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PHARMACOKINETICS OF BROTIZOLAM IN HEALTHY SUBJECTS FOLLOWING INTRAVENOUS AND ORAL ADMINISTRATION

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1 Pharmacokinetics and bioavailability of brotizolam after i.v. and oral administration were studied in healthy young volunteers.

2 Kinetic parameters after i.v. administration were: volume of distribution 0.66 ± 0.19 l/kg, total plasma clearance 113 ± 28 ml/min, distribution half-life 11 ± 6 min, and elimination half-life 4.8 ± 1.4 h (mean values ± s.d.).

3 Kinetic parameters after oral administration were: absorption lag-time 8 ± 12 min, absorption half-life 10 ± 11 min, and elimination half-life 5.1 ± 1.2 h (mean values ± s.d.).

4 Bioavailability of brotizolam was 70 ± 22% when calculated by comparing oral and intravenous area-under-curve values, corrected for intra-individual half-life differences. An alternative calculation method, which is relatively independent of large clearance variations, provided a bioavailability of 70 ± 24% (range: 47–117%).

Keywords brotizolam i.v. and oral administration pharmacokinetics elimination half-life healthy volunteers

Introduction

In recent years, several new benzodiazepine derivatives have been developed in the search for hypnotics with an improved pharmacodynamic and pharmacokinetic profile, i.e. drugs which have satisfactory hypnotic activity but which are free of residual sequelae. Progress in that direction was achieved by the introduction of a heterocyclic ring structure across the 1,2-position of the azepine ring. The first compound with such a ring structure used clinically was triazolam, a triazolo-1,4-benzodiazepine. This drug has proved to be a potent hypnotic with a relatively short duration of action (Pakes *et al.*, 1981)

Another triazolo-derivative is brotizolam, a 1,4-thienodiazepine, which is still in the stage of clinical development. Early human investigations have shown that this drug has good sleep-inducing properties and that no residual effects on performance occur with a dose comparable to that of triazolam (0.25 mg) (Saletu *et al.*, 1979; Nicholson *et al.*, 1980)

The pharmacokinetics of brotizolam have hitherto not been studied extensively. Data on the rate and extent of drug absorption, distribution and elimination are considered highly relevant to the assessment of hypnotic drug treatment (Breimer, 1979; Breimer & Jochemsen, 1981). We have studied the pharmacokinetics and bioavailability of brotizolam following oral and i.v. administration to healthy volunteers, using a specific assay method for the determination of unchanged brotizolam in plasma.

Methods

Experimental design

The subjects were eight healthy young volunteers, aged 21–26 years: five males and three females (Table 1). All subjects had normal renal and hepatic function and, apart from oral contraceptives (combination pills) taken by the females, they used no medication. The protocol was reviewed by an ethical committee and the volunteers gave their written informed consent. They received the drug orally and i.v. with intervals of at least 3 weeks

Table 1 Subject characteristics.

Subject	*Gender*	*Age (years)*	*Weight (kg)*	*Height (m)*	*Cigarettes (number/day)*
1	F	21	52	1.61	>15
2	F	23	58	1.61	5–10
3	F	26	72	1.68	5–10
4	M	24	82	1.86	>15
5	M	24	63	1.80	—
6	M	22	73	1.82	5–10
7	M	23	76	1.80	5–10
8	M	24	76	1.76	>15

between treatments. For i.v. administration, a solution of 0.10 mg brotizolam in 1 ml alcohol was diluted 1 to 10 with a physiological salt solution immediately before injection. The dose (0.25 mg) was given as a bolus injection over 4 min. For oral administration tablets containing 0.50 mg brotizolam (Lendormim^R) were given with 150 ml water.

The studies began at about 09.00 h after the subjects had fasted from the previous night (24.00 h) and no food, fluid or tobacco was allowed for 3 h after drug administration. During this period the subjects remained in a supine position. Venous blood samples were drawn from a flexible cannula during the first day of the experiment and by separate venepunctures during the rest of the experiment at the following times after drug administration: 20, 40, 60, 80, 100 and 120 min and 3, 4, 6, 9, 12, 24, 32 and 48 h. Following i.v. administration additional samples were taken at 15, 30 and 50 min. All blood specimens were collected in heparinized tubes and after separation the plasma samples were stored at −20 °C until analysis.

Analysis of plasma

Concentrations of unchanged brotizolam were determined by a similar capillary g.l.c.-EC method as that described for triazolam (Jochemsen & Breimer, 1981). The column used was a SCOT-column with Tullanox (silanized fumed silica, particle size $\leq 10\,\mu m$) as support layer and with 0.5% PPE-21 and 3%OV-17 as stationary phase. Operating temperatures were: column, 250°C; injection port and detection, 350 °C. The carrier flow (helium) was 10 ml/min and the flow of the auxiliary gas (argon-methane 95 : 5) was 25 ml/min. As for triazolam, a solid injection system was used. Using clonazepam as internal standard (5 ng/ml), calibration graphs were linear in the range 0.1–10 ng/ml. Correlation coefficients were not less than 0.999. The coefficient of variation ($n = 3$) was below 8.6% in the range 0.25–10 ng/ml and 20% for 0.1 ng/ml.

Analysis of data

Pharmacokinetic analysis was performed in two ways. First, compartmental analysis of the data was performed in which the following functions were fitted to plasma concentrations using weighted non-linear regression analysis (Metzler *et al.*, 1974):

$$\text{i.v.:}\quad C = C_1 \cdot e^{-\lambda_1 t} \qquad (1)$$

or

$$C = C_1 \cdot e^{-\lambda_1 t} + C_2 \cdot e^{-\lambda_2 t} \qquad (2)$$

$$\text{oral:}\quad C = C_1(e^{-\lambda_1(t-t_{lag})} - e^{-k_a(t-t_{lag})} \qquad (3)$$

in which t_{lag} is the absorption lag-time. In equations (1) and (3), C_1 is a hybrid intercept term, λ_1 is the apparent first-order elimination rate constant and k_a is the apparent first-order absorption rate constant. In equation (2), C_1 and C_2 are hybrid intercept terms and λ_1 and λ_2 are hybrid coefficients representing rate constants of the initial exponential phase (drug distribution phase) and terminal exponential phase (elimination phase) respectively. Measurements were weighted as the reciprocal of the observed value. The choice between equations (1) and (2) as functions of best fit was based upon the scatter of actual data points about the fitted function and by comparison of weighted residual errors. Coefficients and exponents of the fitted functions were used to calculate the following pharmacokinetic parameters: distribution half-life ($t_{1/2,\lambda_1}$), absorption half-life ($t_{1/2,abs}$), elimination half-life ($t_{1/2,z}$), area under the curve (AUC) and, following i.v. administration, the initial distribution volume (V_1), the total apparent volume of distribution (V) and total clearance (CL).

Second, some parameters were calculated without stringent modelling of the data. Elimination half-lives ($t_{1/2,z}$) were determined by linear regression analysis of the terminal points of the log concentration *vs* time curves. For the calculation of the area under the curves, the trapezoidal rule with extrapolation to infinity was applied. Clearance and apparent volume of distribution were calculated according to:

$$CL = \frac{D}{AUC} \quad (4)$$

$$V = \frac{CL \times t_{1/2,z}}{0.693} \quad (5)$$

The apparent systemic availability (F) of the oral dosage form was determined by two equations:

$$F = \frac{D_{iv} \times AUC_{po} \times t_{1/2,z,iv}}{D_{po} \times AUC_{iv} \times t_{1/2,z,po}} \quad (6)$$

and a recently introduced alternative method, which takes intra-individual differences in drug elimination rates into account (Collier & Riegelman, 1981):

$$F = \frac{D_{iv}}{D_{po}} \times \frac{AUC_{po}}{AUC_{iv}} \times \frac{MRT_{iv}}{\left[MRT_{iv} - \frac{1}{\lambda_{z,iv}} + \frac{1}{\lambda_{z,po}}\right]} \quad (7)$$

in which MRT_{iv} = AUMC/AUC and AUMC = the area under the first moment curve from zero to infinite time.

Results

Representative examples of the plasma concentration-time curves following oral and i.v. administration are given in Figure 1 for subject 8.

I.v. administration

Following i.v. administration, brotizolam concentrations declined rapidly until about 1 h after drug administration. The subsequent plasma concentration decay could be fitted to a straight line on a semi-logarithmic scale, indicating a first-order process of elimination. In six subjects the disappearance of brotizolam from plasma was consistent with equation (2) and in one subject with equation (1). The pharmacokinetic parameters are given in Table 2. The initial distribution phase proceeded with half-lives of less than 20 min. The mean value of the elimination half-life was 4.8 h and the mean apparent volume of distribution (V) was 0.66 l/kg. Total clearance was 113 ml/min. When linear regression was applied to the terminal part of the concentration curves and the AUC determined by the trapezoidal rule with extrapolation to infinity, then the following data were obtained: mean elimination half-life 4.7 ± 1.4 h, apparent volume of distribution 0.63 ± 0.14 l/kg and total clearance 109 ± 29 ml/min. There were no significant differences between the two calculation methods for these three parameters.

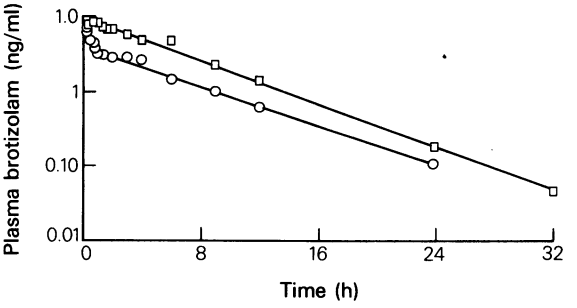

Figure 1 Brotizolam plasma concentrations and fitted functions for subject 8 following i.v. (○, 0.25 mg) and oral (□, 0.50 mg) administration.

Oral administration

The data obtained following oral administration are summarized in Table 3. Following oral administration, absorption was quite rapid with a mean peak time of 1.1 ± 1.0 h. The peak was followed by a concentration decay that could be fitted to a straight line on a semi-logarithmic scale, indicating a first-order process of elimination. There was a good fit to the one-compartment model (equation (3)) of the data points in all eight subjects. In four of the subjects a lag time of 4–36 min elapsed prior to the start of absorption (Table 3). Absorption proceeded as an apparent first-order process with a mean absorption half-life of 11 min. The mean elimination half-life was 5.1 h. Bioavailability of the brotizolam tablets was 70% when calculated by equation (6) on the basis of fitted parameters (Table 4). The mean elimination half-life as calculated by linear regression was 5.0 ± 1.1 h. Bioavailability, when calculated by the trapezoidal rule with extrapolation to infinity, was 70% using both equation (6) and equation (7) (Table 4).

Discussion

This study shows that, in healthy volunteers, the concentration time profile of brotizolam following i.v. administration can in most cases be satisfactorily described by a two compartment open model. Distribution of brotizolam proceeded quite rapidly and elimination was quite fast with elimination half-lives between 3 and 6 h. The volume of distribution was about 40 l (0.66 l/kg), which is approximately the volume of total body water. This is small as compared to other diazepines: the benzodiazepines diazepam and nitrazepam have volumes of distribution of about 1.1–1.5 l/kg (Giles *et al.*, 1981; Greenblatt *et al.*, 1980) and 2 l/kg (Jochemsen *et al.*, 1982a) respectively; the 3-

Table 2 Pharmacokinetic parameters of i.v. brotizolam.

Subject	$t_{1/2,\lambda_1}$ *(min)*	*CL (ml/min)*	V_1 *(l/kg)*	*V (l/kg)*	$t_{1/2,z}$ *(h)*
1	—	145	—	0.86	3.6
2	8.6	87	0.14	0.43	3.3
3	19.6	109	0.22	0.76	5.9
4	—	—	—	—	—
5	4.8	79	0.14	0.73	6.7
6	5.4	126	0.14	0.53	3.5
7	14.4	95	0.34	0.67	6.2
8	10.2	150	0.26	0.78	4.6
Mean ± s.d.	11 ± 6	113 ± 28	0.21 ± 0.08	0.66 ± 0.19	4.8 ± 1.4

* For this subject plasma concentrations could not be measured because of an interfering peak in the gas chromatogram.

hydroxy-benzodiazepines lorazepam and temazepam have volumes of distribution of about 1.3 l/kg (Greenblatt *et al.*, 1979; Divoll *et al.*, 1981; Jochemsen *et al.*, unpublished results) and the short-acting benzodiazepines triazolam (triazolo-derivative) and midazolam (imidazole-derivative) have volumes of distribution of 1.1 l/kg (Jochemsen *et al.*, 1983) and 1.7 l/kg (Greenblatt *et al.*, 1981) respectively. The total clearance of brotizolam was 113 ml/min, indicating that drug elimination is predominantly dependent on drug metabolizing enzyme activity and not on hepatic drug blood flow (low extraction drug). This contrasts with triazolam and midazolam, which have extraction coefficients of about 0.45 to 0.5 (Amrein *et al.*, 1981, Jochemsen *et al.*, 1983).

Table 3 Pharmacokinetic parameters of oral brotizolam.

Subject	t_{lag} *(min)*	$t_{1/2,abs}$ *(min)*	$t_{1/2,z}$ *(h)*
1	0	30	5.2
2	0	1	5.0
3	36	24	5.9
4	11	2	6.7
5	14	15	6.2
6	4	3	3.1
7	0	1	4.1
8	0	7	4.2
Mean ± s.d.	8 ± 12	10 ± 11	5.1 ± 1.2

Following oral administration, the concentration time profile of brotizolam could in all cases satisfactorily be described by a one-compartment model. Lag times ranged from 0–36 min, suggesting that there are substantial differences in dissolution characteristics of the active compound from the dosage form in the gastrointestinal tract of the different subjects. Absorption generally occurred quite rapidly, but there were large inter-individual differences. The mean bioavailability as calculated by all methods was 70%, but again large inter-individual variability was observed. This may be due to the pharmaceutical formulation used and/or to the physico-chemical characteristics of brotizolam itself, which are both important for drug dissolution and drug absorption.

When bioavailability is calculated by the traditional method (equation (6)) it is assumed that intraindividual differences in elimination half-life are related directly to changes in clearance and that the volume of distribution remains constant. In order to avoid this assumption, F was also calculated by a recently proposed alternative

Table 4 Bioavailability (%) of oral brotizolam compared to i.v. administration.

Subject	*AUC determined by compartmental analysis using equation 6*	*AUC determined by trapezoidal rule using equation 6*	*using equation 7*
1	84	90	84
2	66	53	53
3	55	62	62
4	—	—	—
5	46	46	47
6	58	58	63
7	66	60	62
8	112	118	117
Mean ± s.d.	70 ± 22	70 ± 25	70 ± 24

method which is relatively independent of changes in clearance (Collier & Reigelman, 1981). By this method (equation (7)), the same results were obtained as with the traditional method (equation (6)) (Table 4), indicating that the intra-individual differences in half-life were indeed due to changes in clearance.

In conclusion, it has been shown in this study that brotizolam is on average quite rapidly absorbed, but that there are large inter-individual differences in absorption rate and in extent of absorption (bioavailability). The latter is often incomplete. This gives rise to relatively large concentration differences and may have consequences in terms of interindividual differences in onset of drug action. Furthermore, it was demonstrated that brotizolam is rapidly eliminated in the body (short elimination half-life), which is a favourable characteristic with regard to a limited duration of action. Although one of the metabolites of brotizolam probably has about one-half to one-third of the activity of the parent drug, this compound is not present in plasma in measurable amounts following a single dose of brotizolam to healthy young subjects (Jochemsen *et al.*, unpublished results). The results of this kinetic study agree well with those obtained in performance studies with brotizolam (Nicholson *et al.*, 1980).

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Pharmakokinetik von Brotizolam bei gesunden Probanden nach intravenöser und oraler Applikation

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1 Pharmakokinetik und Bioverfügbarkeit von Brotizolam nach intravenöser und oraler Applikation wurden bei gesunden, jungen Probanden untersucht.

2 Die kinetischen Parameter nach intravenöser Applikation waren wie folgt:
Verteilungsvolumen 0,66 ± 0,19 l/kg, Gesamtplasmaclearance 113 ± 28 ml/min, Verteilungshalbwertszeit 11 ± 6,0 min und Eliminationshalbwertszeit 4,8 ± 1,4 Stunden (Mittelwerte ± s.d. (Standardabweichung)).

3 Die kinetischen Parameter nach oraler Applikation waren wie folgt:
Resorptionsverzögerung 8 ± 12 min, Resorptionshalbwertszeit 10 ± 11 min und Eliminationshalbwertszeit 5.1 ± 1.2 Stunden (Mittelwerte ± s.d.).

4 Die Bioverfügbarkeit von Brotizolam betrug 70 ± 22%, berechnet durch Vergleich der oralen und intravenösen Werte der Flächen unter der Kurve, und korrigiert für intraindividuelle Halbwertszeitdifferenzen. Eine alternative Berechnungsmethode, die relativ unabhängig von grossen Clearanceschwankungen ist, lieferte eine Bioverfügbarkeit von 70 ± 24% (Bereich: 47 – 117%).

Pharmacocinétique du brotizolam chez des sujets sains après administration intraveineuse et orale

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1 La pharmacocinétique et la biodisponibilité du brotizolam, après l'administration intraveineuse et orale, ont été étudiées chez de jeunes volontaires en bonne santé.

2 Les paramètres cinétiques après l'administration intraveineuse ont été les suivants: volume de distribution 0,66 ± 0,19 l/kg, clairance plasmatique totale 113 ± 28 ml/min, demi-vie de distribution 11 ± 6,0 min, et demi-vie d'élimination 4,8 ± 1,4 h (valeurs moyennes ± d.s.).

3 Paramètres cinétiques après l'administration orale: retardement d'absorption 8 ± 12 min, demi-vie d'absorption 10 ± 11 min, et demi-vie d'élimination 5,1 ± 1,2 h (valeurs moyennes ± d.s.).

4 La biodisponibilité du brotizolam a été de 70 ± 22% lorsqu'elle a été calculée en comparant les aires sous la courbe après l'administration orale et intraveineuse, corrigée des différences intraindividuelles de la demi-vie. Une autre méthode de calcul, relativement indépendante des larges variations de la clairance, a donné une biodisponibilité de 70 ± 24% (marge: 47 – 117%).

Farmacocinética de brotizolam en voluntarios sanos después de administración intravenosa y oral

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1 En voluntarios jóvenes sanos se estudiaron la farmacocinética y biodisponibilidad de brotizolam después de administración intravenosa y oral.

2 Los parámetros cinéticos después de administración intravenosa fueron: volumen de distribución 0,66 ± 0,19 l/kg, depuración plasmática total 113 ± 28 ml/min, vida media de distribución 11 ± 6,0 min y vida media de eliminación 4,8 ± 1,4 h (promedios ± D.S. (desviación standard)).

3 Los parámetros cinéticos después de administración oral fueron: retardo de absorción 8 ± 12 min, vida media de absorción 10 ± 11 min y vida media de eliminación 5,1 ± 1,2 h (promedios ± D.S.).

4 La biodisponibilidad de brotizolam fue 70 ± 22% al procederse al cálculo comparativo de los valores correspondientes a las áreas bajo la curva tras administración oral e. i.v., con corrección para diferencias intraindividuales de las vidas medias. Un método de cálculo alternativo, que es relativamente independiente de grandes variaciones de la depuración, arrojó una biodisponibilidad de 70 ± 24% (límites: 47 – 117%).