Dexamethasone Concentrations in Bovine Blood Plasma and Milk After Intravenous Injection

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ABSTRACT

Passage of dexamethasone from plasma to milk in five lactating dairy cows after an intravenous injection was evaluated by a high performance liquid chromatographic technique for measurement of concentrations in blood plasma and milk. The dexamethasone ester was 21-isonicotinate as a solution and was administered at .1 mg/kg bodyweight. The drug was detected in milk postinjection from 15 min to 8 h with a peak concentration of 20.6 ng/ml at the second sample time (30 min). Half-times of dexamethasone in plasma and milk were 4.5 and 3.0 h. The ratio of mean concentration for milk/plasma was .39. It is anticipated that no residues of dexamethasone would be detected in milk if normal dairy practices are followed.

INTRODUCTION

Dexamethasone (DXM) is a synthetic glucocorticoid with potent anti-inflammatory properties. Pharmacological properties and therapeutic indications of this drug are well known; however, little is known in both medical and veterinary medical fields about passage of these drugs, if any, into milk. Concentrations of .0016 and .0267 µg/ml of prednisolone and prednisone were in human breast milk 2 h after 10 mg prednisone was given to nursing women (4, 5). In another experiment (6), seven healthy lactating volunteers were given 5 mg of radioisotope-labeled prednisolone, and milk samples were obtained for 48 h after dosing. The delay profile for radioactivity in milk was initially rapid, then asymptotic. An average of .14% of the dose was recovered in a liter of milk. In cattle, a small portion of endogenous glucocorticoids are excreted into the milk (8). In contrast, by a sensitive high pressure liquid chromatography method with a limit of detectability estimated at 5 ng/ml, no residues were in milk of five cows receiving 20 mg of DXM administered intramuscularly (3).

Our experiment was designed to reevaluate with an appropriate experimental design the passage of DXM from blood plasma to milk in lactating dairy cows treated with an intravenous dose of DXM.

MATERIALS AND METHODS

Animal

Five normal adult cows, 5 to 9 yr old, of different breeds (two Friesian, two Aubrac, and one Blonde d'Aquitaine) weighing between 347 and 474 kg, and fed ad libitum hay and oats were selected from the Toulouse Veterinary College herd. Three cows had calved normally, but the two others (equally normal) were delivered by caesarian section to train veterinary students. Each cow and calf was housed in individual stable, and all calves were permitted to suckle ad libitum throughout the experiment. No attempt was made to evaluate milk production. However, a rather normal milk yield (12 to 18 liters/day) was expected as judged by well being of the calves.

Drug Administration and Sampling

The experiment commenced between 2 and 3 wk after calf delivery. Preinjection blood (via tail vein) and milk samples were collected with minimal disturbances to animals. The DXM 21-isonicotinate as a solution (Voren, Boehringer-Ingelheim, Reims, France) then was injected intravenously via the left jugular vein at .1 mg/kg body weight (as 21-isonicotinate; that is, .078 mg DXM/kg). Blood and milk samples for analyses were collected simultaneously from each cow at the following times postadministration: 15 and 30 min, 1, 2, 4, 8,
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12, 24, and 48 h. Blood was collected into heparinized tubes by venipuncture on each occasion via the right jugular vein. Centrifugation followed as soon as possible, and plasma samples were stored at −20°C until chromatographic analysis. The udder, milked by hand, was stripped completely of milk on each occasion. Because of the presence of the suckling calf and the suppressive effect of DXM on milk yield, milk sample size was always small, ranging from between 10 to 20 ml.

Analytical Method

Concentrations of DXM in plasma and milk were measured by an original high performance liquid chromatographic assay technique described with more details in (1). Briefly, corticosteroids were extracted from plasma by dichloromethane after alkalinization with sodium hydroxide (NaOH .1 N). From the milk, after alkalinization and delipidation by isooctane, corticosteroids were extracted by dichlorometane. Chromatographic analysis was by Waters Apparatus (Waters Assoc. Milford, MA) with a radial compression module, a universal injector (U6K), a 254-nm UV detector (M 440), a column filled with the normal phase (Radial pack B), and a Houston omniscribe recorder. Analyses were by a mobile phase of dichloromethane, methanol, and acetic acid (96:4:4, vol/vol/vol) at a flow rate of 1.5 ml/min. Prednisolone was the internal standard, and sensitivity was 2 ng/ml. Retention time for dexamethasone was 5 min.

Pharmacokinetic Analysis

Least squares regression analyses were by a Hewlitt-Packard 41-C programmable calculator. Pharmacokinetic constants were obtained from plasma and milk concentration time profiles of DXM drawn from the pooled means of the five cows. Data were described by a one compartment model; volume of distribution, half-time, and body clearance were calculated by generally accepted equations (9).

RESULTS

Dexamethasone in Plasma

After injection, DXM of plasma declined (Figure 1). Linear regression analysis showed that the time profile of plasma concentration could be described adequately by a monoeXponential expression of the form:

\[
C_p(t) = B e^{-\beta t}
\]

where \(C_p(t)\) (ng/ml) is the concentration at time \(t\), \(B\) (ng/ml) is the initial concentration at \(t = 0\) obtained by extrapolation of the line, and \(\beta\) (h\(^{-1}\)) is the rate constant of elimination. Estimates of pharmacokinetic parameters from pooled data are in Table 1.

Dexamethasone in Milk

With the exception of one cow, DXM was in milk at first sampling (15 min) (Table 2). Peak concentration (20.6 ± 1.47 ng/ml; mean ± SD) occurred at 30 min and thereafter declined at a rate similar to that of plasma (Figure 1). Half-time of DXM in the milk was 3 h, which was slightly shorter than the plasma half-time of 4.5 h. At all sampling times, DXM concentrations of milk were less than concentrations of plasma, and at \(t = 0\) (extrapolation) a concentration ratio of mean DXM milk/plasma was .39. At 12 h postinjection we were unable to detect DXM in milk; however, from the regression line 1.46 ng/ml was calculated, which is slightly less than the sensitivity of our analysis.

DISCUSSION

The pharmacokinetics of DXM in the plasma of dry cattle have been investigated in our laboratory for both intravenous and intramuscular routes of administration (unpublished
### Table 1. Pharmacokinetic measures describing disposition kinetics of dexamethasone 21-isonicotinate (.1 mg/kg) in bovine plasma and milk.

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Plasma</th>
<th>Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial concentration (B), ng/ml</td>
<td>59.11</td>
<td>23.31</td>
</tr>
<tr>
<td>Slope of elimination line (β), h⁻¹</td>
<td>.1528</td>
<td>.2310</td>
</tr>
<tr>
<td>Half time t/2 (β), h</td>
<td>4.54</td>
<td>3.00</td>
</tr>
<tr>
<td>Volume of distribution (Vd), l/kg</td>
<td>1.31</td>
<td>. . .</td>
</tr>
<tr>
<td>Clearance (ClB), liters/kg per h</td>
<td>.20</td>
<td>. . .</td>
</tr>
</tbody>
</table>

### Table 2. Dexamethasone concentration (ng/ml) in milk after intravenous administration of dexamethasone 21-isonicotinate (.1 mg/kg); ND : less than 2 ng/ml.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Cow No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>X (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>.25</td>
<td>...</td>
<td>18</td>
<td>16</td>
<td>16</td>
<td>15</td>
<td>16.2</td>
<td>(1.15)</td>
</tr>
<tr>
<td>.5</td>
<td>20</td>
<td>18.5</td>
<td>20.5</td>
<td>22</td>
<td>22</td>
<td>20.6</td>
<td>(1.47)</td>
</tr>
<tr>
<td>1</td>
<td>18</td>
<td>39</td>
<td>6</td>
<td>18</td>
<td>11</td>
<td>18.4</td>
<td>(12.58)</td>
</tr>
<tr>
<td>2</td>
<td>27</td>
<td>14</td>
<td>10</td>
<td>12</td>
<td>9.5</td>
<td>14.5</td>
<td>(7.21)</td>
</tr>
<tr>
<td>4</td>
<td>17</td>
<td>12</td>
<td>6</td>
<td>3</td>
<td>ND</td>
<td>7.6</td>
<td>(6.87)</td>
</tr>
<tr>
<td>8</td>
<td>7</td>
<td>11</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>3.6</td>
<td>(5.1)</td>
</tr>
<tr>
<td>12</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Calculated on four measurements.*

The plasma half-time (i.v. route) was 4.86 h, which is not significantly different from the half-time of 4.5 h in lactating cattle; this plasma half-time is sufficiently long that DXM may diffuse into the milk. Therefore, it may be of interest to study the half-time of elimination of DXM from milk. For this analysis milk samples are required soon after administration of DXM (i.e., 15, 30 min, 1, 2, 4, and 8 h postinjection). In contrast, experiments are not appropriate that reproduce the normal practice of taking samples from cows milked at 12-h intervals.

In our experiment, the udder was stripped completely of milk on each occasion, and quantities of milk were always low. Reasons for this small quantity are suckling of calves ad libitum and inhibitory effect of DXM on milk production (2). In our allowing ad libitum suckling, each sample consisted of milk most recently secreted, and, thus, drug concentrations in the milk could be related directly to plasma concentrations at each sample time.

The specificity and sensitivity of the analytical method allowed detection of DXM in milk up to and including 8 h postinjection. Because endogenous glucocorticoids in the cow (8) and some corticosteroid drugs in women (4, 5, 6) have been found in milk, it is not surprising that we detected DXM. We found that concentrations of DXM in milk were approximately one-third of those in plasma. Passage of drugs into milk is governed in part by the extent of binding to plasma albumin. Binding of DXM to albumin in the plasma of cows is 73.8% over a range of concentrations between .03 and 4.04 μg/ml (7). We can presume, therefore, that approximately 30% of the total in plasma concentration of DXM will be free to diffuse into milk. This is close to our calculated ratio of DXM in milk/plasma of .39.

In (3), with a different experimental design (milking twice daily), no DXM was in milk after a single i.m. injection of DXM (20 mg).
apparent discrepancy with our results can be related both to the different dosage regimen and to protocol. In our experiments DXM was injected i.v. to provide 100% bioavailability. However, after i.m. administration (.1 mg/kg), maximal concentration in plasma was 40 ng/ml after a delay of 4 h (unpublished observation). Consequently, with a total dosage of 20 mg (approximately .05 mg/kg), maximum plasma and milk concentrations of approximately 20 and 8 ng/ml may be expected. The latter is close to the sensitivity of the analytical technique. In addition, in our protocol milk was obtained briefly and regularly after drug administration; in contrast, in the experiment of De Paolis et al. (3) cows were milked twice a day. In this condition, sufficient time elapsed between drug administration and milking, and DXM was able to diffuse back from milk to plasma and was eliminated systemically.

From this study, with an unconventional method of milking, passage of DXM occurred from plasma to milk after a single i.v. dose of a soluble ester of this compound. However, because of the equilibrium of DXM between plasma and milk, DXM residues in milk are probably not relevant in practice. Indeed, DXM most often is administered i.m., and concentrations in plasma achieved are lower than those after i.v. administration. In addition, the plasma half-time of DXM is relatively short (4.5 h) by comparison with the interval between two milkings. Consequently, after a single injection of DXM, and if sufficient time has elapsed between drug administration and first milking postinjection (e.g., 10 to 12 h), it can be assumed that the greatest fraction of DXM (which was secreted in the milk during the first 3 h) diffused back into the blood and was eliminated. However, if DXM is given i.v. and if cows are milked less than 8 h after DXM injection, residues may be expected.

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REFERENCES