Pharmacokinetics and Amounts of 25-Hydroxycholecalciferol in Sheep Affected by Osteodystrophy

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ABSTRACT

Amounts of 25-hydroxycholecalciferol in plasma were measured in two groups (A and B) of lambs (Experiment 1) and in two groups (C and D) of wethers (Experiment 2). Groups A (eight lambs) and C (nine wethers) consisted of animals born and raised in total confinement; these animals exhibited an osteodystrophic condition. Groups B (four lambs) and D (10 wethers) consisted of healthy animals born and raised in a conventional barn with free access to an open barn yard (i.e. exposure to sunshine).

The 25-hydroxycholecalciferol in plasma of both groups of sick animals, Group A (12.9 ng/ml) and Group C (18.0 ng/ml), were lower than the amounts of the two corresponding groups of healthy animals, Group B (29.2 ng/ml) and Group D (32.5 ng/ml). Pharmacokinetic analysis of 25-hydroxycholecalciferol in affected lambs following intramuscular injection of 1,000,000 IU vitamin D3 indicated that transport of vitamin D3 from the site of injection to the liver and its metabolism to 25-hydroxycholecalciferol were rapid. Peak 25-hydroxycholecalciferol occurred at .6 wk, and half-life was 3.1 wk.

INTRODUCTION

Lambs raised in total confinement are susceptible to vitamin D deficiency disease and, consequently, are the ones for which supplementation is most common. Hidiroglou et al. (5) reported the clinical history of a flock (ARI) kept under total confinement which showed a high incidence (8%) of an osteodystrophic condition, a vitamin D responsive disease. They also reported that a single massive parenteral administration of vitamin D markedly reduced the incidence of this bone disorder. The efficacy of this loading technique in preventing rickets in lambs has been reported (4). 25-Hydroxycholecalciferol (25(OH)CC), the product of the first step in the metabolism of cholecalciferol to its active form (12), currently is regarded as the major circulating form of vitamin D (16), and its concentration in plasma reflects the vitamin D status of the individual. The use of 25(OH)CC values from serum was helped in the clinical evaluation of vitamin D deficiency in humans (11).

The purposes of this study were to compare 25(OH)CC in plasma of sheep with an osteodystrophic condition with 25(OH)CC in plasma of healthy sheep and to estimate the biological half-life of a single massive intramuscular injection of vitamin D3 to lambs affected by the osteodystrophic condition.

METHODS

In the first experiment were two groups of crossbred lambs, 40 to 60 days of age. Group A consisted of eight lambs from a flock born and raised in total confinement. These eight animals exhibited the osteodystrophic condition which manifested itself at 20 to 30 days of age. The management and clinical symptoms of sheep in this flock were described in (5). Group B consisted of four healthy lambs 40 to 60 days old which were born and raised in a conventional barn with free access to an open yard and came from a flock free of the osteodystrophic condition. All the animals from Group A were given a single intramuscular dose of 1,000,000 IU of vitamin D3 dissolved in 1 ml propylene glycol. Blood samples were drawn from the jugular vein into heparinized containers from all animals (Groups A and B) prior to vitamin D3 administration to Group A (time 0). In addi-
tion, blood was taken from four lambs selected at random from Group A at 1, 2, 3, 6 h and at various other times up to 216 h (Group A1); the four remaining osteodystrophic lambs were bled at weekly intervals for 9 wk (Group A2).

In the second experiment, blood was taken from 19 wethers, 90 to 120 days old. Nine of these wethers (Group C), from the same flock as Group A, were affected by the osteodystrophic conditions which manifested itself when they were 20 to 30 days of age; 10 healthy wethers (Group D) originated from a flock free of the osteodystrophic condition.

The plasma in both experiments was separated from the cellular blood components in a refrigerated centrifuge at 4 C and stored at -20 C until assayed for 25(OH)CC by the method of Hollis et al. (6).

The data from both experiments (time 0 data from experiment 1) were used to test differences between sick and healthy animals for significance. Because of a skewed distribution, a logarithmic transformation (base e) was used; however, variances of the two groups were not homogeneous (neither before nor after transformation); therefore, the t-test as modified by Cochran (3) was used. The means of the logarithms were backtransformed by the method of Oldham (10).

As the main purpose of the kinetic study was the estimation of biological half-life (tσ) of 25(OH)D₃ after vitamin D₃ loading, a simplified pharmacokinetic model was used to represent the disposition and metabolism of the injected vitamin. The model assumed that the concentration in plasma of 25(OH)CC at time t could be represented by an equation of three terms: a constant term representing the normal amount in plasma, a term representing the uptake by the plasma of 25(OH)CC arising from the metabolism of the injected vitamin D₃, and a term representing the irreversible removal from the plasma of 25(OH)CC by excretion, metabolic alteration, or binding. Thus, assuming first-order kinetics, the model was

\[ B(t) = a_0 + a_1 (e^{k_2 t} - e^{k_1 t}) \]  

where \( B(t) \) is the 25(OH)CC in plasma at time t, \( a_0 \) is the normal amount in plasma (i.e. expected in this environment if no vitamin D₃ is injected), \( a_1 \) is proportional to the amount of vitamin D₃ injected, and \( k_1 \) and \( k_2 \) are rate constants reflecting the uptake and the excretion of 25(OH)CC.

Estimates of the parameters in [1] were by iterative techniques to minimize:

\[ \sum (\ln y_t - \ln B(t))^2 \]  

where \( \ln y_t \) is the natural logarithm of the observed 25(OH)CC in plasma at time t. Observed were the means at the various sampling times of each of the two subgroups of Group A. Minimization on logarithmic transformation was chosen because preliminary analysis indicated that the variances were related to the means.

The biological half-life (the time required for one-half of the injected material to be excreted) was calculated from the theoretical excretion curve:

\[ E_p(t) = k_2 \int_0^t B^*(t)dt = \frac{1}{k_1 - k_2} \left( \frac{k_1 e^{-k_1 t}}{k_2 e^{-k_2 t}} - 1 \right) \]  

where \( E_p(t) \) is the proportion of the total dose excreted at time t and \( B^*(t) \) is the proportion of the total dose in the plasma at time t. See, e.g. Batschelet (1) for a derivation of the kinetic equations.

RESULTS AND DISCUSSION

The results, from both experiments, of the sick vs. healthy sheep comparisons are in Table 1. Lambs raised in confinement and affected by an osteodystrophic condition (Group A) had lower (\( P<.01 \)) 25(OH)CC in plasma (12.9 ng/ml) than healthy lambs (Group B) exposed to outdoor light (29.2 ng/ml). Also, wethers with affected limbs had (\( P<.01 \)) lower 25(OH)CC than the control animals (18.1 and 32.5 ng/ml).

The higher mean values for normal sheep compared to those affected by the bone disorder probably reflect greater exposure to ultra-violet and, thus, higher dermosynthesis of cholecalciferol, the precursor of 25(OH)CC. Neer et al. (9) reported a definite effect of an artificial light environment on 25(OH)CC in man.

All lambs with bone disorder and 40% of the
TABLE 1. Mean 25(OH)CC in plasma and standard errors (SE) for sick and healthy animals for Experiments 1 (lambs) and 2 (wethers).

<table>
<thead>
<tr>
<th></th>
<th>Lambs</th>
<th>Wethers</th>
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<tbody>
<tr>
<td></td>
<td>Transformed Mean</td>
<td>Transformed Mean</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td>Sick</td>
<td>2.48</td>
<td>.057</td>
</tr>
<tr>
<td>Healthy</td>
<td>3.33</td>
<td>.066</td>
</tr>
<tr>
<td>t*</td>
<td>9.28 **</td>
<td>4.16 **</td>
</tr>
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</table>

*Means calculated from transformed means (see text).
** (P<.01).

affected wethers had 25(OH)CC of 14 ng/ml or lower, which Care et al. (2) considered as the lower limit of normal 25(OH)CC in plasma in goats. It is suggested that affected wethers with 25(OH)CC greater than 14 ng/ml had a mineral imbalance when they were younger, but at the time of the study the bone lesions had healed although the deformity was still present. The clinical history of the osteodystrophic condition in the flock showed that there can be a reversal of the mechanism producing the bone disorder and that some animals appear to pass through the critical period, and then the bone lesions heal.

The differences in 25(OH)CC among wethers with the bone disease may be related to the individual’s ability to convert dietary vitamin D3 to its metabolite in the liver. Various values for 25(OH)CC were reported in sheep’s plasma by Ross et al. (14), e.g. 16.3 ng/ml for neonate lambs and 24.8 ng/ml for ewes. These values are less than the 40.7 ± 9.09 ng/ml in summer and 37.1 ± 8.82 ng/ml in winter reported for sheep by Shany et al. (15).

TABLE 2. Estimates of kinetic parameters.

<table>
<thead>
<tr>
<th>Constant</th>
<th>SE</th>
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<tr>
<td>a0 (ng/ml)</td>
<td>11.7 ± 1.5</td>
</tr>
<tr>
<td>a1 (ng/ml)</td>
<td>157.6 ± 13.9</td>
</tr>
<tr>
<td>k1 (wk⁻¹)</td>
<td>5.2 ± 8.6</td>
</tr>
<tr>
<td>k2 (wk⁻¹)</td>
<td>.24 ± .21</td>
</tr>
<tr>
<td>t1/2 (wk)</td>
<td>3.1</td>
</tr>
<tr>
<td>t at max (wk)</td>
<td>.6</td>
</tr>
</tbody>
</table>

Estimates of the kinetic parameters are in Table 2; Group A means and the curve of equation [1] are plotted in Figure 1. Although the curve is a fairly good fit to the data (the function accounts for 94% of the total variation among means), there is a suggestion of a systematic departure of some data points (e.g. wk 3 to 6) from the curve. However, no pattern is consistent. Various explanations (other than chance) for these departures can be offered, for example: a) the compartmental model may not represent adequately the underlying transport, storage, and metabolic processes (e.g. because the data were not adequate to estimate the additional parameters, the model does not include the exchange of plasma vitamin D3 and 25(OH)CC with a tissue or peripheral compartment), and b) saturation kinetics may apply.

FIG. 1. Semi-logarithmic plot of 25(OH)CC vs. time for the two groups of lambs with bone disease. The curve is plotted from equation [1] from parameter estimates from Table 1. The vertical bar indicates the 95% confidence interval of the means.
of vitamin D3, thus producing a flatter top to the concentration curve of plasma than expected from first order kinetics. During the analysis of the data, we found that certain combinations of values for \( a_1, k_1, \) and \( k_2 \) (in some cases the values differed substantially from the estimates in Table 2) could be used in [1] to produce a curve similar to that in Figure 1. This result may explain the large standard errors for \( k_1 \) and \( k_2 \) (Table 2). However, estimates of \( t_\frac{1}{2} \) from these combinations of values were all close to 3 wk (cf. 3.1 wk, Table 2).

The high 25(OH)CC soon after injection of vitamin D indicates that the transport of D3 from the site of injection to the liver and its metabolism to 25(OH)CC were rapid. These results do not agree with those of Hollis et al. (7), who reported no significant increase in 25(OH)CC of plasma during the first 7 days following the injection of a massive dose of D3 to dairy cows. However, it should be pointed out that the cows were mature, nonlactating, nonpregnant with an adequate supply of vitamin D, and, therefore, with relatively low maintenance requirement for Ca whereas the Ca requirement by our lambs was probably high due to growth. This explains the rapid rise in 25(OH)D3 in this study and suggests that the activity of liver 25 hydroxylase enzyme was increased in the lambs to meet the demand for Ca. Our results agree with those of Rojanasatit and Haddad (13), who observed an almost immediate rise in plasma 25(OH)CC following the administration of a massive dose of D3 to rats.

The rapid increase of 25(OH)CC in plasma to high levels (Figure 1) indicates that mechanisms associated with the absorption of D3 from the site of injection and its conversion to 25(OH)CC were not defective in the lambs with the bone disorder.

Equation [1] predicts that peak 25(OH)CC in plasma occurred at .6 wk following administration of D3. However, Hollis et al. (7) in the study with dairy cows observed peak 25(OH)CC at 25 to 28 days after i.m. vitamin D3 administration. The estimate of \( t_\frac{1}{2} \) from the excretion curve in Figure 2 was 3.2 wk (Table 2) and was similar to that of Hollis et al. (7).

The half-life of 25(OH)CC in plasma has been calculated to be 19.6 days in humans receiving normal vitamin D (16) and 21 days in the rat (13), although Mawer et al. (8) reported that the half-life was longer in patients receiving large doses of vitamin D.

These results show that a single i.m. massive dose of vitamin D3 to the affected lambs is a theoretically sound intervention. According to Fitch (4) the attractiveness of this prophylactic technique is enhanced by its reasonable cost and economy of labor.

REFERENCES

25-HYDROXYCHOLECALCIFEROL IN SHEEP 571


