Comparison of the Pharmacokinetics of Diazepam after Single and Subchronic Doses*

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Summary. In seven healthy male volunteers the effects of the pattern of dosing on the pharmacokinetics of diazepam have been studied. A cross-over design was employed that consisted of three parts: a single intravenous dose (0.1 mg/kg), and oral dosing (10 mg/day) for six days followed by an intravenous bolus (0.1 mg/kg) on the seventh day, followed by re-examination of a single intravenous dose after diazepam (D) and its major metabolite desmethyldiazepam (DD) had been completely eliminated. Plasma levels of D and DD were monitored by a specific, sensitive GLC-method. In younger patients (n = 5, age 29 - 35 years) the elimination half-life, T1/2(β), of D was 33.9 ± 10.6 h (mean ± S.D.) after the single dose. The control study gave an almost identical result (35.7 ± 12.1). After subchronic dosage in all patients T1/2(β) showed a modest but significant prolongation (paired t-test p<0.01) to 52.9 ± 17.4 h. It was caused by a significant decrease (p = 0.016) in total plasma clearance (Cl), from 26.0 ± 10.8 ml/min to 18.2 ± 7.0 ml/min. Older patients (age 43-60 years) showed the same phenomenon. Blood/plasma ratios remained constant indicating no change in protein binding. Biliary excretion of D was measured in five patients with a T-tube. Only negligible amounts (0.3 - 0.4%) of administered D were excreted within 3 days after subchronic dosage, which demonstrates a lack of enterohepatic cycling of D. After multiple administration of D, there was accumulation of DD to levels approximately five times higher than after a single dose. The possibility that the slower elimination of D after subchronic treatment might be caused by DD was also supported by experiments in dogs and rabbits. After pretreating rabbits with DD and maintaining a high DD plasma level, there was prolongation of T1/2(β) from 2.7 h to 5.2 h, with a corresponding decrease of Cl from 101.6 ml/min to 23.4 ml/min. Similar results were obtained in dogs. It is concluded that the disposition of D is altered by subchronic use and may be regulated by the plasma DD concentration.

Key words: Diazepam, pharmacokinetics, subchronic dosage in man, desmethyldiazepam.

Diazepam (D), a drug which is widely used in the management of anxiety and tension, as an anticonvulsant, and as a muscle relaxant has to be biotransformed in several steps before it can be eliminated (1, 2). In the major pathway D is first demethylated to desmethyldiazepam (DD), the major metabolite in plasma, and subsequently hydroxylated to oxazepam. Both metabolites still possess biological activity, about 20 to 50 percent of that of diazepam (3). In a minor pathway diazepam is first hydroxylated and then demethylated. The hydroxylated metabolites are conjugated to their respective glucuronides with oxazepam as the main urinary excretion product. The disposition and elimination of D after a single dose were examined in

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a recent study (4). Since many patients are more commonly treated with D on a subchronic or chronic basis, this study has been extended to establish the pharmacokinetics of D after subchronic administration. The disposition and elimination after both patterns of treatment have been compared, when the pharmacokinetics of D might either remain unchanged, as shown for methaqualone (5), or altered to a prolonged half-life as do tetracyclines (6), or to a shortened half-life, as does carbamazepine (7).

During the investigation it became apparent that the DD formed might have some influence on the elimination rate of D. To elucidate this effect, animal experiments were performed to examine in vivo the effect of DD on the pharmacokinetics of D.

MATERIAL AND METHODS

Patients and Clinical Protocol

After obtaining their informed written consent, seven healthy male volunteers (29 - 60 years) received first a single intravenous dose of 0.1 mg D/kg. Blood samples were collected at frequent intervals during the first few hours and then every 12 or 24 hours for three days. Next the patients were treated with 10 mg oral doses of D daily for six consecutive days. Seven blood samples were collected during the following 24 hours. On Day 7 the subchronic treatment was completed with an intravenous bolus of 0.1 mg/kg. Thereafter seven blood samples were collected within the first 12 hours and one sample daily for six days. The first study was repeated after all D and DD had been eliminated, some 4 - 8 weeks later, and blood samples were collected for up to eight or ten days from certain individuals. During all three parts of the study the blood/plasma ratio was measured in one blood sample.

Five patients with a T-tube after cholecystectomy and normal liver function tests were treated with oral D 10 mg for five consecutive days. Blood was collected for the next three days.

Animal Experiments

Three New Zealand white rabbits and two mongrel dogs received intravenously 2 mg D/kg and 1 mg D/kg, respectively. Frequent blood samples were taken over the first two hours and up to 8 or 10 hours from the rabbits, and for 30 or 36 hours from the dogs. The experiment was repeated after at least 6 weeks, when the rabbits were pretreated intraperitoneally with a dose of DD (3 mg or 10 mg/kg) one hour before the D injection. To maintain high DD plasma levels during the experiment, DD was reinjected two and five hours after an intravenous bolus of D 2 mg/kg. Dogs were pretreated with DD 1 mg/kg sc one hour before injecting D 1 mg/kg iv. In one dog after two and five hours DD 2 mg/kg was re-injected. Blood samples from both species were collected according to the same schedule as in the control experiments.

Analytical Techniques

The concentrations of D and DD in bile, blood and plasma were determined by a specific, sensitive gas-chromatographic procedure using an electron capture detector, as described recently (4).

Pharmacokinetic and Statistical Calculations

Since inspection of the plasma concentration/time curves in man and animals indicated biexponential decline, it appeared that the data could best be described by a two compartment open model. The classical exponential time function $C_p(t) = Ae^{-\alpha t} + Be^{-\beta t}$ was fitted to the curve for each individual by the least squares iterative digital computer program SAAM 25 (8). The kinetics of this model and definitions of its characteristic parameters have been well described (9, 10). Statistical analysis of the cross-over study was performed by the paired Student's $t$-test, for all other studies the two-tailed $t$-test was used, with the minimal level of significance of $p = 0.05$.

RESULTS

All seven healthy volunteers showed the same biexponential decline of plasma levels of D after single and multiple doses of D. The clinical protocol has been illustrated in Fig. 1 by the measured plasma concentration/time profiles of D in one typical individual. After the single intravenous bolus of 0.1 mg/kg (left part of Fig. 1) the decline of D in plasma was fast during the first 8 to 12 hours, with a half-life of distribution, $T_{1/2(a)}$, of 1.5 h. The subsequent, slower phase, which represented mainly the elimination processes, was characterized by an elimination half-life, $T_{1/2(b)}$, of 26.0 h. Treatment of this patient with oral doses of D 10 mg for six consecutive days and monitoring the plasma levels for 24 hours after the sixth dose gave the curve shown in the centre...