PHARMACOKINETICS OF SINGLE AND MULTIPLE DOSES OF CLOBAZAM IN HUMANS

W. RUPP, M. BADIAN, O. CHRIST, P. HAJDÚ,
R.D. KULKARNI, K. TAEUBER, M. UIHLEIN, R. BENDER & O. VANDERBEKE

Hoechst AG, D-6230 Frankfurt/Main 80, Postfach 80 03 20, West Germany, and
Clinical Pharmacology Unit, Grant Medical College and
J.J. Group of Hospitals, Bombay, India (R.D.K.)

- 1 The pharmacokinetics of clobazam and its biotransformation product *N*-desmethyloclobazam were investigated after single and multiple doses in normal subjects.
- 2 The relevant physicochemical properties of clobazam were measured and are presented. Different assay methods (radiochemical, fluorimetric and gas chromatographic) were applied and the results correlated.
- 3 After single doses the pharmacokinetic profile of clobazam includes time to peak levels 1–4 h after dosing, peak levels increasing linearly with the logarithm of dose, and terminal half-lives of about 18 hours. At least 87% of an oral dose is absorbed, as indicated by urinary recovery of labelled material.
- 4 In multiple-dose studies unchanged clobazam levelled off at minimum steady-state concentrations within one week of dosing. During 28 d of medication *N*-desmethyloclobazam accumulated to near steady-state levels about eight times higher than those of the unchanged compound.
- 5 No pharmacokinetic interactions were discovered between clobazam and the antidepressant nomifensine.

Introduction

IT is challenging to elaborate the differences in the pharmacokinetic profile of 1,4-benzodiazepines and 1,5-benzodiazepines as carefully as Kuch (1979) has displayed the differences in the molecular structure. But limited time requires concentration on a few topics of practical interest. All reported studies in this review were carried out in the Clinical Pharmacology facilities of the Hoechst organization.

The single dose and interaction studies were carried out in Frankfurt, whereas the multiple dose experiments were contributed by the Clinical Pharmacology Unit in Bombay. All analytical work was carried out in the laboratories in Frankfurt (Christ & Rupp 1972, 1974; Hajdú, 1975; Rupp, 1975, 1976; Christ *et al.*, 1976; Hajdú & Uihlein, 1977; Rupp *et al.*, 1977).

Relevant physicochemical properties of clobazam

These properties are listed in Table 1. It is obvious from the solubility data that clobazam is distributed predominantly in the non-aqueous phase and therefore dosage forms for intravenous administration are not presently available. The compound is undissociated in the physiological pH range. This fact may exclude problems of absorption from acid and alkaline gastrointestinal fluids. Between the serum concentrations of

100 and 1600 ng/ml at least 10% of clobazam is non-protein-bound.

Comparison of assay methods

The quality of pharmacokinetic information derived from human studies depends largely on the assay methods used. This seemingly oversimplified statement should be kept in mind when results are discussed and

Table 1 Physicochemical properties of clobazam relevant to pharmacokinetics

Molecular weight	300.74
Solubility (g/100 ml)	
in water (pH 3–11)	0.008
in chloroform	15–20
in acetone	15
in ethanol	2
Distribution	Quantitatively in ether phase (pH 3–11)
Dissociation	Nil (in physiological pH range)
Protein binding	87–90% (range 100–1600 ng/ml)

Figure 1 Information produced by radiochemical (¹⁴C), fluorimetric and GLC assay methods, Serum samples were analyzed up to 96 h after administration of [¹⁴C]-labelled clobazam 40 mg. Open bars indicate unchanged clobazam, hatched bars show fluorogenic and non-fluorogenic biotransformation products (metabolites). AUCs were calculated using the trapezoidal rule.

compared. As shown in Figure 1, measurements were made in identical serum samples at various times after administration of [¹⁴C]-labelled clobazam 40 mg to three normal subjects. Radiochemical, fluorimetric and gas-liquid chromatographic (GLC) assays produced the following information. During the first 24 h after dosing the mean areas under the serum level time curves (AUCs) were 11.2 µg/ml.h when measured by total [¹⁴C]-radioactivity, 10.3 area units using the fluorimetric and 6.4 area units using the gas-liquid chromatographic (GLC) method.

As the measured radioactivity represents the sum of unchanged clobazam and its biotransformation products these data were used as the 100% reference point for comparison. On the other hand, it had been shown that the GLC method is specific for the unchanged compound. Within the first 24 h 57% of the total [¹⁴C]-AUC correspond to unchanged clobazam and 35% to fluorogenic metabolites. Between 24 and 96 h the relative amount of unchanged compound decreases to 25%, whereas the percentage of fluorogenic metabolites remains practically the same. In addition, there is an increase from 8 to 38% of non-fluorogenic biotransformation products.

The correlation between the radiochemical and GLC data within the first 24 h and 24–96 h after dosing is displayed in Figure 2. There is a close correlation within the first 24 h ($r = 0.929$), the slope of the estimated regression line being 0.70. Later, the correlation is less tight ($r = 0.667$) and the slope less steep (0.52). Furthermore, it can be calculated that about

Figure 2 Correlation of serum concentrations of unchanged clobazam and metabolites measured using radiochemical (¹⁴C) and GLC assays in the first 24 h (▲) after dosing and later (△). Doses as in Figure 1. ▲: $\hat{y} = 0.02 + 0.70x$; $r = 0.929$; △: $\hat{y} = 0.09 + 0.52x$; $r = 0.667$.

170 ng of labelled products of metabolism may be present per ml serum when the GLC result is zero.

Similarly, the correlation between radiochemical and fluorimetric data was also close, the coefficients of correlation being 0.987 and 0.899. The slope of the estimated regression line was almost unity (Hajdú, 1975; Rupp, 1976).

From this comparison of assay methods it may be concluded that the GLC method is more adequate to

measure the unchanged compound. But if global information is required and labelling is not feasible the fluorimetric assay may be used (Christ *et al.*, 1976).

Single dose studies

Single doses of clobazam 20, 30 and 40 mg were given to normal subjects. They were male employees at Hoechst AG, who had volunteered to participate in the studies. All of them underwent regular medical and clinical chemistry examinations. After explanation of the nature of the study and its potential risks by the principal investigator, the subjects signed a consent form.

A history of accidental drug intake unrelated to clinical pharmacology studies revealed no potentially interfering compounds. Before the administration of clobazam each subject had abstained for at least 11 h from food, tobacco, and alcoholic and xanthine-containing beverages.

Before and after dosing, blood samples were drawn from a cubital vein at regular intervals. In studies with [^{14}C]-labelled clobazam, urine and faeces were collected quantitatively for 21 days (Christ & Rupp, 1972, 1974).

The basic pharmacokinetic profile of unchanged clobazam in serum as derived from these studies includes time to peak levels unrelated to dose 1–4 h after

Figure 3 Relationship between oral dose of clobazam and its mean peak serum level. Single doses of 20 mg were given to eight subjects, of 30 mg to ten subjects, and 40 mg to another three subjects. Serum concentrations were measured using the GLC assay.

dosing; peak levels increasing linearly with the logarithm of the dose (Figure 3) from 244 ± 31 (s.e.m. ng/ml after 20 mg to 430 ± 26 ng/ml after 30 mg, and to 527 ± 78 ng/ml after 40 mg; terminal half-lives of about 18 h; (range 9.7–30.3 h (Figure 4); AUCs of

Figure 4 Half-lives of elimination from serum of unchanged clobazam measured using the GLC assay after single doses of 15 mg and 30 mg. Mean $\pm$ s.e. of ten subjects (reprinted from Rupp *et al.*, 1977; in Figure 4 of this paper the correct dimension of time is hours, not minutes as printed on page 144S).

3.63 ± 0.53 $\mu\text{g/ml.h}$ after 15 mg and of 6.76 ± 0.51 $\mu\text{g/ml.h}$ after 30 mg for the time period 0–24 h after dosing (Figure 5) (Rupp *et al.*, 1977).

Figure 5 AUCs 0–24 h after administration of clobazam 15 mg and 30 mg to ten subjects (Rupp *et al.*, 1977).

In studies with [^{14}C]-labelled clobazam 92–99% of the administered radioactivity were recovered from urine and faeces. The mean recovery from the urine was 87% (range 81–97%) of the dose, indicating the minimum fraction of absorption (Table 2) (Christ & Rupp, 1974).

Table 2 Renal elimination of clobazam and its metabolites after oral administration to seven normal subjects: results of study with [^{14}C]-labelled clobazam

Recovery (days 1–17)	81–97
	Percentage of dose (^{14}C activity)
Terminal half-life (d)	2.7–5.9

For specification of metabolites, see
Volz *et al.* (1979)

The results of radiochromatographic separation and identification of the radioactive material excreted in urine have been reported by Volz *et al.* (1979). The terminal half-life of renal elimination of the total

radioactivity in the range of 2.7–5.9 d suggests the presence of a metabolic product having a much longer half-life than the unchanged clobazam.

Single dose studies with *N*-desmethyclobazam

These studies were carried out using three subjects of the Frankfurt volunteer panel in 1972. After oral administration of *N*-desmethyclobazam 40 mg, the peak serum levels and AUCs (0–24 h) in the range 7.05–10.8 $\mu\text{g}/\text{ml}\cdot\text{h}$ indicated rather uniform absorption. The half-lives of elimination from serum were 36–46 h in these three subjects (Figure 6), which is about twice the half-life of unchanged clobazam.

Multiple dose studies with clobazam

Based on the kinetic information derived from single dose studies, accumulation was to be expected of both unchanged compound and metabolite(s) when

Figure 6 Half-lives of elimination from serum after single oral dose of *N*-desmethyclobazam 40 mg to subjects I (a), II (b) and III (c). Serum levels were measured using the fluorimetric method. Subject I: $t_{1/2} = 36$ h; $r = -0.999$; subject II: $t_{1/2} = 46$ h; $r = -0.981$; subject III: $t_{1/2} = 45$ h; $r = -0.988$.

Figure 7 Serum levels of unchanged clobazam and *N*-desmethyclobazam after first, seventh and seventeenth dose of clobazam 10 mg in the morning and 20 mg in the evening daily for 10 days. Means $\pm$ s.e. of seven subjects. Open bars indicate unchanged clobazam, hatched bars show *N*-desmethyclobazam levels as mean of all seven subjects. Dotted bars refer to the mean levels of the five subjects not having the highest serum concentrations. GLC assay method.

clobazam is administered once a day or more often. The question of accumulation is not one which may simply be answered in absolute terms, but a quantitative problem which has to be solved by pharmacokinetic means, for example, multiple dose studies.

In the first study carried out by the Clinical Pharmacology Unit, Bombay, seven subjects received clobazam 10 mg in the morning and 20 mg in the evening for 10 days. Figure 7 shows the mean serum levels of unchanged clobazam and of *N*-desmethyclobazam 24 h after doses 1, 7 and 17. Mean clobazam levels increased from 550 ng/ml on day 2 to 940 ng/ml on day 5.

Obviously, steady-state conditions were achieved at an accumulation factor of about 2 within the study period. However, levels of *N*-desmethyclobazam increased with no evidence of steady state being achieved. In addition, the levels of two of the seven subjects were consistently higher on day 5 and day 10,

Figure 8 Minimum serum levels of unchanged clobazam (▲) and *N*-desmethyclobazam (△) after clobazam 10 mg twice daily for 28 days. Means of ten subjects. GLC assay method.

indicating disparity in elimination of this metabolite (Uihlein, 1976; Kulkarni *et al.*, 1977). The second study carried out by the Bombay team was therefore extended to 28 days. Ten subjects were given clobazam 10 mg twice daily for a total of 56 doses.

In Figure 8 the minimum serum levels of unchanged clobazam and of *N*-desmethyloclobazam are shown during the medication period of 28 d and 7 d after cessation of clobazam dosing. Minimum steady-state concentrations of unchanged clobazam were achieved within 1 week at 333 ± 61 ng/ml. After the last dose the serum levels of clobazam decreased relatively quickly. Seven days after the end of dosing the level was 20 ± 10 ng/ml. *N*-desmethyloclobazam accumulated during the study period to mean levels of 2811 ± 540 ng/ml on the final day of medication. Three days after the end of dosing *N*-desmethyloclobazam levels began to decrease slowly reaching 2031 ± 489 ng/ml 7 d after cessation of dosing.

From these results it may be concluded that clobazam 10 mg twice daily for 28 d seems to produce serum levels of *N*-desmethyloclobazam which are about eight times higher than the steady-state levels of unchanged clobazam.

Interaction studies

The Frankfurt team investigated potential interactions between clobazam and nomifensine, a new antidepressant, in ten normal subjects. Results showed no relevant differences in the classical criteria for bio-availability (peak serum levels, time to peak, AUC) and in the half-life of elimination from the serum after administration of each drug alone or in combination. In Figure 9 the peak serum levels of unchanged clobazam as assayed in this study are shown (Rupp *et al.*, 1977).

Figure 9 Peak serum levels of unchanged clobazam (hatched bars) and nomifensine (open bars) after sole and combined medication of clobazam 15 mg and 30 mg with nomifensine 75 mg. Means $\pm$ s.e. of ten subjects. Latin square design. Clobazam was assayed using the GLC method, and nomifensine using radioimmunoassay (Rupp *et al.*, 1977).

Of special interest in the practice of pharmacotherapy are potential interactions of a drug with alcohol. The results of our simultaneous kinetic and dynamic study on clobazam alone and in combination with alcohol are reported here by Taeuber *et al.* (1979).

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Discussion

PROFESSOR D. LAWSON (Glasgow) asked whether there was any information about the effects of smoking on the metabolism or elimination of clobazam. In particular, were the smoking habits of the subjects involved in the pharmacokinetic studies known, and could the substantially higher blood levels in one or two individuals be related to their being non-smokers?

DR RUPP replied that this information was not available.

PROFESSOR LAWSON asked about the renal elimination of the metabolite desmethylclobazam.

DR RUPP in reply referred to the paper by Volz *et al.* (1979), who showed that all the metabolites M₁ to M₉ (N-desmethylclobazam) appeared in the urine together with relatively little of the unchanged drug. The elimination half-life of the metabolites was considerably longer (approximately 3 d) than that of the parent compound (18 hours).

PROFESSOR LAWSON wondered whether this would cause problems in patients with significant renal impairment.

DR RUPP replied that this question could not yet be answered but that a study was currently in progress in patients with various degrees of renal impairment.