Short Communication

A Double-Peak Phenomenon in the Pharmacokinetics of Alprazolam after Oral Administration

(Received December 22, 1998; accepted April 12, 1999)

This paper is available online at http://www.dmd.org

ABSTRACT:

The pharmacokinetics of alprazolam (ALP) after i.v. and p.o. administration in rats were characterized. ALP decayed biexponentially after the i.v. dose (1.25 mg/kg), but the concentration-time profiles after the p.o. doses (7 and 12.5 mg/kg) exhibited a double-peak phenomenon. The presence of two peaks was confirmed by statistical analysis of the serum concentration data of ALP, as well as by observed double peaks in the serum concentration-time profiles of the two active metabolites (α-hydroxyalprazolam and 4-hydroxyalprazolam). An absorption model incorporating a delay site is proposed to describe the data, and the absolute oral bioavailability is estimated to be about 30%. The two peaks were ~80 to 115 min apart, and there was a delay in the absorption of close to 80% of oral ALP, regardless of dose. We hypothesize that the mechanism underlying the double-peak phenomenon is due to reduction in gastric motility caused by the muscle relaxant effect of ALP. This hypothesis is supported by the observed longer delay in the appearance of the second peak at the higher p.o. dose. Enterohepatic recycling is precluded from being the underlying mechanism, because of the presence of double peaks after the p.o. doses but not after the i.v. dose. This is the first reported case of double peaks for oral ALP, and this phenomenon has not been reported for other benzodiazepines. The double-peak phenomenon caused by the hypothesized mechanism may have important therapeutic and drug interaction implications, especially because benzodiazepines are commonly coadministered with other drugs.

Alprazolam (ALP), a triazolobenzodiazepine, is the most widely prescribed benzodiazepine (BZ) and is used as an anxiolytic, antipanic, and antidepressant agent (Fawcett and Kravitz, 1982; Dawson et al., 1984). In humans, ALP is rapidly and completely absorbed after oral administration, with an elimination half-life of 6 to 16 h and volume of distribution of 1 l/kg, respectively (Greenblatt et al., 1983; Miller and Jamali, 1997), but such phenomenon has not been reported for other benzodiazepines. The double-peak phenomenon caused by the hypothesized mechanism may have important therapeutic and drug interaction implications, especially because benzodiazepines are commonly coadministered with other drugs.

This research was supported by National Institute on Drug Abuse Grant R37 DA03117 (J.L.F.).

Abbreviations used are: ALP, alprazolam; AIC, Akaike’s information criterion; AUC, area under the curve; BZ, benzodiazepine; Cmax, maximum concentration for the first peak; C2max, maximum concentration for the second peak; Cmin, minimum concentration; Cl, clearance; F, bioavailability; f, fraction of bioavailable ALP that enters the delay site; N, number of delay compartments; PK, pharmacokinetics; T2, delay time; T1max, time at which C1max occurred; T2max, time at which C2max occurred; Tmax, time at which Cmax occurred; Vc, volume of distribution in the central compartment; Vss, volume of distribution at steady state; α-OHALP, α-hydroxyalprazolam; 4-OHALP, 4-hydroxyalprazolam; RM, repeated-measures.

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Drugs

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Materials and Methods

Animals. Four male, albino, Sprague-Dawley rats from Harlan Sprague-Dawley, Inc. (Indianapolis, IN) were used. They were housed individually in a temperature-regulated room with a daily cycle of illumination from 7:00 AM to 7:00 PM. They were reduced to 80% of their initial, adult free-feeding body weights (mean = 381 g; range: 380–382 g) by receiving limited daily food rations (5 g for the first day, 10 g for the next 5 days) and were then maintained at their weights with a daily food supplement (range: 14–16 g). Water was continuously available in the living cages. They were held at these weights for 2 to 3 months before starting the experiment, a time period usually needed for training, establishing baseline, and examining drug dose-response relations for operant behavior. Experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication 85-23, revised 1985).
Drugs. ALP and its metabolites, α-OHALP and 4-OHALP, were obtained from Upjohn Laboratories (Kalamazoo, MI). ALP (5 mg) was dissolved in 50 μl of 1.2 N HCl and diluted with 0.9% NaCl and administered either i.v. as a bolus or p.o. by gavage in an injection volume of 1 ml/kg body weight. When an i.v. ALP bolus dose was administered, drug solution was delivered in 15 s and was followed by 0.3 ml 0.9% saline in 15 s.

Catheterization. Right jugular vein cannulation was performed under sterile conditions and has been described previously (Lau et al., 1996). The proximal end of the silastic catheter was inserted into the jugular vein, and the distal end of the catheter was threaded s.c. and exited through a small incision in the back of the animal. The catheter was flushed with 0.9% saline containing 50 units of heparin per milliliter and sealed with fishing line when not in use.

Reagents and HPLC. Reagents were obtained from standard commercial sources. The serum microsample HPLC method for the determination of ALP and its metabolites has been described previously (Jin and Lau, 1994).

Drug Administration and Blood Sampling. Animals were allowed to recover for at least 2 days from jugular vein catheterization before the administration of ALP. The animals initially received an i.v. dose of ALP (1.25 mg/kg) via the jugular vein catheter, followed on other days by p.o. administration of 7 and 12.5 mg/kg ALP in a random order. Drug doses were separated by 3 to 5 days.

Blood samples (100 μl) from the jugular catheter were obtained after ALP administration at 2, 5, 10, 15, 20, 30, 45, 60, 90, and 120 min postinjection; for the two oral doses (7 and 12.5 mg/kg), blood samples also were obtained at 180, 240, and 360 min. After each blood sampling, 0.2 ml of sterile 0.9% NaCl solution was administered to replace the blood sample. To maintain the feeding regimen and avoid the effect of food on ALP PK, drug doses were given 6 h before the feeding time. Thus, the daily food supplements were given immediately after the last blood samples.

PK Analysis. We performed PK data analysis using the SAAM II software system (SAAM Institute, 1997). Model parameters were estimated by numerical optimization using Akaike’s information criterion (AIC) as the objective function (Akaite, 1974). ALP serum concentrations after i.v. bolus administration is described by an open two-compartment model, with elimination from the central compartment (Fig. 1, top). The compartmental model parameters \( k_{0,1}, k_{1,2}, k_{2,1}, \) and \( V_{1} \) are used to calculate the model-independent parameters in the equation, \( C_{p} = Ae^{-\alpha t} + Be^{-\beta t} \), using standard formulae (Gabrielsson and Weiner, 1997).

Two models are considered to describe the serum ALP concentrations after p.o. administration: 1) the conventional first-order absorption model and 2) a proposed modification of the first-order absorption model that incorporates a delay site (Fig. 1, bottom). The delay site is characterized by two parameters that are estimated from the data: \( T_{d} \) the delay time; and \( N \), the number of delay compartments. Mass entering the delay site passes through each of the \( N \) compartments before entering the central compartment. The mass transfer coefficient between the delay stages and between the delay site and the central compartment is \( k_{1,4} = N/T_{d} \). Additional parameters in the proposed absorption model are: \( k_{a} \), a first-order absorption rate constant; \( F \), the bioavailable fraction of a p.o. dose of ALP; and \( f \), the fraction of bioavailable ALP that enters the delay site. The differential equations specifying the model are presented below:

\[
\begin{align*}
\frac{dA_1}{dT} &= -(k_{0,1} + k_{1,2})A_1 + k_{1,2}A_2 \\
&+ F(1-f)k_{a}A_1 + k_{1,4}A_1N
\end{align*}
\]

\[
\begin{align*}
\frac{dA_2}{dT} &= k_{1,2}A_1 - k_{1,2}A_2 \\
\frac{dA_3}{dT} &= -(F(1-f)k_{a} + F \cdot f \cdot k_{a})A_1 + k_{1,4}A_1N
\end{align*}
\]

\[
\begin{align*}
\frac{dA_{4,1}}{dT} &= F \cdot f \cdot k_{a} \cdot A_1 - k_{1,4} \cdot A_{4,1} \\
\frac{dA_{4,2}}{dT} &= k_{1,4} \cdot A_{4,1} - k_{1,4} \cdot A_{4,2}
\end{align*}
\]

\[
\begin{align*}
\frac{dA_{4,N}}{dT} &= k_{1,4} \cdot A_{4,N-1} - k_{1,4} \cdot A_{4,N}
\end{align*}
\]

The parameters in the absorption models (\( F, f, k_{a}, N, \) and \( T_{d} \)) were estimated by numerical optimization using the two-compartment open model parameters previously estimated from the i.v. data. Absorption model parameters are estimated independently for each animal at each p.o. dose level. The \( f \) parameter is constrained to be between 0 and 1 and the number of delay compartments to integer values. Statistical analyses were performed by repeated-measures (RM) one-way ANOVA by using SigmaStat (Jandel, San Rafael, CA). The bioavailability of ALP (\( F \)) is also calculated from the i.v. and p.o. areas under the curve (AUCs) of ALP using noncompartmental analysis (Gabrielsson and Weiner, 1997). Additional noncompartmental parameters describing the concentration and location of the two peaks (\( C_{1,\text{max}}, C_{2,\text{max}} \) and \( T_{1,\text{max}}, T_{2,\text{max}} \)) and the trough between the peaks (\( C_{\text{min}} \) and \( T_{\text{min}} \)) are also reported as observed values.

Results and Discussion

The mean serum concentrations (±S.E.) of ALP after the i.v. (1.25 mg/kg) and p.o. doses (7 and 12.5 mg/kg) are shown in Fig. 2, A–C, respectively. ALP decayed biexponentially after i.v. administration, with an initial half-life (\( T_{1/2,\text{ini}} \)) of 3.22 ± 0.72 min and a terminal half-life (\( T_{1/2,\text{ter}} \)) of 23.1 ± 3.85 min. The estimated values of the coefficients in the biexponential equation corresponding to these half-lives are 2.917 ± 0.384 nmol/ml and 0.602 ± 0.104 nmol/ml, respectively. The microconstants specifying the open two-compartment model (Fig. 1, top) are presented in Table 1. These values closely followed those reported previously (Lau et al., 1997a).

A qualitative visual examination of the data indicated the presence of double peaks in the concentration-time profiles of oral ALP and its two metabolites for each of the four animals regardless of dose (Fig. 2, B and C). Quantitative analysis of ALP serum concentration data for the two p.o. doses is conducted to compare the location (\( T_{1,\text{max}} \) and \( T_{2,\text{max}} \)) and magnitude (\( C_{1,\text{max}} \) and \( C_{2,\text{max}} \)) of the two peaks, and is presented in Table 1. Although the \( T_{2,\text{max}} \) for the second peak was significantly greater (\( p < 0.01 \)) than that for the first, the values of \( C_{1,\text{max}} \) and \( C_{2,\text{max}} \) were similar for both peaks (\( p > 0.1 \)) at a given dose, as judged by RM one-way ANOVA. However, the values of the \( C_{1,\text{max}} \) and \( C_{2,\text{max}} \) for the 12.5 mg/kg dose were 2-fold greater than
those for the 7 mg/kg dose. The location ($T_{\text{min}}$) and magnitude ($C_{\text{min}}$) of the concentration in the trough between the two peaks is also presented in Table 1. Although the $T_{\text{min}}$ values were significantly different for the two oral doses, the $C_{\text{min}}$ values were not significantly different.

The goodness of fit of the two absorption models described in Materials and Methods is assessed by using AIC. Analysis of data from each animal, at each p.o. dose level, indicates that the proposed absorption model with a delay site is more appropriate than the conventional first-order absorption model. The mean ($\pm$ S.E.) AIC value for the conventional absorption model is $20.108 \pm 0.208$ and that for the proposed absorption model is $25.255 \pm 0.367$. The mean ($\pm$ S.E.) of the parameters in the proposed absorption model are reported in Table 1. The values of $N$ ranged from 3 to 11 and from 9

### Table 1

<table>
<thead>
<tr>
<th>Parameters</th>
<th>ALP (i.v.)</th>
<th>ALP (p.o.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_c$ (l/kg)</td>
<td>1.25 $\pm$ 0.16</td>
<td>0.138 $\pm$ 0.051</td>
</tr>
<tr>
<td>$V_{ss}$ (l/kg)</td>
<td>2.65 $\pm$ 0.24</td>
<td>0.103 $\pm$ 0.041</td>
</tr>
<tr>
<td>$Cl$ (l/kg/h)</td>
<td>7.98 $\pm$ 0.61</td>
<td>0.009 $\pm$ 0.004</td>
</tr>
<tr>
<td>$k_{0,1}$ (min$^{-1}$)</td>
<td>0.112 $\pm$ 0.018</td>
<td>0.026 $\pm$ 0.009</td>
</tr>
<tr>
<td>$k_{1,2}$ (min$^{-1}$)</td>
<td>0.060 $\pm$ 0.018</td>
<td>0.073 $\pm$ 0.0021</td>
</tr>
<tr>
<td>$k_{2,1}$ (min$^{-1}$)</td>
<td>0.073 $\pm$ 0.026</td>
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<table>
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<tr>
<th>Parameters specifying proposed absorption model</th>
<th>7 mg/kg/22.67 $\mu$mol/kg</th>
<th>12.5 mg/kg/40.5 $\mu$mol/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_a$ (min$^{-1}$)</td>
<td>0.138 $\pm$ 0.051</td>
<td>0.216 $\pm$ 0.147</td>
</tr>
<tr>
<td>$k_{0,2}$ (min$^{-1}$)</td>
<td>0.103 $\pm$ 0.041</td>
<td>0.140 $\pm$ 0.097</td>
</tr>
<tr>
<td>$k_{1,3}$ (min$^{-1}$)</td>
<td>0.009 $\pm$ 0.004</td>
<td>0.014 $\pm$ 0.009</td>
</tr>
<tr>
<td>$k_{1,4}$ (min$^{-1}$)</td>
<td>0.026 $\pm$ 0.009</td>
<td>0.062 $\pm$ 0.042</td>
</tr>
<tr>
<td>$k_{2,3}$ (min$^{-1}$)</td>
<td>0.073 $\pm$ 0.021</td>
<td>0.095 $\pm$ 0.013</td>
</tr>
<tr>
<td>Delay (min)</td>
<td>98.55 $\pm$ 19.91</td>
<td>128.25 $\pm$ 7.04</td>
</tr>
<tr>
<td>$f$ (%)</td>
<td>78.90 $\pm$ 7.35</td>
<td>81.55 $\pm$ 1.56</td>
</tr>
<tr>
<td>$F$ [absorption model] (%)</td>
<td>27.36 $\pm$ 7.17</td>
<td>34.39 $\pm$ 10.06</td>
</tr>
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</table>

<table>
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<tr>
<th>Noncompartmental parameters</th>
<th>30.88 $\pm$ 2.47</th>
</tr>
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<tbody>
<tr>
<td>AUC$_{0-\infty}$ (nmol $\cdot$ min/ml)</td>
<td>48.31 $\pm$ 13.46</td>
</tr>
<tr>
<td>$T_{1\text{max}}$ (min)</td>
<td>27.94 $\pm$ 0.56</td>
</tr>
<tr>
<td>$C_{1\text{max}}$ (nmol/ml)</td>
<td>11.25 $\pm$ 4.44</td>
</tr>
<tr>
<td>$Cl_{\text{max}}$ (nmol/ml)</td>
<td>3.50 $\pm$ 0.155</td>
</tr>
<tr>
<td>$T_{\text{min}}$ (min)</td>
<td>37.50 $\pm$ 20.62</td>
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<tr>
<td>$C_{\text{min}}$ (nmol/ml)</td>
<td>0.146 $\pm$ 0.053</td>
</tr>
<tr>
<td>$T_{2\text{max}}$ (min)</td>
<td>90.00 $\pm$ 14.14</td>
</tr>
<tr>
<td>$C_{2\text{max}}$ (nmol/ml)</td>
<td>0.306 $\pm$ 0.089</td>
</tr>
</tbody>
</table>

FIG. 2. Mean serum ALP and its metabolite (4-OHALP and α-OHALP) concentration-time profiles after ALP administration. A, i.v. 1.25 mg/kg; B, p.o. 7 mg/kg; C, p.o. 12.5 mg/kg.
effect of ALP. BZs (e.g., diazepam, flunitrazepam, midazolam) have been found not only to relax airway muscle by a direct action on airway smooth muscle in guinea pigs (Koga et al., 1992) but also to alter the gastrointestinal motility in conscious dogs (Fargeas et al., 1984). It is likely that gastric emptying played a role in producing the double-peak phenomenon because ALP has been reported to alter the oral absorption of coadministered caffeine (Lau et al., 1997a), whereas no such interaction was observed when ALP and caffeine were simultaneously administered i.v.

The similarity in the $C_{\text{min}}$ values at both p.o. doses is also consistent with the hypothesis that the double-peak phenomenon is caused by the muscle relaxant effect of ALP. The $C_{\text{min}}$ can be considered as a threshold concentration, above which ALP exerts its muscle relaxant effect effectively on gastric motility. The value of $T_{\text{min}}$ is 30 min greater for the 12.5 mg/kg dose in comparison with that for the 7 mg/kg dose (Table 1), because it takes longer for the serum concentration of ALP to fall to $C_{\text{min}}$ for the higher oral dose.

A possible explanation for why the double-peak phenomenon has not been reported in humans is that the therapeutic dose (range: 0.75–3 mg/day) was much lower than those (7–12.5 mg/kg) used in the present study. After a single oral dose (1 mg) in humans, serum ALP concentrations were below 20 ng/ml or 0.065 nmol/ml (Greenblatt et al., 1983), which were much lower than those $C_{\text{min}}$ (~0.15 nmol/ml) reported in the present study. Moreover, multiple-peak phenomenon has been reported for other drugs independent of food intake in rats (Piquette-Miller and Jamali, 1997), and therefore it is unlikely that food regimen plays a role in producing the double-peak phenomenon for oral ALP.

The double-peak phenomenon in ALP serum concentration-time profile after p.o. doses was observed in all of the four animals used in the study. Double peaks in the serum concentration-time profiles of both ALP metabolites provides evidence for the existence of the double-peak phenomena for the parent compound (Fig. 2, B and C). We have hypothesized that the double-peak phenomenon is caused by the muscle relaxant effect of ALP, which can have important therapeutic and drug interaction implications, especially because BZs are commonly coadministered with other drugs. Further studies are needed to investigate the mechanism underlying the double-peak phenomenon for ALP and other muscle relaxants.

Acknowledgments. We thank Dr. B.E. Williams (Upjohn Co., Kalamazoo, MI) for generous supplies of ALP and its two metabolites.

References


