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The pharmacokinetics of a new benzamide drug, clebopride, in the rat and the dog

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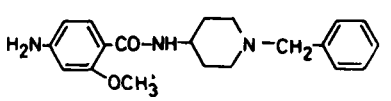
After intravenous, intramuscular and oral administration of clebopride in the rat and the dog its apparent volume of distribution is high (1·6–3·2 l kg^{-1}) and it has a longer biological half-life than metoclopramide in both species. High clearance values and concentrations of metabolites in plasma after oral administration indicate that the drug is subjected to an extensive first pass metabolism in the rat. Thus, clebopride administered orally gives relatively low concentrations in the systemic circulation in rats even though it is rapidly absorbed. The metabolic processes appear to become saturated at high doses which is reflected in dose-dependent kinetics. Linear kinetics were observed in the dog, although enterohepatic recycling could occur.

We have previously reported plasma concentration profiles of the benzamide drugs metoclopramide and sulpiride in animals given large single doses (Bakke & Segura 1976; Segura et al 1976). The most recent addition to this group of drugs is clebopride (Prieto et al 1977) which in a variety of pharmacological tests for antidopaminergic and related activities has proved, both in vitro and in vivo, to be more potent than the earlier members of the group (Elliott et al 1977; Jenner et al 1978; Magistretti & Schorderet 1978; Massó et al 1978; Mishra 1978; Roberts et al 1978; Salazar et al 1978; Roberts 1979).

Animal studies showed that metoclopramide had a large volume of distribution, was rapidly absorbed and probably suffered a first pass effect (Bakke & Segura 1976). Subsequent work in man (Teng et al 1977; Bateman et al 1978) indicated the usefulness of these animal models. In the present work with clebopride, the same animal species and protocols were used.



Clebopride

Clebopride

Compounds

The synthesis and properties of clebopride acid malate have been described (Prieto et al 1977). Purity (>99%) was demonstrated by thin layer chromatography in the following solvent systems: system 1, 1,2-dichloroethane–ethanol–24% ammonia solution (70:15:2); system 2, butanol–acetic acid–water (4:1:1); and solvent 3, isopropanol–24% ammonia solution–water (80:4:5).

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MATERIALS AND METHODS

Animal experiments

Male albino Wistar rats (200–300 g) and mongrel and beagle dogs (11·0–23·0 kg) of either sex were fasted overnight before experiments.

For oral administration, clebopride acid malate was dissolved in water and 10 ml kg^{-1} of the solution was given by stomach tube. For intravenous and intramuscular administration, a solution of clebopride acid malate was made in lactic acid, water, 1:50 (by weight). The pH was then adjusted to 4·6 with NaOH and 1 ml kg^{-1} was injected. The sites of the intravenous injections were the tail vein in the rat and a superficial vein of the forelimbs in the dog. The intramuscular injection to dogs was given deep into the gluteal muscle. The doses of clebopride acid malate corresponded to 10 mg kg^{-1} of the base intravenously, 10, 25, 50 and 100 mg kg^{-1} orally and 10 mg kg^{-1} intramuscularly.

Heparinized blood was obtained at various times after administration of the drug as reported by Bakke & Segura (1976).

Analytical methods

When the expected clebopride concentrations were higher than 1 $\mu g\ ml^{-1}$, the drug was extracted with chloroform from plasma adjusted to pH 8·0 with

0·1 M borate buffer. Concentrated extracts were applied to precoated thin-layer plates that were developed in solvent system 1. Clebopride (R_F 0·65) was assayed by photodensitometry according to Bakke & Segura (1976) and Segura et al (1976). The coefficient of variation observed by analysis of plasma samples (n = 21) containing 5 μg ml^{-1} of clebopride was 8·2%. Solvent system 2 was also used as eluent for chromatography (R_F of clebopride 0·57). The specificity of the method was investigated by analysing aliquots of plasma by two dimensional thin layer chromatography in solvent 1 or solvent 2 followed by solvent 3. The R_F value of clebopride in solvent 3 was 0·63.

Concentrations lower than 1 μg ml^{-1} were analysed by photodensitometry on the thin layer plates after in situ diazotization followed by coupling with *N*-(1-naphthyl)-ethylene-diammonium dichloride according to Huizing et al (1979 a). Extracted metabolites could be estimated by this method. In the absence of authentic reference compounds, a standard curve prepared with known amounts of clebopride was used for the assay of metabolites.

Pharmacokinetics

The intravenous data were analysed according to a one or two compartment open pharmacokinetic model by using a programmable desk calculator (Compucorp model 445 Statistician) connected online to an automatic plotter (Compucorp model 493). Linear least squares regression analysis was carried out in both cases. The back projection technique was used to estimate the α phase of the two compartments model. The pharmacokinetic parameters were calculated using conventional equations (Wagner 1975).

RESULTS

The plasma concentrations of unchanged clebopride in the rat after intravenous or oral administration are shown in Figs 1A and 1B. To facilitate comparison between species and with other benzamides (Bakke & Segura 1976; Segura et al 1976) the pharmacokinetics of intravenously administered clebopride is also summarized in Table 1. Plasma concentrations of approximately 7 μg ml^{-1} of clebopride after 5 min falling to 0·5 μg ml^{-1} after 120 min were found after intravenous administration of 10 mg kg^{-1} to the rat. After oral administration of 100 mg kg^{-1}, a bi-modal concentration-time curve in plasma was observed; the first peak (7·5 μg ml^{-1}) occurred after 30 min and the second 60 min after dosing. The drug then slowly disappeared from plasma with a half-life of approximately 84 min. When 10 mg kg^{-1} was given orally a plasma concentration of clebopride less than one tenth of that found with the higher dose (100 mg kg^{-1}) was observed. Peak concentrations in plasma appeared between 15 and 45 min (0·3–0·35 μg ml^{-1}), the elimination half-life beyond 1 h was about 27 min, and no drug was detected 3 h after dosing. The area under the concentration-time curve (AUC) was 0·319 μg h ml^{-1}, 5 times lower than expected when compared with the high dose (15·58 μg h ml^{-1}) if linear kinetics were followed.

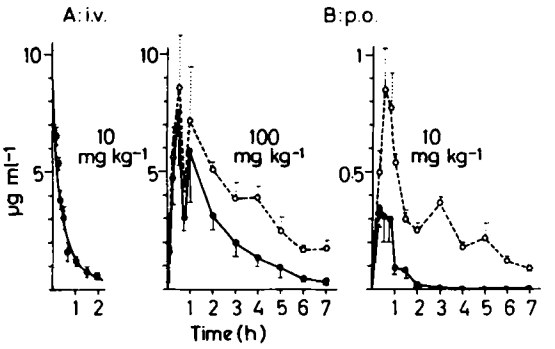

FIG. 1. Concentrations of clebopride (●) and the sum of the unchanged drug plus its metabolites (○) in plasma from individual rats killed at different times after intravenous injection of the drug (A) or after administration by stomach tube (B). Each point represents the mean value of four animals. Vertical bars are units of s.e.m. The curve in (A) is the theoretical line for the two compartment open model according to the parameters presented in Table 1.

In previous studies (Huizing et al 1979b, 1980; Segura et al 1980a) the following metabolic products

Table 1. Pharmacokinetic parameters for unchanged clebopride after intravenous injection of 13·6 μg kg^{-1} of clebopride acid malate (equivalent to 10 mg kg^{-1} of the base) in Wistar rats and beagle dogs.

Parameters[a]		Rat	Dog
A	μg ml^{-1}	5·86	1·13
B	μg ml^{-1}	2·98	2·80
C_0	μg ml^{-1}	8·84	3·93
$t\frac{1}{2}\alpha$	min.	12·89	17·48
$t\frac{1}{2}\beta$	min.	46·80	104·40
V_1	l kg^{-1}	1·13	2·54
V_2	l kg^{-1}	0·49	0·63
V_d (ss)	l kg^{-1}	1·62	3·17
k_{12}	h^{-1}	0·72	0·45
k_{21}	h^{-1}	1·68	1·81
k_{el}	h^{-1}	1·72	0·52
Clearance	ml min^{-1} kg^{-1}	32·36	22·14
Total area	μg h ml^{-1}	5·15	7·53

[a] According to the two compartment open model of Riegelman et al (1968) and expressed by conventional symbols (Greenblatt & Kock-Weser 1975).

of clebopride had been identified: *N*-(4′-piperidyl)-2-methoxy-4-amino-5-chloro benzamide (metabolite III), *N*-(1′-α-hydroxybenzyl-4′-piperidyl)-2-methoxy-4-amino-5-chloro benzamide (tentative structure for metabolite IV) and *N*-(4′-piperidyl-2′-one)-4-amino-5-chloro-2-methoxy benzamide (metabolite V). The chromatograms also permitted evaluation of metabolites extracted from plasma. Small amounts of metabolites III, IV and V were detected after intravenous injection to rats. However, after oral administration of the drug, as many as eleven coloured spots were detected by diazotization and coupling. The main spots in addition to that of the unchanged compound corresponded to metabolite III and metabolite V. Another unidentified compound (R_F 0·49 in solvent system 2) was also an important metabolite in plasma. When the sum of unchanged clebopride plus its metabolites in plasma were considered (Fig. 1B), the total bioavailability obtained after giving 10 mg kg^{-1} (AUC 2·01 μg h ml^{-1}) was approximately equivalent to one tenth of that obtained when 100 mg kg^{-1} (AUC 27·1 μg h ml^{-1}) was administered.

Intravenous administration to beagle dogs (Fig 2A, Table 1) also gave low concentrations of clebopride in plasma and consequently its apparent volume of distribution is high (3·17 l kg^{-1}). The elimination half-life is much longer in the dog (104 min) than in the rat (47 min) although the half-life of the distribution phase was similar in both species. In all of the dogs given clebopride orally (Fig 2B), the absorption was rapid, i.e. high concentrations were detected 15–30 min after dosing; elimination from the plasma was slower than after intravenous injection. Plasma concentrations were maintained for 3–5 h and began to decrease thereafter. The animals given higher doses (100 mg kg^{-1}) showed signs of serious toxicity. Both the blood concentrations and the areas under the concentration-time curves were in reasonable agreement with linear pharmacokinetics for the drug in this species.

Only traces of metabolites III, IV and V were detected after intravenous injection of the drug to dogs, and the metabolic profile after oral dosing was much simpler than in the rat. Two compounds (metabolite III and IV) appeared in all samples although they never exceeded 1 μg ml^{-1}. Higher doses were associated with prolonged appearance of metabolite IV. The levels of metabolite III were relatively constant in all samples at a given dose, and traces of metabolite V were also detected on the chromatograms.

Clebopride by intravenous injection was compared with metoclopramide in a cross-over experiment in four mongrel dogs. The results with metoclopramide were presented by Bakke & Segura (1976) and those obtained with clebopride are now presented in Fig. 3A. Plasma concentrations were not significantly different from those obtained in beagle dogs (Fig. 2A) although to compare the pharmacokinetics a single compartment open pharmacokinetic model

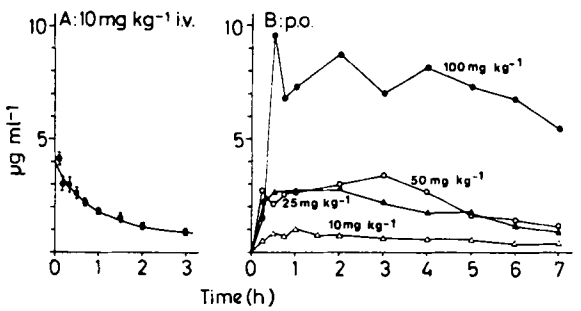

FIG. 2. Concentrations of clebopride in plasma from beagle dogs given the drug by intravenous injection (A) or by stomach tube (B). Mean values with s.e.m. (n = 4) are shown in (A). Mean values of three (25 and 50 mg kg^{-1}) or two (10 and 100 mg kg^{-1}) dogs are presented in (B). The curve in (A) is the theoretical line for the two compartments open model according to the parameters presented in Table 1.

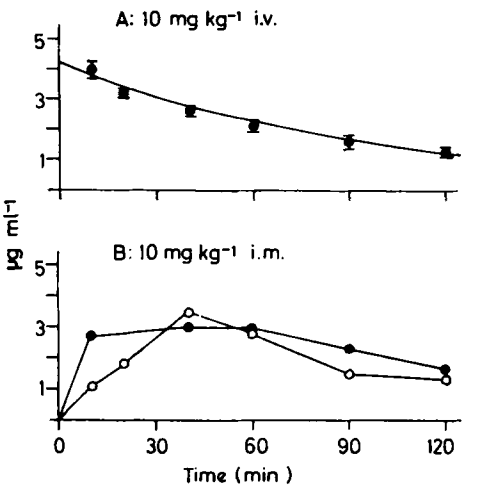

FIG. 3. Concentrations of clebopride in plasma from mongrel dogs given the drug by intravenous (A) or intramuscular injection (B). Mean values with s.e.m. (n = 4) are shown in (A), whereas individual values are shown in (B). The curve in (A) is the theoretical line for the one compartment open model according to the parameters presented in the text.

was used. The main pharmacokinetic parameters for clebopride were (mean ± s.e.m.): V_d 2·43 ± 0·18 l kg^{-1}; half-life 69·5 ± 9·5 min, and clearance 25·4 ± 3·4 ml $min\ kg^{-1}$. Intramuscular injection of clebopride in the dog (10 mg kg^{-1}) gave plasma concentrations similar to those observed after intravenous administration of the same dose (Fig. 3B).

DISCUSSION

The metabolites of clebopride did not interfere with the detection of clebopride as was demonstrated by two dimensional t.l.c. of plasma extracts; consequently, the analytical methods appear specific. However, when diminution of background fluorescence was used, interindividual differences in blank plasma were a complicating factor. In the rat, concentrations of 0·5 $\mu g\ ml^{-1}$ or higher could be accurately determined, whereas concentrations of 0·2 $\mu g\ ml^{-1}$ could be estimated when blank plasma was available from the same animal (dog). For its higher sensitivity and selectivity, the colorimetric method of Huizing et al (1979a) is recommended when the expected concentrations are lower than 1 μg clebopride ml^{-1}.

The apparent volume of distribution of clebopride is higher in rats and dogs than that of metoclopramide and sulpiride in both species (Bakke & Segura 1976; Segura et al 1976). In a cross-over study in mongrel dogs, the mean apparent volume of distribution of metoclopramide (1·27 l kg^{-1}) was significantly different ($P < 0·02$, paired t-test) from clebopride (2·43 l kg^{-1}) in a one compartment open pharmacokinetic model. In both species, however, the clebopride data are pharmacokinetically better described when an initial distributive phase (half-life 12–18 min) is considered (Table 1). The use of a two compartments open model to describe the pharmacokinetics of metoclopramide in the rat has been suggested by other authors (Tuñón et al 1974; Tam & Axelson 1978). The figures for the volumes of distribution cited above should therefore be cautiously taken into account in their absolute value. An underestimation has been produced by using the monocompartimental approach. Correct pharmacokinetic parameters for clebopride are presented in Table 1.

As observed with metoclopramide and with sulpiride, the clearance of clebopride was high in the rat (Table 1). Since little clebopride is excreted unchanged (Segura et al 1980a), metabolism accounts for the major part of the total clearance calculated. A high metabolic clearance approaching the hepatic blood flow is a characteristic feature of drugs undergoing extensive first pass metabolism (Nies et al 1976). The rapid appearance of metabolites in rat plasma after oral administration of clebopride confirms the existence of a first pass effect. The metabolic processes appear to be saturated at high doses leading to dose-dependent kinetics, though this is not the case when the metabolites are taken into account (Fig. 1B). Optimal absorption of total radioactivity was demonstrated after giving 0·5 mg kg^{-1} of [^{14}C]clebopride orally to rats (Segura & Borja, to be published); therefore, in spite of the low systemic bioavailability of unchanged clebopride in this species, the absorption of the drug when given by the oral route is high at any dosage level.

In other benzamides, e.g. metoclopramide and sulpiride (Teng et al 1977; Sugnaux & Benakis 1978; Imondi et al 1978), the importance of biotransformation processes decrease in the order rat >dog>monkey>man. Metabolism of clebopride in the dog is far less important than in the rat and linear pharmacokinetics appear to apply (Fig. 2B). The half-life of clebopride after oral administration to the dog is high and, as extensive biliary excretion of radioactivity has been shown in the rat and the dog after administration of [C^{14}]clebopride acid malate (Segura & Borja, to be published), it is probable that enterohepatic recycling may play an important part in maintaining plasma concentrations. The biliary excretion in the dog is supported by the fact that as much as 64–76% of total radioactivity is found in faeces from dogs given 0·2 mg kg^{-1} of [^{14}C]clebopride either orally or intravenously (Segura and Borja, to be published). The bi-modal concentration-time curve obtained at 100 mg kg^{-1} p.o. in the rat (Fig. 1B) also confirms that an enterohepatic circulation probably occurs in this species.

In conclusion, clebopride is a new benzamide drug with some pharmacokinetic features in common with related compounds. As with other benzamides, the drug concentration in the systemic circulation is low after oral administration to rats as a result of an extensive first pass metabolism. Minor interspecies variations in the apparent volume of distribution after intravenous injection have been observed. However, as the size of the animal species (rat; rabbit, Segura et al 1980b; and dog) increases, the half-life of clebopride and sulpiride also increase when compared to metoclopramide. It is to be expected therefore that clebopride will have a longer half-life and duration of effect than metoclopramide in man. Nevertheless, currently available methods of analysis are not sufficiently sensitive to measure clebopride in man because of the high volume of distribution of the drug and the low clinical dose.

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