Pharmacokinetics in lactating women: prediction of alprazolam transfer into milk

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1 Alprazolam, a triazolobenzodiazepine, is extensively prescribed for the treatment of anxiety disorders, which predominantly affect women of child-bearing age. The purpose of the present study was to assess the pharmacokinetics of alprazolam and its two hydroxylated metabolites: 4-hydroxy-alprazolam and α-hydroxy-alprazolam in lactating human volunteers and to test the predictability of four recently reported models for drug transfer into milk based on physicochemical properties.

2 Multiple milk and serum samples in eight lactating subjects were collected up to 36 h following single oral doses of 0.5 mg alprazolam; suckling of the infant was discontinued after drug administration. 4-Hydroxy-alprazolam was the predominant metabolite in serum samples while α-hydroxy-alprazolam was not detected.

3 The mean oral clearance of alprazolam was 1.15 ± 0.32 ml min⁻¹ kg⁻¹. The time course of alprazolam in milk roughly paralleled the respective plasma time profile (mean serum residence time = 16.42 ± 4.69 h; mean milk residence time = 18.93 ± 7.03 h). The mean terminal half-life in serum was 12.52 ± 3.53 h.

4 Observed milk/serum concentration ratios were determined in vivo as AUCmilk/AUCserum (mean M/Sobs = 0.36 ± 0.11). Predicted M/S ratios were calculated from the in vitro measures of the unbound fractions of alprazolam in serum and skim milk (mean fₛ = 0.18 ± 0.02, mean fₘ = 0.74 ± 0.05 respectively); the unionized fractions in serum and whole milk (both values approached unity); the skim to whole milk drug concentration ratio (mean S/W = 0.86 ± 0.09); cematocrin (mean Cr = 0.06 ± 0.02), and assuming the milk lipid:ultrafiltrate partition coefficient, Pₘ = 5.48. The diffusion based models using in vitro measurements adequately predicted M/Sobs.

5 In conclusion, the neonatal dose of alprazolam in breast milk is low [between 0.3–5 µg kg⁻¹ day⁻¹; or 3% (body weight adjusted) of the maternal dose] and its transfer into milk was consistent with a passive diffusion mechanism.

Keywords alprazolam pharmacokinetics blood-milk transfer prediction model breast feeding

Introduction

Benzodiazepines as a class, are the most commonly prescribed anxiolytics in the United States. Alprazolam (Xanax®) a triazolo-benzodiazepine introduced in 1980, has replaced diazepam as the most widely prescribed benzodiazepine; partly due to its shorter half-life resulting in less cumulative drowsiness [1, 2]. It is commonly prescribed for the treatment of generalized anxiety disorder and panic disorder which are twice as frequently manifested among women than among men [3, 4]. The age of onset is usually 18–45 years old which coincides with age of child bearing. The incidence of psychiatric illness is higher in the first 12 weeks of postpartum than at any other time in a women’s life [5]. Breast feeding has its inherent

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advantages in terms of milk composition, immunoprotection, and bonding [6]. Benzodiazepines such as diazepam, oxazepam, lorazepam, lormetazepam, quazepam, midazolam, nitrazepam, flunitrazepam, metacezepam and lormetzapam have been found in breast milk to varying degrees, with milk:serum (M/S) ratios ranging from 0.1–3 [7–16]. These reports show that neonatal levels were generally low and not associated with obvious adverse effects, with the exception of sedation [16]. However, the impaired ability of the neonate to oxidize and conjugate benzodiazepines prior to their renal elimination continues to be a clinical concern [17]. Faced with limited treatment option, clinicians have a propensity to advise lactating mothers suffering from anxiety or panic disorders to refrain from breast feeding.

Very little is known about the blood-milk transfer of alprazolam, or its two major metabolites: α-hydroxy-alprazolam and 4-hydroxy-alprazolam. In a cohort study, mild drowsiness in an infant which resolved spontaneously despite continued therapy was reported in one of the five cases in which alprazolam was being consumed by the breast feeding mothers [18]. However, an estimate of the infant’s dose could not be made as no plasma or milk concentrations were assayed. A more rigorous study design is needed to address the potential exposure of alprazolam in view of its prevalent use in women of child bearing age.

Previously, we have proposed a diffusion model to predict the transfer of drug into milk based on physicochemical properties of the drug [19–21]. The model incorporates pH partition theory, binding to serum and milk proteins, and partitioning into milk fat to make prediction of M/S ratio. A direct comparison of the model predictions with in vivo measurements for a number of model compounds were made using lactating New Zealand White rabbits [20, 21]. Three other diffusional models have been proposed by Atkinson & Begg [22], Begg & Atkinson [23], and by Stebler & Guentert [24]. To date, none of these models has been prospectively tested in humans. The availability of a good model is appealing because of its potential to make useful clinical prediction from the physicochemical data.

The purpose of the study was to study the blood-milk transfer of alprazolam in lactating women and to validate model prediction of M/S ratio from the in vitro determinations.

Methods

Subjects and study design

The study population consisted of eight healthy, non-obese, lactating human volunteers 6–28 weeks postpartum who had decided to stop nursing or suspend nursing for the duration of the study. All subjects signed the informed consent forms according to the University Institutional Review Board prior to their participation in the study. The subjects did not receive any known enzyme inducing or inhibiting agents for a period of 30 days, or any other medication for a period of 7 days, or alcohol 2 days preceding their participation in this study. An electronic breast pump (Egnell LACT-E, Cary IL) was used for the collection of milk samples. Each subject received a single oral dose of alprazolam (two 0.25 mg Xanax® Tablets). Multiple blood (7 ml) and milk (15–60 ml) samples were obtained at 0.0, 0.25, 0.50, 1.0 2.0, 4.0 6.0, 8.0, 12.0, 24.0 and 36 h. Prior to dosing, blank serum and milk samples were obtained for in vitro determinations of pH, skim to whole milk concentration ratio, protein binding and crematocrit. Following collection, milk samples and serum samples were harvested and immediately stored at −20°C until drug analysis.

In vitro determinations

Measurements of milk pH were performed anaerobically at 37°C within 1 h of collection using a clinical blood gas analyzer. Fresh whole milk (spiked with [14C]-alprazolam) was vortexed gently for 1 min. An aliquot of 100 μl of whole milk was removed (in triplicate) for analysis. The remaining whole milk was centrifuged at 15,000 rev min⁻¹ for 5 min. Aliquots of the skim milk (100 μl, triplicates) were analyzed. Subsequently, skim to whole milk concentration ratio (S/W) was calculated. Skim milk samples (300 μl), in triplicates (spiked with [14C]-alprazolam) were dialyzed in plexiglass cells (separated by Spectropor-2, dialysis membrane with M.W. cutoff of 12,000–14,000) for 6 h against pH 7.1 phosphate buffer at 37°C. At the end of dialysis, 5 ml of scintillation fluid was added to each of the 200 μl aliquots of buffer and skim milk, which were then counted in a scintillation counter. Volume shift in each chamber, as well as pH of the buffer were measured. The ratio of the buffer to milk is the free fraction in skim milk (f2). Similarly, the free fraction in serum (f1) was determined with serum dialyzed against pH 7.2 phosphate buffer; which increased to approximately 7.4 after 6 h dialysis. Fresh, well mixed milk sample was drawn into a sealed haematocrit tube and centrifuged for 10 min. The length of the creamy layer was measured with a magnifying glass with imprinted micro-scale. The percentage of the length of the creamy layer to the whole length was defined as the crematocrit.

Analytical assay

To 2 ml of whole milk, 50 μl of standard solution (15.6 to 250 ng ml⁻¹ of alprazolam, 4-hydroxy-alprazolam and α-hydroxy-alprazolam) and 25 μl of internal standard (206 ng ml⁻¹ diazepam) was added. Acetonitrile (5 ml) was added dropwise while vortexing gently to precipitate the milk proteins. After standing for 5 min in a −20°C freezer, the supernatants were transferred and evaporated under nitrogen gas until about 0.5 ml remained. Water (1 ml) was added to the remaining supernatant and then loaded into a Bond Elut CN solid-phase extraction
Oral clearance posed to unionized be

Data analysis

Serum and milk drug concentration vs time data were analyzed by fitting a triexponential equation to these profiles using nonlinear regression analysis (RSTRIP, MicroMath, Salt Lake City, UT). Area under the drug concentration-time curve (AUC) and area under the first moment curve (AUMC) were determined from the coefficients and exponents of these fitted relationships. The subscripts s and m refer to serum and milk, respectively. Peak alprazolam concentrations ($C_{\text{max}}$) and the corresponding times ($t_{\text{max}}$) were noted directly from the data.

$$M/S_{\text{obs}} = \frac{\text{AUC}_m}{\text{AUC}_s} \quad \text{Equation 1}$$

Oral clearance ($CL_{po}$) was calculated from

$$CL_{po} = \frac{\text{Dose}}{\text{AUC}_s} \quad \text{Equation 2}$$

Mean residence time was determined as

$$\text{MRT} = \frac{\text{AUMC}}{\text{AUC}} \quad \text{Equation 3}$$

Four recently published models have been proposed to predict the distribution of drugs between milk and plasma. Predicted M/S value ($M/S_{\text{pred}}$) can be calculated from the in vitro physicochemical properties of the drug, assuming a passive simple diffusion.

Predicted M/S values of Model 1 (Fleishaker et al., 1987) [19] were derived by assuming only the free, unionized xenobiotic will be able to cross the blood-milk barrier.

$$M/S_{\text{pred1}} = \frac{f_{s}^{\text{un}} f_s}{f_{m}^{\text{un}} f_m} \quad (S/W) \quad \text{Equation 4}$$

where $f_{s}^{\text{un}}$ and $f_s$ values refer to fractions unionized and unbound in the respective fluids of milk or serum and S/W is the skim to whole milk partition ratio.

Predicted M/S values of Model 2 (Atkinson & Begg, 1990) [22] were calculated from the summation of the contribution of the skim milk portion and the milk fat portion:

$$M/S_{\text{pred2}} = \frac{f_{s}^{\text{un}} f_s}{f_{m}^{\text{un}} f_m} K \quad \text{Equation 5}$$

where $K$ is defined by

$$K = \left( \frac{1-Cr}{f_m} + Cr \frac{P_m}{f_m} \right) \quad \text{Equation 6}$$

and Cr is the crematocrit; $P_m$ is the milk lipid:ultrafiltrate partition coefficient which is estimated from the derived relationship with octanol: water partition coefficient: $\log P_m = -0.88 + 1.29 \log (O/W)$ [25]. The O/W value of alprazolam is 18 [26].

Predicted M/S values of Model 3 as modified via In transformation and regression analysis for basic drugs (Begg & Atkinson, 1993) [23] yields

In $M/S_{\text{pred3}} = 0.025 + 2.28 \ln \left( \frac{f_{s}^{\text{un}} f_s}{f_{m}^{\text{un}} f_m} \right) + 0.886 \ln f_s + 0.505 \ln K \quad \text{Equation 7}$

Predicted M/S values of Model 4 (Stebler & Guentert, 1992) [24] used extended Model 2 by incorporating measured S/W ratio:

$$M/S_{\text{pred4}} = \frac{f_{s}^{\text{un}} f_s}{f_{m}^{\text{un}} f_m} \left( \frac{1-Cr}{S/W} + Cr \frac{P_m}{(1-S/W)} \right) \quad \text{Equation 8}$$

The regression coefficients ($r^2$) of the four models were used in testing significant difference from one another (Z-test) at $P < 0.05$ [27]. In addition, the model with the lowest absolute mean error (ME), mean square error (MSE) and root mean square error (RMSE) would be deemed superior [28].

Results

The mean weight of the eight subjects was 62.7 ± 8.0 kg. The mean postpartum period was 11.8 ± 2.3 weeks. The mean pH of milk prior to drug administration was 7.13 ± 0.12. 4-Hydroxy-alprazolam was found in serum, but not milk, at the detection limit of 0.5–1 ng ml$^{-1}$ or less; $\alpha$-hydroxy-alprazolam was not detected in any of the samples. The serum and milk concentration-time profile of alprazolam for a representative subject following the oral administration of alprazolam is presented in Figure 1. Alprazolam concentrations in serum and milk peaked and declined in
roughly a parallel fashion. The pharmacokinetic analysis of the data is given in Table 1. As reflective of the peak concentration and the mean residence time in milk were equivalent to their respective values in serum. Milk concentrations were lower than the serum concentrations as shown by the lower values for peak concentrations and AUC values.

The in vitro parameters used to estimate the predicted M/S values are presented in Table 2. Fractions unionized for both serum and milk are unity as the pKa of alprazolam is 2.8. The predicted M/S values of the four models and the observed M/S values are presented in Table 3. From the mean M/S predicted value, Model 1 (0.296 ± 0.042), Model 2 (0.300 ± 0.037) and Model 3 (0.293 ± 0.025) were found to be closer to the mean M/S observed (0.360 ± 0.113) than Model 4 (0.213 ± 0.029). Although not statistically significantly different from one another (P > 0.05), Model 1 has the highest regression coefficient (r² = 0.604, 0.049, 0.029, 0.009, respectively), the lowest sum of square error (MSE = 0.014, 0.019, 0.020, 0.044, respectively), and the lowest root mean square error compared with Model 2, Model 3 and Model 4 (0.117, 0.138, 0.143, 0.209, respectively) (Table 3).

Model 4 has a higher mean error (−0.146) than Model 1, Model 2 and Model 3 which have similar mean error (−0.063, −0.060, −0.066, respectively) (Table 3).

### Discussion

Lactation did not change the serum pharmacokinetic parameters of alprazolam except the free serum fraction (fₐ = 18%; literature reported fₐ = 24–31% [2]). The reason for a lower free serum fraction for the study is unclear. Low concentrations of 4-hydroxy-alprazolam were detected in serum while α-hydroxy-alprazolam was not detected at all following an oral dose of 0.5 mg alprazolam. This finding is consistent with the literature in that less than 10% of the dose is detected as metabolites in serum, with 4-hydroxy-alprazolam as the major metabolite [29–32]. As with other benzodiazepines [7–16], alprazolam is rapidly absorbed and distributed into milk, with the time of peak concentration of both serum and milk around 60 min. The parallel blood and milk concentration-time profile suggests that alprazolam can readily diffuse across the blood-milk barrier in a

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**Figure 1** Concentration-time profile for alprazolam in serum (●) and milk (○) following an oral dose of 0.5 mg alprazolam in a representative lactating human volunteer.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Serum</th>
<th>Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_max (ng ml⁻¹)</td>
<td>8.88 ± 2.69</td>
<td>3.70 ± 1.59</td>
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<tr>
<td>t_max (h)</td>
<td>0.60</td>
<td>1.10</td>
</tr>
<tr>
<td>(0.45–2.65)</td>
<td>(0.47–3.83)</td>
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<tr>
<td>MRT (h)</td>
<td>16.42 ± 4.69</td>
<td>18.93 ± 7.03</td>
</tr>
<tr>
<td>t½₀ (h)</td>
<td>12.52 ± 3.53</td>
<td>14.46 ± 6.27</td>
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<tr>
<td>CLpo (ml min⁻¹ kg⁻¹)</td>
<td>1.15 ± 0.32</td>
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<tr>
<th>Patient</th>
<th>fₐ</th>
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<th>S/W</th>
<th>Cr</th>
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<td>2</td>
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<td>3</td>
<td>0.209 0.809</td>
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<td>0.097</td>
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<td>4</td>
<td>0.160 0.689</td>
<td>0.886</td>
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<tr>
<td>5</td>
<td>0.176 0.701</td>
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<td>0.048</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.214 0.701</td>
<td>0.905</td>
<td>0.042</td>
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<tr>
<td>7</td>
<td>0.188 0.760</td>
<td>0.898</td>
<td>0.065</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.170 0.784</td>
<td>0.924</td>
<td>0.036</td>
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<tr>
<td>Mean</td>
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<td>0.859</td>
<td>0.062</td>
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<tr>
<td>s.d.</td>
<td>0.018 0.054</td>
<td>0.090</td>
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<tr>
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<th>Model 2</th>
<th>Model 3</th>
<th>Model 4</th>
<th>M/Sobs</th>
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<td>8</td>
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<tr>
<td>RMSE</td>
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<td>0.138</td>
<td>0.143</td>
<td>0.209</td>
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<tr>
<td>ME</td>
<td>−0.063</td>
<td>−0.060</td>
<td>−0.066</td>
<td>−0.146</td>
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</table>

ME, Mean error (bias indicator).

MSE, Mean Square Error.

RMSE, Root Mean Square Error (precision indicator).
References


20 Fleishaker JC, McNamara PJ. In vivo evaluation in the lactating rabbit of a model for xenobiotic distribution into breast milk. J Pharmacol Exp Ther 1988; 244: 919–924.
30 Smith RB, Kroboth PD. Influence of dosing regimen on alprazolam and metabolite serum concentrations and tolerance to sedative and psychomotor effects. Psychopharmacol 1987; 93: 105–112.

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