Communications to the Editor

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PLASMA CONCENTRATION PROFILE OF DIAZEPAM AFTER ORAL ADMINISTRATION OF THE OPEN-RING FORM OF DIAZEPAM TO MAN

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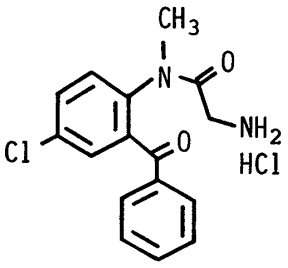
Diazepam was detected in plasma after oral administration of the open-ring form of the compound. A plasma concentration profile after the administration was quite similar to that after administration of diazepam itself. These observations show that the reversible ring-opening reaction of diazepam, which has been demonstrated *in vitro*, takes place *in vivo*.

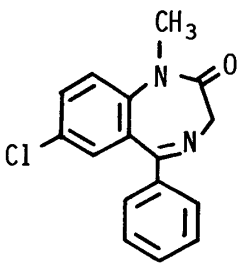
KEYWORDS —— 2-N-glycyl-N-methylamino-5-chlorobenzophenone; diazepam; benzodiazepine; plasma level; open-ring form; cyclization; ring closure

Recently, the hydrolytic ring-opening reactions of various benzodiazepines[1-3] including diazepam[2] and thienodiazepines[4,5] in acidic solutions at body temperature have been studied. These reactions apparently take place at the azomethine bond and the corresponding open-ring compounds produced are in equilibrium with the parent closed-ring compounds, but their degrees of reactivity are quite dependent on both the chemical structure and the nature of the substituents. We observed *in vitro* that open-ring forms of N-substituted benzodiazepin-2-one including diazepam[2] cyclize readily in solution at a neutral pH range.

Here we describe the plasma concentration profiles in man of diazepam after oral administration of 2-N-glycyl-N-methylamino-5-chlorobenzophenone hydrochloride (gift from Sumitomo Chemical Co., Osaka), the open-ring form of diazepam (I), and diazepam itself (II). Identity and purity of I were confirmed by its NMR spectrum (JEOL FX



(I)



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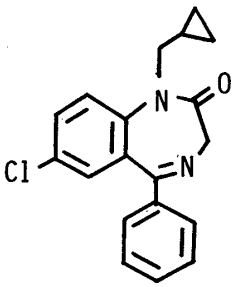

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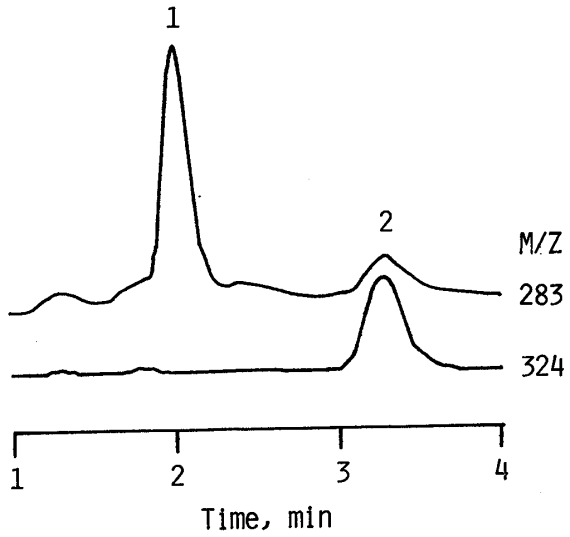

Fig. 1. SIM Chromatograms of the Extract from Plasma Containing 200 ng/ml of Diazepam. 1: diazepam; 2: prazepam (internal standard).

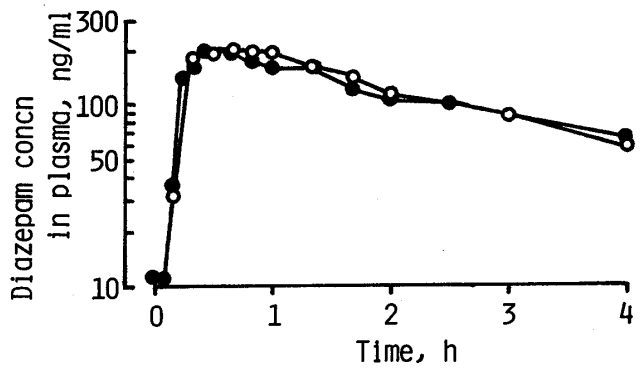

Fig. 2. Plasma Diazepam Concentration Profiles after Oral Administration of 5 mg Equivalent of Diazepam (○) and its Open-Ring Form (●).

Table I. Pharmacokinetic Parameters after Oral Administration of Diazepam and its Open-Ring Form to a Healthy Volunteer

Adminitered form	$AUC_{0-\infty}/10^3$ (ng/ml)·min	$MRT_{0-\infty}$ min	k_a min^{-1}	k_{el} min^{-1}	Vd l	Lag time min
Diazepam	37.8	170	0.117	0.00667	20.1	8.7
Open-ring form of diazepam	37.6	200	0.0911	0.00678	21.9	5.9

100) in a DCl - D_2O system.

A healthy subject, 28 years old, 53 kg, received with a glass of water 5 mg equivalent of II as a commercially available tablet (Cercine, Takeda Pharmaceutical Industries, Osaka) and I as a tablet. The tablet of I, prepared in our laboratory, dissolved rapidly in the 1st fluid of the disintegration test, JP X. There was a washout period of 2 weeks between tests. The subject fasted for 12 h before and during the study but was allowed a light meal 4 h after dosing. He abstained from alcoholic beverages for 24 h before and during the study.

Venous blood samples (5 ml) for determination of diazepam were drawn just before

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dosing and frequently during the ensuing 4 h. All samples were collected in heparinized tubes. The plasma was separated by centrifugation and stored at -20°C pending analysis.

Plasma diazepam concentrations were determined on a Shimadzu gas chromatograph - mass spectrometer (GC-MS) system, model QP-1000. To 0.5 ml aliquots of plasma, were added 1 ml of a saturated sodium chloride solution and 5 ml of benzene containing 500 ng of prazepam (III, gift from Kowa Co., Nagoya) as an internal standard. After mixing for 10 min, the mixture was centrifuged, then the benzene layer was removed and the residue was evaporated *in vacuo* at 60°C. The residue was then dissolved in 50 μl of benzene and 5 μl aliquots of the benzene solution were injected into the GC-MS system.

The GC part of the GC-MS system was equipped with glass columns of 3% OV-17 on Chromosorb Q. The flow rate of helium was 50 ml/min. The temperatures of the injection port, column, transfer line to MS system, and ion source of the MS system were 275, 260, 260, and 280 °C, respectively. Mass spectra were obtained in the electron impact (EI) mode at an electron energy of 70 eV. The selected ion monitoring (SIM) technique was performed at m/z 283 and 324 for II and III, respectively. Typical SIM chromatograms of II and III are shown in Fig. 1. Peak height ratios of II to III in a plasma sample spiked with various amounts of II to a blank plasma were calculated to obtain the standard curve. A linear calibration curve was obtained from 10 ng/ml to 4 μg/ml of II with a correlation coefficient of 0.9998. Presence of a closed-ring form in plasma is expected, based on *in vitro* studies of quantitative ring-closure under the pH conditions of plasma.[2] Identification of the closed-ring form (II) in plasma following oral administration of I was made in the following way. A MS fragmentation pattern identical to that of authentic diazepam was obtained by GC-MS analysis of the extract of plasma samples obtained after oral administration of I. This confirms the presence of II in plasma and hence the *in vivo* cyclization of I to II.

Figure 2 shows the plasma concentration patterns of II after oral administration of 5 mg of II, and of I equivalent to 5 mg of II. II was administered first followed 2 weeks later by I. In spite of this time interval between dosing the plasma level of II before administration of I was 11.5 ng/ml because of the long half-life of II in the terminal phase.[7] Thus, the plasma levels of II after administration of I was corrected for this effect before the calculation of pharmacokinetic parameters. Table I lists the pharmacokinetic parameters calculated by fitting to the one compartment model with lag time by non-linear regression analysis because the minimum AIC value[8] was obtained in this model. Similar plasma level profiles of II were obtained after administration of I and II. The AUC value for II obtained after oral administration of I was very similar to that after administration of II. So were other pharmacokinetic parameters. Although quite short periods of elimination after oral administration of both compounds used to calculate the elimination half-lives, these values may be considered to be the half-lives at a distribution phase,[9] since the elimination half-lives of II at the terminal phase were reported to be 20 - 45 h in healthy adults.[7]

Thus, the ring closure of I to II in the gastrointestinal tract was confirmed by the presence of II in plasma after oral administration of I to a human volunteer, although a statistical analysis was not possible. Several investigators[10 - 11] reported that peptidoaminobenzophenones could serve as pro-drug forms of various

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benzodiazepines. Peptide groups are cleaved by peptidases to produce 2-N-glycyl-aminobenzophenones which spontaneously cyclize to form the corresponding benzodiazepines upon injection. Our results also indicate that I cyclizes to form II after administration.

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