad (1)$$

where V_{max} is the maximum rate of substrate consumption, K_m represents the Michaelis constant and S_0 is the initial (and almost constant) substrate concentration. The Michaelis-Menten parameters K_m and V_{max} were determined from Lineweaver-Burk plots of Eqn 1 (an example is shown in Fig. 4) and are listed in Table 2.

It should be noted that the saliva used in the kinetic runs was obtained under conditions favouring a high esterase activity (Hansen et al., 1992).

Inspection of the data in Table 2 shows that the ester **III** is the best substrate, having the greatest V_{max} value and the lowest K_m value (tightest binding). The most stable compound is the 3,3-dimethylbutyrate ester (**II**) which may be ascribed to steric hindrance by the bulky alkyl side chain. The low reactivity of ester **II** may also partly be ascribed to a decrease in pH of the saliva upon addition of the ester which was used in the form of its fumarate salt. Thus, whereas addition of the esters **III** and **IV** (hydrochloric acid salts) at a concentration of 1.5×10^{-2} M to saliva did not change the pH from that in a saliva sample without ester (pH 6.9–7.0), addition of ester **II** at a similar concentration afforded a pH value of 4.7. We have previously found that the esterase activity of saliva at pH 4.5 is about 1.6-times lower than at pH 7 (Hansen et al., 1992).

The rate data obtained along with Eqn 1 permit the calculation of the extent of saliva-catalyzed degradation of the ketobemidone esters under clinically relevant conditions. Thus, the

TABLE 1

Weight loss of sublingual or buccal tablets withdrawn 20 min after administration to two subjects (mean ± SD, n = 3)

Type of tablet (disintegration)	Flavouring agent added [a]	Site of administration	Weight loss (%)	
			Subject 1	Subject 2
Fast [b]	yes	sublingual	100	100
Fast [b]	no	sublingual	100	100
Slow	yes	sublingual	35 ± 11	59 ± 14
Slow	no	sublingual	44 ± 7	58 ± 16
Slow	yes	buccal	9 ± 1	16 ± 3
Slow	no	buccal	11 ± 3	13 ± 3

[a] 0.5 mg peppermint oil/tablet.

[b] Time of disintegration was 1.5–3.5 min

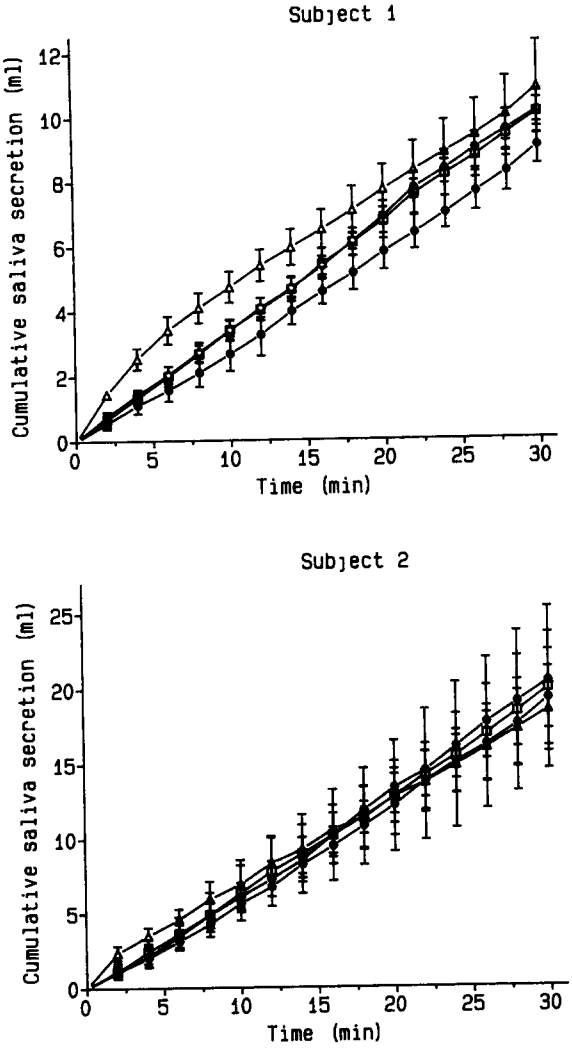


Fig 1. Plots of cumulative saliva secretion against time after administration of rapidly (△) and slowly (□) disintegrating sublingual tablets, and administration of slowly disintegrating buccal tablets (○) to two subjects; (●) control data (i.e., saliva secretion when no tablets were given) Error bars represent S.D ($n = 3$–6).

amount of ester degraded in 5 min can be expressed as:

$$\text{Degraded ester } (\%) = \frac{V_{max} \times 5}{K_m + S_0} \times 100 \qquad (2)$$

The results of such calculations as a function of saliva volume are shown in Fig. 5 for ester pro-

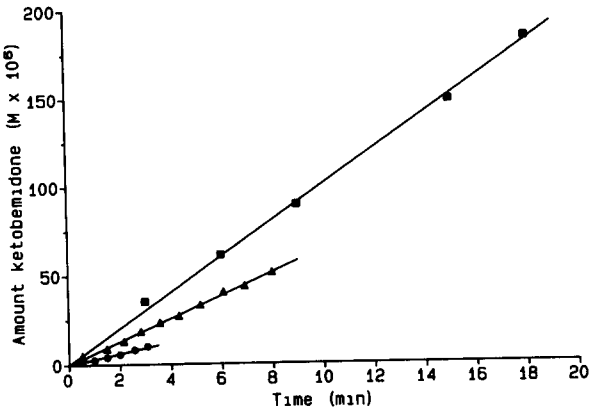


Fig. 2. Plots showing the rate of formation of ketobemidone upon hydrolysis of compound **IV** in whole human saliva (37°C) at initial concentrations of compound **IV** of 4.0×10^{-4} M (●), 1.3×10^{-3} M (▲) and 1.3×10^{-2} M (■)

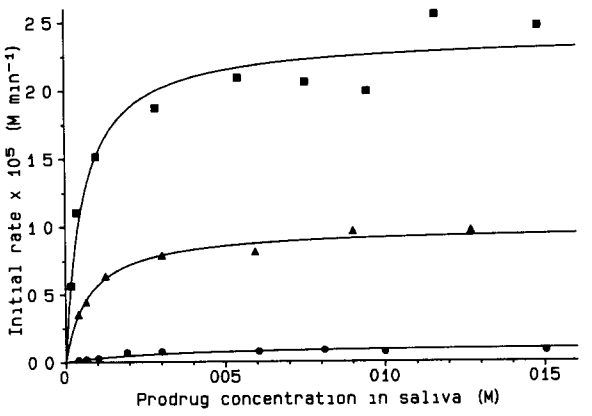


Fig 3. The effect of substrate concentration on the initial rate of the saliva catalyzed hydrolysis of compound **II** (●), **III** (■) and **IV** (▲). The full lines have been calculated from Eqn 1 and the K_m and V_{max} values in Table 2.

TABLE 2

Michaelis-Menten parameters K_m and V_{max} for the hydrolysis of various ketobemidone ester prodrugs at 37°C in human whole saliva

Compound	K_m (M)	V_{max} (M min^{-1})
II	2.9×10^{-3}	1.2×10^{-6}
III	5.5×10^{-4}	23.9×10^{-6}
IV	7.4×10^{-4}	9.8×10^{-6}

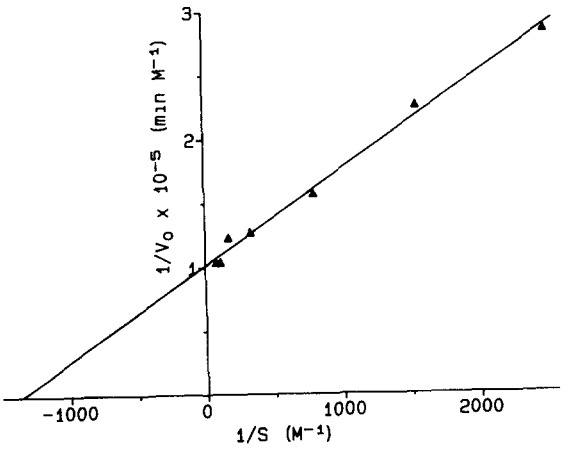


Fig 4 Lineweaver-Burk plot of the rate data for the saliva catalyzed hydrolysis of ketobemidone ester IV

drug doses corresponding to 5 or 10 mg ketobemidone hydrochloride (normal doses). In the calculations the assumption was made that the doses

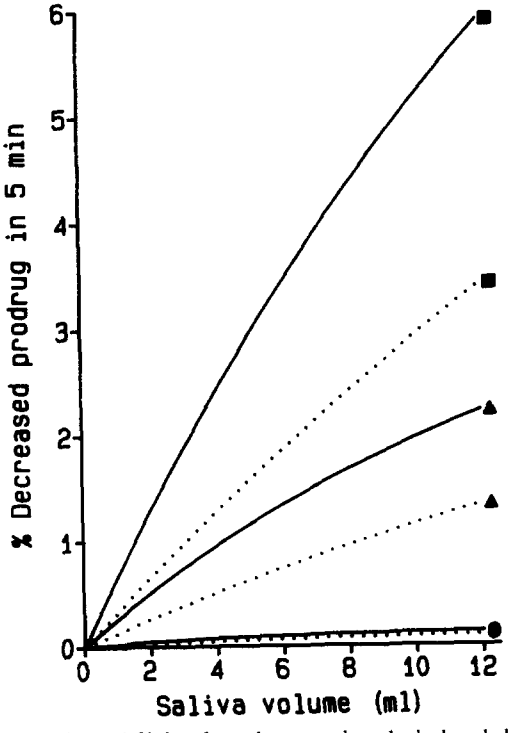


Fig 5. Plots of % ketobemidone prodrug hydrolyzed during incubation for 5 min in human saliva against saliva volume at prodrug concentrations equivalent to 5 mg (———) or 10 mg (·) ketobemidone hydrochloride. Ester II (●), III (■) and IV (▲) The curves were calculated from Eqn 2

applied were dissolved immediately and homogenously distributed in the saliva, the volume of which remained constant during the 5 min period. The amount of saliva normally secreted during 5 min is in the range of 1.5–4 ml. As can be seen from Fig. 5, hydrolysis of a ketobemidone ester prodrug by saliva enzymes at pharmacologically relevant concentrations is only expected to occur to a very minor extent which in turn is due to the occurrence of zero-order kinetics at these ester concentrations.

Acknowledgements

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