CLOBAZAM PLASMA CONCENTRATIONS: PHARMACOKINETIC STUDY IN HEALTHY VOLUNTEERS AND DATA IN EPILEPTIC PATIENTS

Clobazam (CLB) is a 1, 5 benzodiazepine with anxiolytic properties (Brogden, Heel, Speight & Avery, 1980) which has been recently used in the treatment of various forms of epilepsy (Escobedo, Otero, Chaparro, Flores & Rubio, 1978; Gastaut & Low, 1979).

The pharmacokinetics and metabolism of CLB have been investigated in different animal species (Volz, Christ, Kellner, Fehlhaber, Gantz, Hajdu & Cavagna, 1979) and in man (Rupp, Badian, Christ, Hajd, Kulkarni, Taeuber, Viehlein, Bender & Vanderbeke, 1979; Vallner, Needham, Jun, Brown, Stewart, Kotzan & Honigberg, 1978) using different techniques. However, in the first reported human study (Vallner *et al.*, 1978) the fluorometric method used was not able to distinguish between CLB and its desmethylmetabolite and in another study (Rupp *et al.*, 1979) no detailed information was given regarding the subjects, the plasma sampling and the kinetic analysis. Moreover, there have been no published studies concerning CLB plasma levels in epileptic patients undergoing long-term therapy.

For these reasons we have investigated the pharmacokinetics of CLB after a 10 mg single oral dose, and also the plasma concentrations of CLB in 30 epileptic out-patients taking CLB in association with other antiepileptic drugs in long-term therapy.

CLB was measured using a gas-chromatographic method developed in our laboratory (Riva, Tedeschi, Albani & Baruzzi, 1981). In brief, 1 ml plasma was acidified, the internal standard added and the sample extracted with benzene. The organic phase was evaporated to dryness and the residue reconstituted in a small volume of acetone, an aliquot of which was injected into the chromatographic column. CLB was measured non-derivatized by an electron capture detector. The sensitivity of the assay was 5 ng/ml.

Six healthy volunteers participated in the study (Table 1). They were instructed to take no medications for at least 1 week before the study. Each subject received one 10 mg capsule of CLB (Frisium, Hoechst AG, Italy) with a glass of water at 08.00 h. Blood samples (5 ml) were collected at various time intervals in heparinized tubes for the 72 following drug intake. The plasma was immediately separated and stored at −20°C prior to analysis.

The CLB plasma concentration-time data were fitted to a tri-exponential equation associated with a two-compartment open pharmacokinetic model using the non-linear regression program BMD X 85 (Biomedical Health Sciences Computing Facility, 1969) on a Hewlett-Packard 1000 computer. The area under the plasma concentration/time curve from 0 to the time of the last sample ($AUC_{o\text{-}t}$; ng ml^{-1} h) was calculated using the trapezoidal rule. The extrapolation of this value to the infinite (AUC ∞; ng ml^{-1} h) was done according to the formula; Correction area $= C_t/\beta$.

Thirty epileptic out-patients of both sexes participated in the study: 16 children aged between 2 and 15 years (mean ± s.e. mean 6.6 ± 0.7) and 14 adolescents or adults aged between 16 and 67 years (mean ± s.e. mean 30.0 ± 4.3). The subjects were receiving CLB chronically, in combination with other antiepileptic drugs (21 were receiving phenobarbitone, 12 carbamazepine, 14 valproic acid and 14 phenytoin). CLB was administered twice or three times daily (at 08.00 h, 14.00h and 20.00 h). The daily dose of CLB ranged from 0.3 to 1.6 mg/kg. Venous blood samples were drawn into heparinized tubes at 08.00 h (before

Table 1 Pharmacokinetic parameters of clobazam in six healthy subjects after a single oral administration (10 mg dose)

Subject	Sex	*Age (years)*	*Dose (mg/kg)*	*Peak time (h)*	*Peak concentration (ng/ml)*	$T_{½}\alpha$ *(h)*	$T_{½}\beta$ *(h)*	$AUC_{o\rightarrow\infty}$ *(ng ml^{-1} h)*
1	F	26	0.16	1.0	256	0.62	15.7	2646
2	F	23	0.20	0.5	305	0.45	28.9	5531
3	M	24	0.16	1.0	227	0.46	18.0	4259
4	M	27	0.13	2.0	164	0.94	21.0	2605
5	M	25	0.17	2.0	290	0.49	10.2	4123
6	F	26	0.19	2.0	325	1.17	57.9	8220
	Mean	25	0.17	1.4	261	0.69	25.3	4564
± s.e. mean		±0.6	±0.01	±0.3	±24	±0.12	±7.0	±859

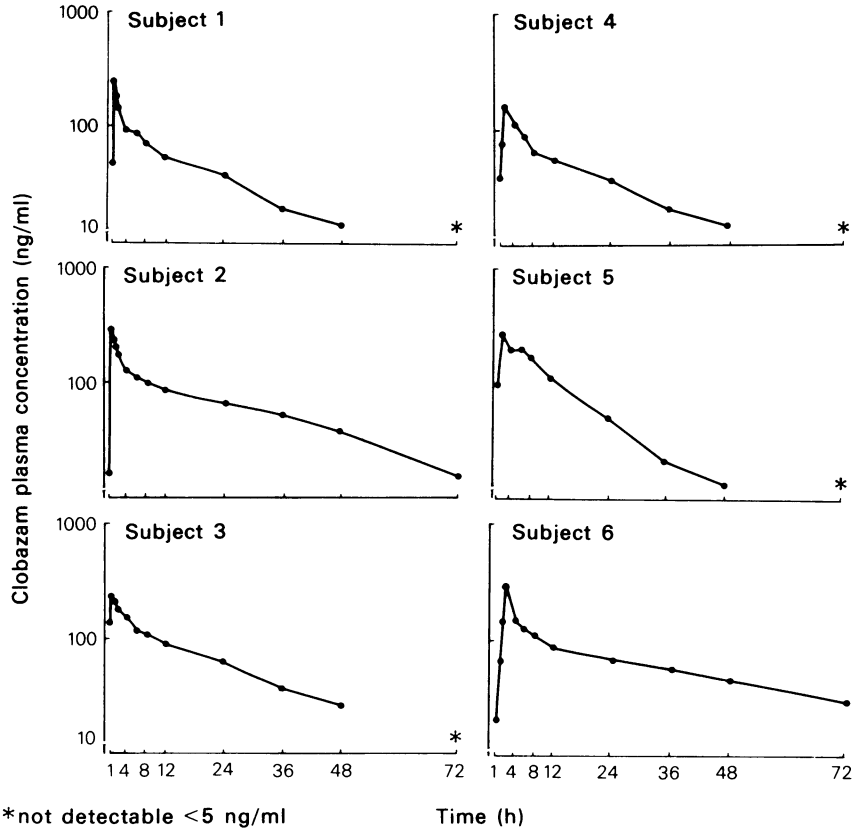

Figure 1 CLB plasma levels (ng/ml) in six healthy volunteers after a 10 mg single oral dose. * not detectable < 5 ng/ml.

the morning dose) and at 12.00 h (4 h after the morning dose).

The CLB plasma concentration-time curves after oral administration of 10 mg in the six subjects are shown in Figure 1. The calculated pharmacokinetic parameters are reported in Table 1. It could be seen that CLB was rapidly absorbed from the gastrointestinal tract, peaks time of maximal concentration being obtained between 0.5 and 2 h. Peaks plasma concentrations ranged from 164 to 325 ng/ml and appeared to be linearly related to the dose ($y = 2228.6x - 114.0$, $r = 0.936$, $P < 0.01$). Following the absorption peak, CLB plasma concentrations appeared to decay bi-exponentially with a $T_{1/2}$ of the distribution phase of 0.45 – 1.17 h. A 5 fold inter-individual variation (from 10.2 to 37.9 h) was found in the terminal elimination half-life.

In the patients group CLB plasma levels of between 20 and 240 ng/ml for the first daily sample (before the morning dose) and between 56 and 290 ng/ml for the second daily sample were found. The correlation between the daily dose and the plasma levels (Figure 2) was statistically significant ($P < 0.05$) only for the adults group. Nevertheless a variability up to about 10 fold was observed for the same dose. For the children an about 8 fold variability was observed for the same dose and no statistical correlation was found between the daily dose and CLB plasma levels.

The mean ± s.e. mean level/dose ratio before the morning dose was 0.078 ± 0.010 for children and 0.147 ± 0.019 for adults. The same ratio calculated 4 h after the morning dose was 0.174 ± 0.026 and 0.251 ± 0.027 for children and adults respectively.

In the pharmacokinetic study CLB appeared to be regularly and rapidly absorbed after oral administration. These data are in agreement with a previously reported study (Rupp *et al.*, 1979). After a rapid absorption CLB decreased bi-exponentially and a two-compartment open model appeared to adequately describe the pharmacokinetic behaviour of the drug. The $T_{1/2}$ of the terminal elimination phase (10.2 – 57.9 h) was in the same range as that found by Rupp *et al.* (1979). In the patients, the great inter-

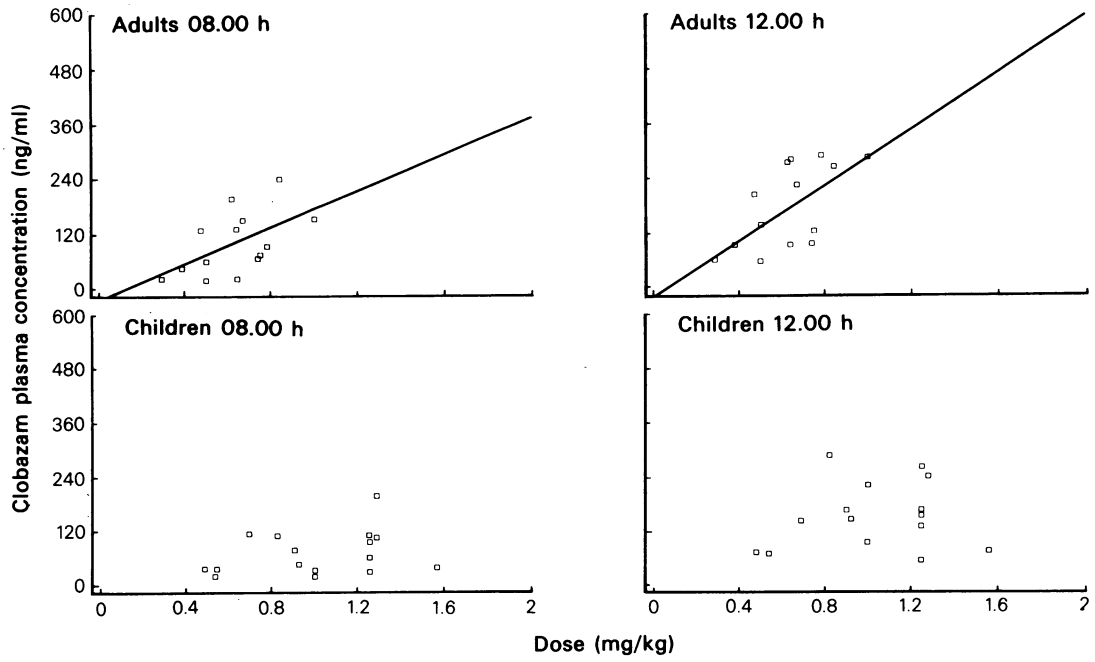

Figure 2 Correlation between dose (mg/kg) and CLB plasma concentrations (ng/ml) for children and adults in the first (08.00 h) and second (12.00 h) daily samples. *Children*, at 08.00 h $y = 39.07 \times + 31.43$ ($r = 0.27$, $P > 0.05$), at 12.00 h $y = 90.42 \times + 84.14$ ($r = 0.25$, $P > 0.05$); *Adults* at 08.00 h $y = 200.36 \times - 27.25$ ($r = 0.55$, $P < 0.05$), at 12.00 h $y = 312.13 \times - 21.61$ ($r = 0.61$, $P < 0.05$).

individual variability of CLB plasma levels might be explained by differences in metabolic activity, as it is known for other drugs, and by a poor compliance of same patients on the other hand.

The linear correlation between oral dose (mg/kg) and CLB plasma levels (ng/ml) for adults, even if statistically significant, cannot be used to predict the daily dosage of CLB because of the great inter-individual variability (up to about 10 fold) for the same dose.

The finding on the different level/dose ratio between children and adults appears to be interesting. This difference suggests that CLB, as many other drugs (Morselli, 1977), is more extensively metabolized in children than in adults.

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HYDRALAZINE-INDUCED PERIPHERAL NEUROPATHY SEEN IN A JAPANESE SLOW ACETYLATOR PATIENT

We report a case of hydralazine (Apresoline®)-induced peripheral neuropathy which, to our knowledge, has never been described in relation to the genetically determined acetylator phenotype.

A 61-year-old, well-nourished (76.5 kg) man was admitted to our hospital because of difficulty in walking, numbness, and weakness of both lower extremities. His clinical course before and after admission is illustrated in Figure 1. Six months before admission, hydralazine 150 mg and reserpine 1 mg daily were prescribed for controlling moderate hypertension at another hospital. The dose of hydralazine was titrated up to 300 mg daily in the following 2 months. When he visited our out-patient department

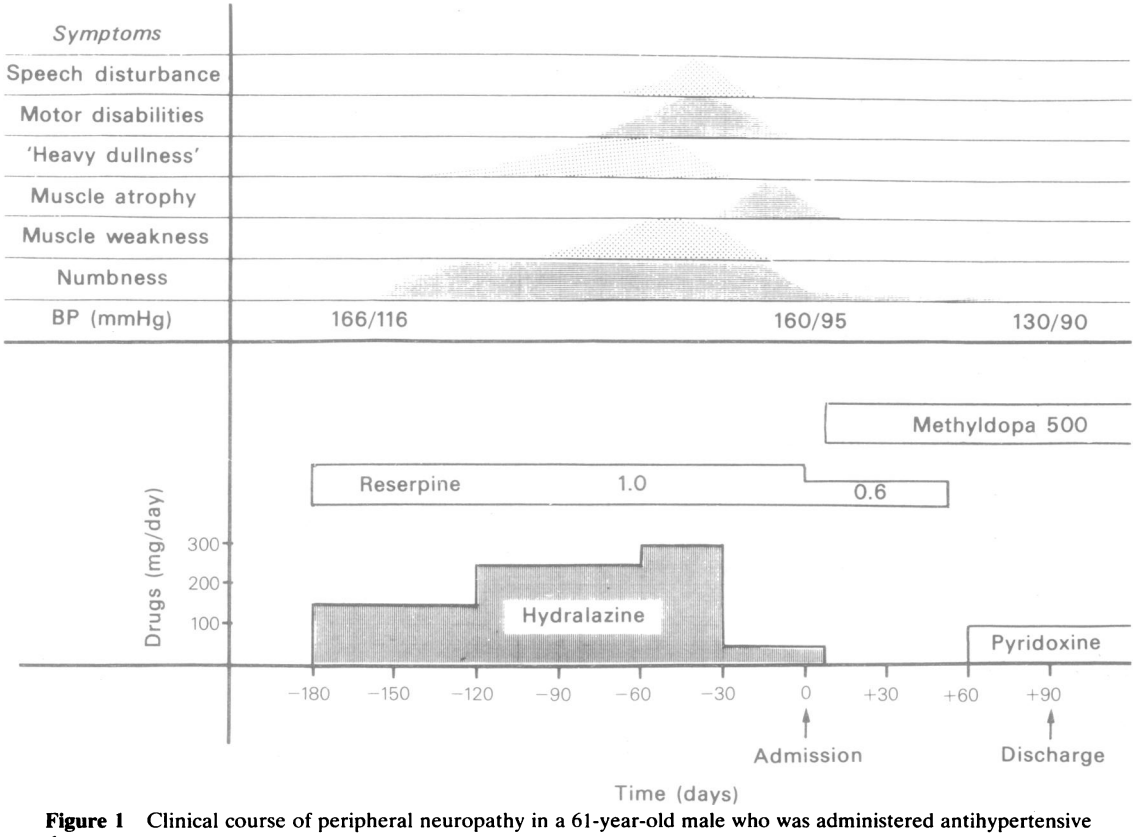

Figure 1 Clinical course of peripheral neuropathy in a 61-year-old male who was administered antihypertensive drugs.

The neurological symptoms appeared approximately one month after the administration of hydralazine 150 mg daily and aggravated gradually after increasing the dose up to 300 mg daily. The symptoms subsided in response to the reduced dose and withdrawal of hydralazine with pryidoxine supplement.