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Buccal absorption of ketobemidone and various ester prodrugs in the rat

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Summary

The buccal absorption of ketobemidone, a strong narcotic analgesic, and various carboxylate and carbonate ester prodrugs was studied in rats The compounds were administered in the form of aqueous solutions of pH 7.4. The absolute bioavailability of ketobemidone following buccal dosing was 26% whereas the bioavailability of ketobemidone following buccal administration of the prodrugs ranged from 37 to 98% The highest bioavailability was obtained with the ethyl carbonate ester. An apparent parabolic correlation between bioavailability and lipophilicity of the compounds was seen All esters were rapidly hydrolyzed to ketobemidone after both buccal and intravenous administration The acute toxicity of the esters after i.v. administration to mice and rats was similar to that of the parent drug. It is concluded that esterification of the phenolic hydroxyl group in ketobemidone to give a more lipophilic prodrug may be a useful approach to improve the buccal delivery of this analgesic.

Introduction

Ketobemidone is a strong narcotic analgesic usually administered intramuscularly, perorally or rectally. Due to a pronounced first-pass metabolism its peroral and rectal bioavailability is, however, incomplete and variable (Bondesson et al., 1980; Anderson et al., 1981).

Studies have been initiated in our laboratories to develop a ketobemidone preparation for buccal or sublingual administration in order to improve its bioavailability and achieve a more rapid onset of action relative to the peroral preparation. Initial studies using the in vivo buccal absorption model of Beckett and Triggs (1967) showed that ketobemidone did not penetrate the human oral mucosa, presumably because of its limited lipophilicity at physiological pH (Hansen et al., 1991). Esterification of the phenolic hydroxyl group in ketobemidone was hence shown to result in derivatives with increased lipophilicity and considerable susceptibility to undergoing plasma-catalyzed hydrolysis to the parent drug (Hansen et al., 1991). Subsequent studies showed that such ester prodrugs may be promising candidates for buccal or sublingual delivery of ketobemidone as they possessed an increased perme-

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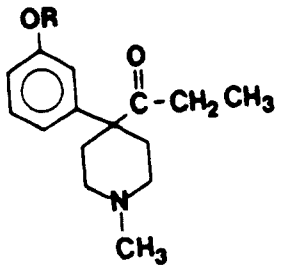
ability through porcine buccal mucosa in vitro relative to the parent compound (Hansen et al., 1992c). Furthermore, it was found that although the esters are susceptible to undergoing saliva-catalyzed hydrolysis (Hansen et al., 1991, 1992a) this phenomenon was predicted to be of minor importance for buccal absorption under clinically relevant conditions (Hansen et al., 1992b).

The purpose of the present study was to determine the absolute bioavailability of ketobemidone and some ester prodrugs following buccal administration to rats. In addition, the acute toxicity of various ketobemidone esters following intravenous administration to mice and rats was determined and compared with that of ketobemidone. The structures of ketobemidone (**I**) and the carboxylic acid and carbonate esters (**II–VIII**) studied are shown in Scheme 1.



I	$-H$
II	$-COCH_2C(CH_3)_3$
III	$-COC_6H_5$
IV	$-COOCH_3$
V	$-COOCH_2CH_3$
VI	$-COOCH(CH_3)_2$
VII	$-COO(CH_2)_3CH_3$
VIII	$-COO(CH_2)_5CH_3$

Scheme 1

Materials and Methods

Apparatus

High-performance liquid chromatography (HPLC) was carried out using a system consisting of a Waters (Milford, U.S.A.) 6000A pump, a Rheodyne Model 7125 injection valve with a precolumn (7 × 4 mm) packed with RP-8 (LiChroprep, 40–63 μm, Merck, Darmstadt, Germany) instead of the sample loop, and a Perkin Elmer Bioanalytical System (BAS, West Lafayette, U.S.A.) Model LC4B/17 electrochemical detector. The electrode was glassy carbon with an Ag-AgCl electrode as reference, the applied potential being 1.1 V. A column packed with Sperisorb C-18 (5 μm, 250 × 4 mm i.d.) was used. A HP-3390A integrator (Hewlett-Packard, Avondale, U.S.A.) was used to measure the peak heights. Electro-cardiogram (ECG) recordings were performed using a Cardiostat T instrument (Siemens, Søderberg). Ventilation of the rats was performed with a small animal respiratory pump (Citenco Motors, Herts, U.K.).

Chemicals

Ketobemidone hydrochloride was obtained from H. Lundbeck A/S, Copenhagen, Denmark. The carboxylate esters **II** and **III** and the carbonate esters **IV–VIII** of ketobemidone were prepared as previously described (Hansen et al., 1991). The ester **II** was isolated as the fumarate salt whereas the other esters were used in form of hydrochloric acid salts. The glucose-Ringer solution (pH 7.4) used had a composition as previously described (Hansen et al., 1992c). Buffer substances and solvents used were of reagent grade.

Toxicity studies

The acute toxicity of ketobemidone and some prodrugs following intravenous administration was determined in NMRI mice and Sprague-Dawley rats. The animals were fasted for 20 h

prior to dosing. The body weight of the mice and rats was 24–32 and 165–190 g, respectively, at the time of dosing. After dosing the animals were placed in room wire cages with free access to food and water. Each compound was administered to five animals per dose in at least three different doses, in aqueous solution injected into the lateral veins of the tail (10 ml/kg) over 30 s. The symptoms following administration were observed 1, 3 and 6 h after dosing. The LD_{50} values after 24 h were calculated by probit analysis. Values of 0 or 100% dead animals were not used in the probit analysis; values of 5 and 95% were used instead.

Bioavailability studies in rats

Ketobemidone and the ester prodrugs **II**, **V**, **VI** and **VIII** were administered to separate groups of male Sprague-Dawley rats intravenously and buccally. The rats (320–430 g) were not fasted prior to drug administration. During the experiments the rats were anaesthetized with urethane (1.25

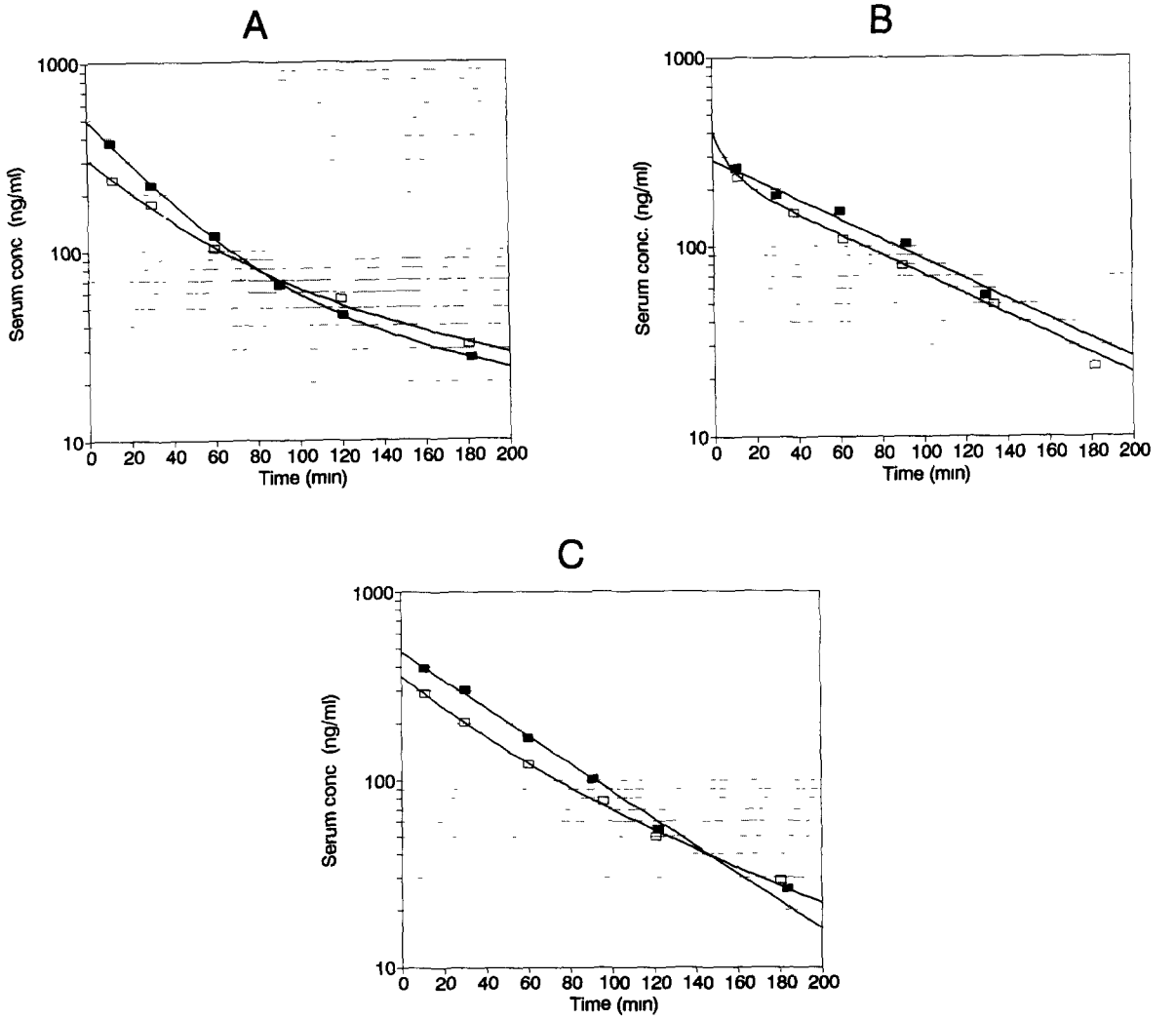


Fig. 1. Serum concentrations of ketobemidone following intravenous administration to two rats of ketobemidone (**I**) (A), prodrug **II** (B) and prodrug **V** (C) in amounts corresponding to 1.5 mg of ketobemidone hydrochloride per kg. The solid lines represent the fitted concentration.

g/kg, i.p.). Both buccal and intravenous administration was performed in rats in which the esophagus was ligated through a small incision in the breast. This ligation prevents the solution administered buccally from being swallowed. Rats were maintained on their back with their head raised. It was ensured that no leakage from the oral cavity occurred. During the experiments the rats were ventilated by room temperature atmospheric air (2 ml, 75 times per min). ECG was recorded prior to blood sampling. After administration, blood samples of 0.5 ml were withdrawn from a catheter in the jugularis vein into non-heparinized test tubes. The serum samples obtained after centrifugation for 30 min at 2000 rpm were stored at −20°C until analysis.

For buccal administration solutions of the compounds in glucose-Ringer solution (pH 7.4) were used. For ketobemidone and the prodrugs **II**, **V** and **VI**, the buccal dose was equivalent to 1.5 mg of ketobemidone hydrochloride/kg whereas the dose for the ester **VIII** was equivalent to 0.43 mg/kg. The dosing solution (0.25 ml/kg) was applied between the cheek and lower gum with a pipette equipped with a prolonged tip. There were two rats in each group.

For i.v. administration aqueous solutions of the compounds (equivalent to 1.5 mg of ketobemidone hydrochloride/kg) were given in the tail vein in a volume of 10 ml/kg over a period of 30 s. There were two rats in each group.

Analysis of serum samples

The serum samples were analyzed using an HPLC method with electrochemical detection as described by Nielsen (1986). Serum samples (200 μl) with 50 ng of morphine added as an internal standard and water (500 μl) added to increase the sample volume were injected directly on a pre-column used in the backflush mode, washed with 10 ml of water and then switched over to the chromatographic system. The detection limit was 1 ng ketobemidone base per sample injected, corresponding to 5 ng/ml serum.

Pharmacokinetic calculations

Intravenous administration. An open two-compartment model:

$$C_{se} = A_1 e^{-\alpha t} + A_2 e^{-\beta t} \quad (1)$$

was fitted to the serum concentrations (C_{se}) following i.v. administration of the substances. In Eqn 1, α is the distribution rate constant, β denotes the elimination rate constant and t is the time after administration of the compounds. The fitting was performed by an iterative non-linear least-squares regression procedure using the SIMPLEX method (Nelder and Mead, 1965). The program was rewritten from the program MULTI published by Yamaoka et al. (1981).

TABLE 1

Pharmacokinetic parameters for ketobemidone and various ketobemidone prodrugs after intravenous administration to rats of equimolar doses corresponding to 1 5 mg of ketobemidone hydrochloride per kg

Parameter		Compound **I**		Compound **II**		Compound **V**		Compound **VI**		Compound **VIII**	
		Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	Rat 7	Rat 8	Rat 9	Rat 10
A	(μg/l)	433	224		168		201	27304	157	321	107
B	(μg/l)	60	82	287	238	480	157	302	138	91	226
α	(min^{-1})	0 030	0 027		0.14		0.028	0 56	0.027	0 027	0 034
β	(min^{-1})	0 005	0 005	0 012	0.012	0.017	0.010	0.019	0.014	0.005	0 013
$t_{1/2}(\alpha)$	(min)	23	26		5 1		24	1 3	26	26	20
$t_{1/2}(\beta)$	(min)	146	129	58	56	41	37	70	51	127	55
V_1	(l/kg)	2 65	2 28	4.54	3.22	2.73	3.66	0 05	4 44	3 18	3 93
$(V_d)_{AUC}$	(kg)	10 2	10.3		5 2		5 8	1.1	6.0	8.4	4 9
V_{dSS}	(l/kg)	5.6	7 4		4 9		4.6	0.29	4 9	5 6	4.5
AUC	(μg l^{-1} min)	27059	23600	24073	20571	28130	23000	15966	16034	28447	20989
Cl_B	(l kg^{-1} min^{-1})	0.048	0.055	0.054	0.064	0.047	0.057	0.082	0.082	0.046	0.062

Buccal administration. The absolute bioavailability (F) after buccal administration was calculated using Eqn 2:

$$F(\%) = \frac{AUC_{buccal}}{AUC_{i.v.}} \times \frac{dose_{i.v.}}{dose_{buccal}} \times 100 \qquad (2)$$

where AUC is the area under the serum concentration-time curves of ketobemidone. AUC_{buccal} was determined by using the trapezoidal rule, and the residual area to time infinity was estimated using the concentration in the last serum sample divided by the terminal elimination rate constant β. $AUC_{i.v.}$ was obtained from the fitted curve by non-linear regression. The clearance (Cl_B) was calculated using the relation between dose (D) and AUC ($AUC_{i.v.} = D/Cl$).

Results and Discussion

Pharmacokinetics following intravenous administration

The pharmacokinetics of ketobemidone in rats has not been reported previously. To obtain information on this and to compare it with the pharmacokinetics of four ketobemidone prodrugs (**II**, **V**, **VI** and **VIII**), five groups of rats (two rats per group) were given the compounds by i.v. injection. Intact prodrug could not be detected in any of the serum samples in accordance with the rapid prodrug-to-drug conversion. The half-lives of hydrolysis of these esters in 80% human plasma at 37°C have previously been shown to be in the range 0.03–1.8 min (Hansen et al., 1991). Essentially the same serum levels of ketobemidone were obtained after i.v. administration of the esters and ketobemidone itself. Examples of serum concentration-time curves are shown in Fig. 1.

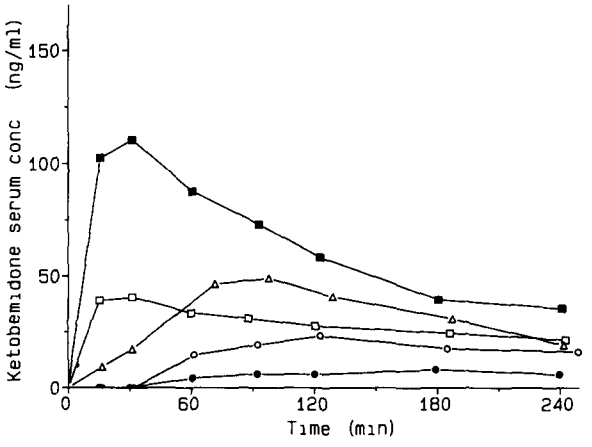


Fig 2. Mean serum concentrations of ketobemidone following buccal administration to rats ($n = 1$–2) of ketobemidone (**I**) (○), prodrug **II** (△), prodrug **V** (■), prodrug **VI** (□) in amounts corresponding to 1.5 mg of ketobemidone hydrochloride per kg and prodrug **VIII** (●) in an amount corresponding to 0 43 mg of ketobemidone hydrochloride per kg.

In eight out of the 10 rats the i.v. data could be well described by a two-compartment open pharmacokinetic model. In the remaining two rats the serum concentration-time curves were best described by a one-compartment open pharmacokinetic model as expressed by the following mono-exponential equation:

$$C_{se} = \frac{D}{V_d} \times e^{-k_e t} \qquad (3)$$

where V_d is the volume of distribution and k_e denotes the elimination rate constant.

The use of the different models may, however, be a result of differences in the frequency of serum sampling at early times after administration. Due to a very rapid distribution phase this phase may easily be overlooked. The pharmacokinetic parameters determined by curve-fitting are listed in Table 1. The mean AUC value (±S.D.) observed for the five compounds in 10 rats was 22 775 ± 4 490 $\mu g\ l^{-1}$ min.

Studies on the pharmacokinetics of ketobemidone after intravenous dosing in humans have previously been reported (Bondesson et al., 1980, 1982; Anderson et al., 1981, 1982; Tamsen et al., 1982) and both two-compartment and three-compartment models have been proposed. In these studies terminal elimination half-lives of 2.1–3.8 h, apparent distribution volumes ($(V_d)_{AUC}$) of 1.8–5.8 l kg^{-1} and plasma clearances (Cl_B) of 0.55–1.30 l kg^{-1} h^{-1} were reported. The discrepancies between the studies may be explained by the choice of different patient groups, by the use of general anaesthesia as opposed to epidural

analgesia and by the use of different protocols for blood sampling and pharmacokinetic calculations.

Buccal absorption

The major objective of the present study was to identify ketobemidone prodrugs showing increased bioavailability of the parent drug following buccal administration. Therefore, in order to establish the most promising derivative, the absorption characteristics of ketobemidone and four ester prodrugs were assessed in rats. All compounds were given in the form of aqueous solutions (glucose-Ringer solution pH 7.4) in amounts equivalent to 1.5 mg of ketobemidone hydrochloride per kg except for the ester **VIII** where the amount given was equivalent to 0.43 mg of ketobemidone hydrochloride per kg due to its lower solubility in the glucose-Ringer solution. The ketobemidone serum concentration-time curves obtained following buccal administration are shown in Fig. 2. As was the case for the i.v. administration, no intact prodrug was detected in any serum sample. The absolute bioavailabilities of the compounds calculated by using Eqn 2 are listed in Table 2. The AUC_{IV} value used in the calculations was the mean value observed in all 10 rats for the five compounds.

The results obtained show that the rate as well as the extent of absorption of the compounds varies widely. Most of the prodrugs are absorbed more rapidly than ketobemidone and all prodrugs show an increased extent of absorption relative to that of the parent drug. Lipophilicity is known to be a major determinant of buccal absorption (Hansen et al., 1992c, and references cited therein) and the increased absorption of the prodrugs may be ascribed to their higher lipophilicities relative to ketobemidone. In Fig. 3 the absolute bioavailabilities of the compounds have been plotted against their lipophilicity as expressed in terms of octanol-pH 7.4 aqueous buffer partition coefficients (Table 2).

Apparently, the bioavailability varies parabolically with the lipophilicity. Compound **V** with a log *P* value of 1.11 shows a markedly higher bioavailability than the more lipophilic esters **II**, **VI** and **VIII**. In the previous study on the in vitro permeation of the compounds through the porcine buccal membrane (Hansen et al., 1992c), the prodrugs also showed a greater permeability than ketobemidone. However, no significant differences in the permeability coefficients were seen for ketobemidone esters having log *P* values in the range 1.5–2.5.

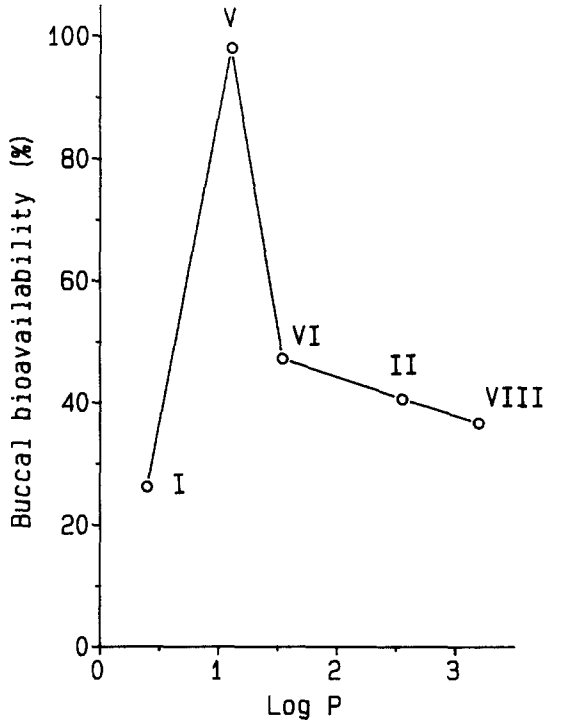


Fig 3. Bioavailability in rats of ketobemidone after buccal administration of ketobemidone (**I**) and various prodrugs plotted against log *P* for the compounds.

TABLE 2

Absolute bioavailability of ketobemidone after buccal administration of ketobemidone and various ester prodrugs to rats (n = 1–2) and partition coefficients (P) for the compounds

Compound	AUC ($\mu g\,l^{-1}$ min)		Bioavailability (%) mean	log *P* [a]
I	5968	–	26.2	0 40
II	9120	9369	40 6	2 55
V	25013	19668	98 0	1.11
VI	12939	8671	47.2	1 54
VIII	2391	–	36 6	3 20

[a] *P*: partition coefficient between octanol and 0 05 M phosphate buffer (pH 7.4) (from Hansen et al., 1991)

TABLE 3

Acute toxicity of ketobemidone and some ketobemidone ester prodrugs after i.v. administration to mice and rats

Compound	LD_{50} (mol kg^{-1}) ($\times 10^{-5}$): Mean (95% confidence limits)	
	Mice	Rats
I	24 (20–29)	3 8 (2 0–7.4)
II	16 (14–20)	6 2 (4 3–8 8)
III	10 (8–14)	
IV	16 (14–19)	
VI	17 (13–21)	3 7 (2 1–6 6)
VIII	17 (14–21)	

Toxicity

The acute toxicity of the ketobemidone esters **II–IV**, **VI** and **VIII** after i.v. injection in mice and rats (only **II** and **VI**) was determined and compared with that of ketobemidone itself. The LD_{50} values obtained are shown in Table 3. The results show that both ketobemidone and the ester prodrugs produce lower LD_{50} values after i.v. dosing to rats than to mice. This is in agreement with the findings for many other drugs. Morphine, for example, has LD_{50} values after i.v. dosing of 275 mg/kg mouse and 237 mg/kg rat (Barnes and Eltherington, 1973). The LD_{50} value found for ketobemidone after i.v. dosing in this study is of the same magnitude as that (67 mg/kg) reported after subcutaneous dosing to albino mice (Petersen, 1951). The results obtained show that there are only small differences between the LD_{50} values for the ester prodrugs and ketobemidone in the two species.

The symptoms following administration of toxic doses of the esters were similar to those following administration of ketobemidone: 'Straub-Hermann' tail reaction, increased muscular tone, convulsion and depression.

Acknowledgements

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