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Oral, Intraperitoneal and Intravenous Pharmacokinetics of Deramciclane and its *N*-desmethyl Metabolite in the Rat

KATALIN BALOGH NEMES, MIKLÓS ABERMANN, ERZSÉBET BOJTI, GYULA GRÉZAL, SAMAR AL-BEHAISI AND IMRE KLEBOVICH

Department of Pharmacokinetics, EGIS Pharmaceuticals Ltd, Budapest, Hungary

Abstract

The pharmacokinetic properties of deramciclane fumarate (EGIS-3886), a new potential anxiolitic agent, and its *N*-desmethyl metabolite have been investigated in Wistar rats after $10\,mg\,kg^{-1}$ deramciclane fumarate was administered orally, intraperitoneally or intravenously.

A highly sensitive, validated and optimized gas chromatographic method with nitrogen selective detection (GC-NPD) using a solid-phase extraction technique was used to determine plasma levels of the parent compound and its *N*-desmethyl metabolite.

After oral administration the absorption of the parent compound was very fast (t_{max} 0·5 h). The maximum plasma concentration (C_{max}) was detected at 44·9, $\geq$177·8 and $\geq$2643·0 ng mL^{-1} after oral, intraperitoneal and intravenous administration of deramciclane, respectively. For the metabolite the respective C_{max} values were 32·0, $\geq$25·4 and 51·0 ng mL^{-1}. The pharmacokinetic curves of both the parent compound and its metabolite showed enterohepatic recirculation for all administration routes. The biological half-life ($t_{\beta 1/2}$) for deramciclane ranged from 3·42 to 5·44 h and for the *N*-desmethyl metabolite the range was 2·90–5·44 h, after administration of the drug by the three different routes. After intravenous administration $AUC_{0-\infty}$ of deramciclane was 29·2- and 5·4-times higher than that observed after oral and intraperitoneal treatment, respectively. These $AUC_{0-\infty}$ ratios were only 2·1- and 1·5-times higher for the metabolite. The absolute bioavailability of deramciclane in rats was 3·42% after oral and 18·49% after intraperitoneal administration.

The comparative pharmacokinetic study of deramciclane in rat after the different administration routes showed fast absorption. Furthermore, plasma levels were found to be administration route-dependent, low bioavailability of the parent compound indicated an extremely fast and strong first-pass metabolism. The apparent volume of distribution suggested strong tissue binding after administration of the drug by any of the three routes studied.

Deramciclane fumarate (EGIS-3886; (1*R*, 2*S*, 4*R*)-(−)-*N*,*N*-dimethyl-2-{(1,7,7-trimethyl-2-phenylbicyclo-[2,2,1]-hept-2-yl)oxy}-ethanamine-2-(E)-butendioate (1 : 1)); Figure 1) is a new anxiolytic compound with high affinity for 5-HT_{2A} and 5-HT_{2C} receptors (Gacsályi et al 1996, 1997; Pälvimäki et al 1998).

Absorption of the drug was found to be very fast and complete from various isolated small intestine sections (Lengyel et al 1998). After oral administration of the ^{3}H-labelled drug in rats, a dominant enterohepatic recirculation was observed (Pátfalusi et al 1997). Investigations using ^{3}H- and ^{14}C-labelled radioisomers in rats indicated that elimination of deramciclane was not dose-dependent and showed lack of linearity between dose and $AUC_{0-\infty}$ value (Bojti et al 1998).

Klebovich et al (1998) conducted extensive pharmacokinetic studies in several animal species and in man after the administration of a single oral dose of $3\,mg\,kg^{-1}$ of the drug. Deramciclane is subject to intense metabolism in different species,

Correspondence: I. Klebovich, EGIS Pharmaceuticals Ltd., Department of Pharmacokinetics, H-1475 Budapest, PO Box 100, Hungary.

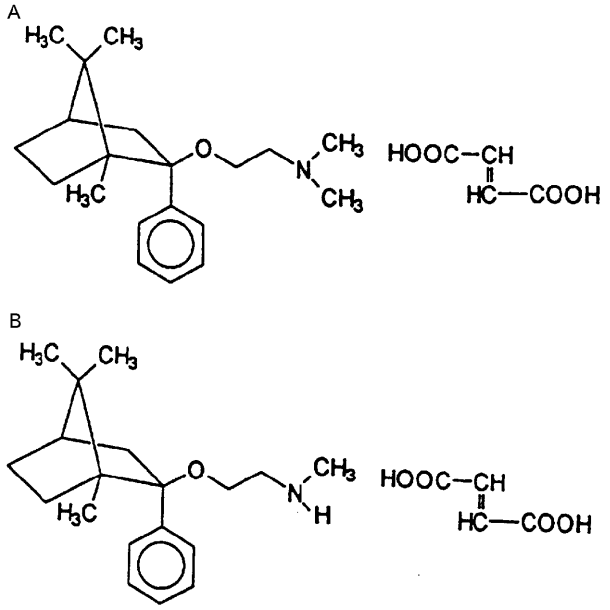

Figure 1. Chemical structure of deramciclane fumarate (A) and *N*-desmethyl-deramciclane fumarate metabolite (B).

as has been demonstrated in-vitro (Monostory et al 1994) and in a pilot in-vivo study (Hazai et al 1995), with the rate and extent of its metabolism varying between species. Moreover, whole-body autoradiography and quantitative organ-level studies of deramciclane in rats demonstrated no specific organ targeting of the drug with no sex difference in fast drug distribution (Hazai et al 1999). The *N*-desmethyl metabolite (EGIS-7056) appeared in the plasma samples of each species. Although the levels of metabolite were different in the various species, the plasma level of *N*-desmethyl metabolite was always significant as compared with that of the parent compound (Klebovich et al 1998). Deramciclane was highly (95–98%) bound to plasma proteins in mouse, rat, dog, rabbit and man (Visy et al 1996).

In this study, comparative pharmacokinetics of deramciclane fumarate have been investigated after a single $10\,mg\,kg^{-1}$ oral, intraperitoneal or intravenous dose in Wistar rats using a gas chromatographic method with nitrogen selective detection (GC-NPD).

Materials and Methods

Standards and chemicals

Deramciclane fumarate (EGIS-3886), *N*-desmethyl-deramciclane fumarate (EGIS-7056) metabolite standard (Figure 1), and bencyclane fumarate (internal standard for the bioanalytical work) were all synthesized by EGIS Pharmaceuticals Ltd (Budapest, Hungary). For extraction of rat plasma, the following chemicals were used: water, HPLC grade; acetic acid, analytical grade; 2-propanol, methanol, acetone, for residue analysis grade (Merck, Darmstadt, Germany); acetonitrile, analytical grade (J. T. Baker & B. V. Deventer, The Netherlands). For the extraction of plasma samples, LiChrolut solid phase extraction column with 200 mg RP-18 packing (Merck) was used.

Animals and administration

The pharmacokinetic experiments were performed on healthy male Wistar rats, in three independent studies. General parameters of experimental animals are described in Table 1. Rats were kept under standardized conditions (temperature $22 \pm 2°C$; relative humidity 40–60%) and acclimatized for a minimum of five days before treatment. They were fed pelleted Altromin rat feed (Lati Kft, Gödöllõ, Hungary) and had free access to water throughout the experiment. After fasting for 16 h, rats were treated with a single $10\,mg\,kg^{-1}$ deramciclane fumarate dose. For each experiment, animals received the drug via a different route i.e. oral, intraperitoneal or intravenous, at the same dose level.

For studying the pharmacokinetics of deramciclane in rats, six animals were treated for each sampling time. Blood sampling times were: before treatment, 0·167, 0·333, 0·5, 0·75, 1, 1·5, 2, 2·5, 3, 4, 6, 8, and 12 h following treatment in all experiments. The intraperitoneal experiment had an additional sampling time 16 h following treatment. The intravenous experiment had additional sampling times at 0·0167, 0·0833, and 16 h following treatment. An anticoagulant, 0·5 mL 3% Na_2–EDTA, was added to the 5-mL blood samples. Plasma was separated by centrifugation and stored below $-18°C$ until further processing.

Table 1. Data relating to the Wistar rats used in the comparative study.

Parameters	Route of administration		
	Oral	Intraperitoneal	Intravenous
Sex	Male	Male	Male
Age (weeks)	7–9	6–8	7–9
Range of body weight (g)	140–213	117–186	145–200
Mean body weight ± s.d. (g)	167·5 ± 16·36	161·5 ± 14·71	168·3 ± 11·07

Bioanalytical methods

Deramciclane, its metabolite and the internal standard (bencyclane fumarate) were extracted from rat plasma with LiChrolut solid-phase extraction columns. Determination of plasma concentration of the analytes was performed using a Hewlett-Packard HP 5890 Series II gas chromatograph equipped with nitrogen–phosphorous selective detector (GC-NPD), an electronic pressure controller (EPC), and an HP 7673A autosampler (Hewlett-Packard, Palo Alto, CA). The bioanalytical extraction method was optimized by ^{14}C-labelled deramciclane, and the EPC and temperature programming were optimized for separation (Klebovich et al 1995; Balogh Nemes et al 1996). The lower limit of quantification of the validated bioanalytical method was 0·5 and 3 ng mL^{-1} for the parent compound and *N*-desmethyl metabolite, respectively. The inter-day precision and accuracy of deramciclane and *N*-desmethyl metabolite were measured at seven or six concentration levels on six days. The mean values (n = 42) for the parent compound amounted to 9·14% and 3·23% for the precision and accuracy, respectively. For the *N*-desmethyl metabolite, these mean values (n = 42) amounted to 12·27% and 0·13%, respectively.

Pharmacokinetic analysis

The pharmacokinetic parameters were determined on the basis of average plasma concentration–time data using SIPHAR/WIN ver. 1·12 (SIMED SA. Biostatics and Data Processing, Créteil Cedex, France) validated pharmacokinetic software package. For maximum plasma concentration (C_{max}) and time to reach maximum plasma concentration (t_{max}), measured and observed values were presented for oral and intraperitoneal administration. For intravenous administration, the value detected at the first blood sampling point (1 min after administration) was denoted as C_{max}. A two-compartment open model was used to calculate the pharmacokinetic parameters. The trapezoidal rule (AUC_{0-t}) and extrapolation to infinity ($AUC_{0-\infty}$) were used to determine the area under the plasma concentration–time curve. AUC_{Rest} values are the percentage of the AUC that is extrapolated. The terminal elimination rate constant (β) was calculated by linear regression using the least-squares method, which fitted a straight line to the terminal phase of the semi-logarithmic plot of the plasma concentration–time data. Biological half-life of elimination ($t_{\beta 1/2}$) was calculated using the formula $0{\cdot}693/\beta$. The volume of total body clearance (CL_{Tot}) and those of the apparent volume of distribution (Vd) were calculated as Dose/$AUC_{0-\infty}$ and as Dose/$\beta AUC_{0-\infty}$, respectively. The mean residence time (MRT) was determined using the formula $AUMC/AUC_{0-\infty}$.

Results and Discussion

The results of the bioanalytical analysis of average deramciclane plasma levels obtained after oral, intraperitoneal or intravenous administration are compared in Figure 2. The plasma concentration curves of the *N*-desmethyl metabolite obtained after the administration of a single 10 mg kg^{-1} deramciclane fumarate dose to rats were closely similar regardless of route of administration (Figure 3). The individual plasma concentrations showed an inter-subject variability similar to other studied species. Extremely rapid absorption was detected ($t_{max} = 0{\cdot}5$ h) for deramciclane after oral administration (Figure 2). For the metabolite the same t_{max} value was observed after oral administration of the parent compound (Figure 3). Maximum plasma concentration (C_{max}) values of deramciclane were 44·94, ≥177·80 and ≥26430 ng mL^{-1} after oral, intraperitoneal and intravenous administration of the parent compound, respectively (Table 2). C_{max} values for *N*-desmethyl metabolite were 31·99, 25·40 and ≥51·0 ng mL^{-1} after oral, intraperitoneal and intravenous deramciclane treatment, respectively (Table 3). The elimination half-life ($t_{\beta 1/2}$) of deramciclane was almost similar after oral, intraperitoneal or intravenous administration (Table 2). Deramciclane could be traced in rat plasma 16 h after either intravenous or intraperitoneal dosing of the drug, as compared with 12 h after oral treatment (Figure 2). Plasma concentrations of *N*-desmethyl metabolite showed similar tendencies (Figure 3). Since the parent compound and the *N*-desmethyl

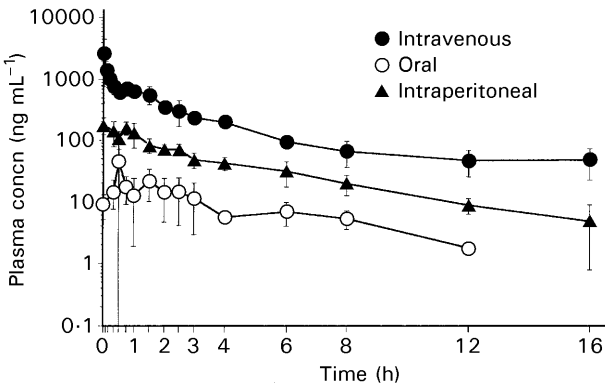

Figure 2. Pharmacokinetic curves of deramciclane in rats. Animals were administered a single dose of 10 mg kg^{-1} deramciclane fumarate by either the oral, intravenous or intraperitoneal route. Values are mean ± s.d. (n = 6 p.o., i.v.; n = 8 i.p.).

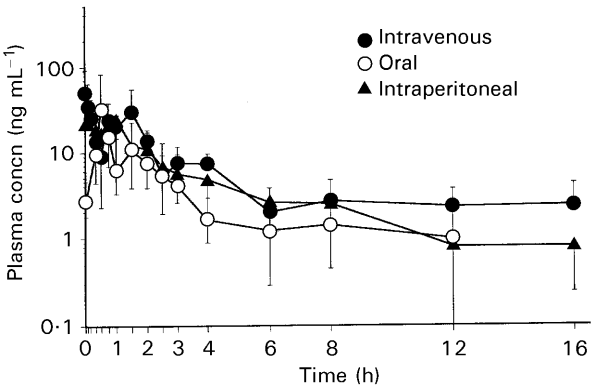

Figure 3. Pharmacokinetic profile of *N*-desmethyl deramciclane in rats. Animals were administered a single dose of 10 mg kg^{-1} deramciclane fumarate by either the oral, intraperitoneal or intravenous route. Values are mean ± s.d. (n = 6 p.o., i.v.; n = 8 i.p.).

Table 2. Pharmacokinetic parameters of deramciclane in rat after a single dose of 10 mg kg^{-1} deramciclane fumarate administered by either the oral, intraperitoneal or intravenous route.

Pharmacokinetic parameter	Route of administration		
	Oral	Intraperitoneal	Intravenous
C_{max} (ng mL^{-1})	44·94	≥177·80	≥2643·00
t_{max} (h)	0·50	≥0·166	–
AUC_{0-t} (ng h mL^{-1})	97·06	540·49	2844·92
$AUC_{0-\infty}$ (ng h mL^{-1})	106·95	578·18	3127·53
AUC_{Rest} (%)	9·24	6·97	9·04
$t_{\beta 1/2}$ (h)	3·89	5·44	3·42
CL_{Tot} kg^{-1} (L h^{-1} kg^{-1})	93·50	17·30	3·20
Vd kg^{-1} (L kg^{-1})	525·20	135·80	15·80
MRT (h)	5·10	5·17	5·15
F (%)	3·42	18·49	–

metabolite appeared in the systemic circulation at the same time, a very fast first-pass metabolism seemed probable (Figure 2 and 3).

On the average pharmacokinetic curve of deramciclane, enterohepatic recirculation could be seen (Figure 2) after oral, intraperitoneal or intravenous administration. Similar enterohepatic recirculation was observed for the *N*-desmethyl metabolite for all three routes of administration (Figure 3). This pharmacokinetic phenomenon was observed after the oral and intravenous dose of 3 mg kg^{-1} [^{3}H]deramciclane (Pátfalusi et al 1997), and the bile excretion study of Lengyel et al (1998). It was suggested that the parent compound and its metabolite have a significant enterohepatic recirculation in rat, which could not be observed in dog, rabbit or man (Klebovich et al 1998).

Table 3. Pharmacokinetic parameters of *N*-desmethylderamciclane in rat after a single dose of 10 mg kg^{-1} deramciclane fumarate administered by either the oral, intraperitoneal or intravenous route.

Pharmacokinetic parameter	Route of administration		
	Oral	Intraperitoneal	Intravenous
C_{max} (ng mL^{-1})	31·99	25·40	≥51·00
t_{max} (h)	0·50	1·00	–
AUC_{0-t} (ng h mL^{-1})	41·37	65·88	95·02
$AUC_{0-\infty}$ (ng h mL^{-1})	49·07	69·60	107·56
AUC_{Rest} (%)	16·13	8·02	12·51
$t_{\beta 1/2}$ (h)	5·44	3·23	2·90

$AUC_{0-\infty}$ values found were 106·95, 578·18 and 3127·53 ng h mL^{-1} after 10 mg kg^{-1} oral, intraperitoneal and intravenous deramciclane treatment, respectively. After single intravenous administration the $AUC_{0-\infty}$ value calculated for the unchanged compound was 29·2-times higher than the value after oral treatment. This ratio was 5·4 when the intravenous administration was compared with the intraperitoneal treatment. The calculated $AUC_{0-\infty}$ value for the *N*-desmethyl metabolite showed minor differences between the three administration routes (Table 3). For the metabolite the $AUC_{0-\infty}$ ratios of intravenous/oral and intravenous/intraperitoneal were found to be 2·1- and 1·5-times higher, respectively (Table 3).

The absolute bioavailability (F,%) of deramciclane in rats was relatively low after oral (3·42%) and after intraperitoneal (18·49%) administration. This indicates an intensive first-pass metabolism and biotransformation since previous absorption studies demonstrated that [^{14}C]- and [^{3}H]deramciclane were completely absorbed from various sections of the small intestine (Lengyel et al 1998).

Total body clearance (CL_{Tot} kg^{-1}) values after deramciclane administration by the different routes (oral, intraperitoneal, intravenous) were found to be 93·5, 17·3, 3·2 L^{-1} kg^{-1}, respectively. The values of the apparent volume of distribution (Vd kg^{-1}) after oral administration of deramciclane indicated strong tissue binding in rat, similar to that seen in dog, rabbit and man (Klebovich et al 1998). MRT values were almost the same regardless of route of deramciclane administration (Table 2).

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