Pharmacokinetic Study of Ascorbic Acid in Sheep

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ABSTRACT

Four groups of sheep (5/group) were used in the experiment. Group 1 sheep were given 1 g of ascorbic acid (AA) intravenously (IV), group 2 were given 3 g IV, group 3 were given 1 g intramuscularly (IM) and group 4 received 3 g IM. Blood was collected for 7 h after IV administration and for 48 h following IM administration. Plasma was analyzed for AA using HPLC techniques. After IV administration the rate of elimination was greater at the high dose than the low (0.8560 vs 0.5231 h⁻¹) but the area under the curve (AUC) parameter was proportional to the dosage (127.9 vs 39.7 mcg*h/mL). After IM administration AUC parameters were higher than following the IV injections. When the times that AA levels were ≥ 5 mcg/mL after IM injection were compared there was no significant difference between the 1 and 3 g dosages. Times that levels were ≥ 10 mcg/mL were significantly longer for the 3 g dose. Using the AUC (area under the curve) parameter as an index of drug exposure, supplementation of adult sheep with AA by the IM route should have a greater effect on the animal than IV administration.

INTRODUCTION

It is known that ascorbic acid has an important function as an antioxidant due largely to its redox properties (1). Rose and Bode (2) recently demonstrated that AA is the primary defence in whole blood against free radicals and its importance has led to pharmacokinetic studies in man (3) and Guinea pigs (4). Muggli (5) reported recently that ascorbate influenced phagocytic cell mobility and chemotaxis.

A number of species of animals, including ruminants (cattle, sheep, etc.), dogs, rats and mice are capable of synthesizing their own ascorbic acid (AA) (7). They have the enzyme L-gulanolactone oxidase responsible for the conversion of gulonic acid to gulanolactone in AA synthesis (6).

From time to time ruminants appear to need increased AA availability and scurvy-like skin lesions have been reported in calves (7,8). The importance of AA in increased resistance to respiratory disease has been described previously (9). Hidiroglou et al (10) demonstrated the importance of AA in the acclimatization of ruminant animals to cold and noted that metabolism of this substance could be affected by cold stress. Bouda et al (11) indicated that young ruminants require more AA during cold stress. Hutchens have been shown to provide enough protection to significantly increase plasma AA levels when compared to steel pens in an open sided barn (12). The observations of Hemingway seem to indicate that AA may be important in preventing scours in calves (13).

Supplementation of ruminants with AA must be done by injection since adult ruminants are incapable of using the orally administered material to increase serum concentrations (14,15).

The situation in very young ruminants, eg. calves and lambs under one month of age, is different; they begin life as mono-gastric animals able to utilize exogenous oral AA. In fact calves depend on high AA levels in milk and colostrum (15); as the rumen micro-flora develops they depend more and more on their own synthesis.

Extensive studies have been carried out in humans and guinea pigs on the metabolism and pharmacokinetics of AA by different routes of administration (16,17,18,19). Quantitative data on AA body pools in species such as sheep and cattle, which produce their own AA are not available.
The purpose of the present experiment was to study the plasma profile and pharmacokinetic parameters of vitamin C in adult sheep following parenteral administration using standard pharmacokinetic techniques. The sheep were used as a model of the adult ruminant.

MATERIALS AND METHODS

ANIMALS

Twenty yearling crossbred wethers, weighing 50–55 kg were used. All originated from a flock raised in confinement. For 6 months prior to the experiment, and while it was in progress, the animals were fed a diet consisting of (g/kg) grain silage 400, hay 400 and corn silage 200. They were assigned to 1 of the following 4 L-ascorbic acid (AA) treatment groups:

1) 1 g AA intravenously (IV),
2) 1 g AA intramuscularly (IM),
3) 3 g AA IV,
4) 3 g AA IM.

The approximate per weight dosages, 18–20 mg/kg for groups 1 and 2 and 54–60 mg/kg for groups 3 and 4, were based on animal weights ranging from 50 to 55 kg.

Zero time blood samples were obtained at 8:00 AM by jugular vein venipuncture. Blood samples after IV administration were taken at 5, 10 and 30 min, 1, 2, 3, 4, 5, 6 and 7 h. After the IM administration into the gluteal muscles, blood samples were taken from the jugular vein at 15 and 30 min, 1, 2, 3, 4, 5, 7, 8, 24, 32, and 48 h. The samples were collected using 10 mL heparinized vacutainer tubes and centrifuged in a refrigerated centrifuge. Immediately after centrifuging, a 2.55% solution of metaphosphoric acid was added to the sample (2:1 plasma to acid) and the sample was frozen until thawed just prior to analysis (the frozen storage period was less than 1 wk).

Method of Analysis — AA was detected in the plasma using high pressure liquid chromatography and electrochemical detection according to the method of Behrens and Madere (20). The limit of detection for AA was 0.5 ± 0.08 mcg/mL and interassay variation was 6.1%.

PHARMACOKINETIC ANALYSIS

For the pharmacokinetic analysis of AA movement in sheep following IV and IM administration, background concentrations of this chemical were not subtracted from plasma AA observations.

To establish the models for the IV treatments, mean plasma AA concentrations were plotted in a semi-log fashion and the terminal linear (elimination) components of the AA decay curves determined (21). Least squares regression analysis was carried out on this terminal decay component and it was extrapolated to time 0 (22). Using the method of residuals, the initial rapid decay/s (distribution component/s) of plasma AA were determined (21,23). To determine whether a 2 or 3 compartment model best fit the mean data both models were used and the goodness of fit to the model was tested using the Akaike’s information criterion and the F test at 0.05 (21,24). The residuals were calculated using (Ct – Ct); Ct were the observations at each sample interval and Ct were the calculated values at each interval using the equation for the 2 or 3 compartment model. The residuals were weighted equally (normalized) using the relationship W*(Ct – Ct) where W equals 1/Ct. When the F comparison did not indicate a significant difference between residuals, the simpler model was selected. Once the model was selected from the mean data points, the individual animal data was analyzed and slopes, intercepts, and correlation coefficients from the curve were determined using that model. The zero time intercept on the y axis of the AA concentration/time curve after IV administration equals the sum of the y axis intercepts of distribution (A) plus elimination (B). Plasma concentration (C) at any time (t) can be calculated using the relationship C = Ae^-alpha(t) + Be^-beta(t) where e is the base of natural logarithms. Volumes of distribution were estimated using relationships:

\[ V(d)_{area} = \frac{Dose}{\text{AUC}\times\text{beta}} \]  
\[ V(d)_{apparent} = \frac{Dose}{B\times\text{AUC}} \]  

AUC (area under the curve) was determined by the trapezoidal method with the terminal observation extrapolated to infinity (21) and by the equation AUC = A/alpha + B/beta for IV treatments. Half-lives of distribution and elimination were calculated using the relationship \( \ln t_{1/2}/\text{decay rate} \) of distribution or elimination. Clearance was obtained from beta*Vd_area. Exact volumes of distribution could not be made because all sheep in a group received the same amount of AA; estimates were included instead. Variations in the weight of the animals however, were very small, and thus the mg/kg dose
for each animal was very close to the 20 mg/kg used in the pharmacokinetic calculations.

Standard pharmacokinetic parameters were not determined for AA after IM administration; only the terminal slope was calculated using regression analysis (22). Parameters such as time that AA levels were ≥ 5 mcg/mL or 10 mcg/mL, as well as Tmax and Cmax were plotted directly on spline plots (31) of the individual time vs concentration data.

The bioavailability of AA given IM was estimated as a percentage of IV availability using the relationship (AUC_{IM}/AUC_{IV})*100 (21, 23). AUC parameters used in these calculations were calculated using the trapezoidal method estimated to infinity.

Statistical significance in differences between 1 g and 3 g dosages were determined using Student’s t test (22).

RESULTS

The pharmacokinetic model used to fit the IV administered AA was a 2 compartment open model. The weighted residuals were not significantly different at F = 0.05 and 1/18 degrees, therefore the 2 compartment model was used to describe the data. The correlation parameters for the mean data, fit to the 2 compartment model were for the 1 g dose 0.9998 (rapid decay) and 0.9752 (slow decay); for the 3 g dose 0.9851 and 0.9661 rapid and slow decays, respectively. Parameters obtained when a 2 compartment model was fit to the individual plasma concentration/time data are shown in Table I.

Figures 1 and 2 indicate that the 2 compartment open model selected closely fit the mean plasma AA levels after IV administration. The lines describing the model after IV administration were obtained from the mean parameters in Table I used in the equation C = Ae^{-alpha(t)} + Be^{-beta(t)}

After IV administration AA was eliminated very rapidly from the blood. The mean half-life of elimination, volume of distribution and clearance rate after the low IV dose were 1.68 h, 1012.1 (mL/kg), and 412.2 (mL/min/kg), respectively; and after the high dose, 1.014 h, 652.6 (mL/kg), and 448 (mL/min/kg). AUC differed slightly for the high dose according to the method of calculation however the ratio of dose to AUC was virtually identical with both methods. Mean AUC’s generated from the equation were 47.8 (mcg*h/mL) and 146.2 (mcg*h/mL), low vs high dose and for the method of trapezoids (extrapolated to infinity) 47.7 (mcg*h/mL) and 139.3 (mcg*h/mL), low vs high. The relationship of high dose:low dose was virtually identical to the AUC high dose:low dose.

Theoretical initial concentration (Co) of AA after IV administration was 82.7 (mcg/mL) after the 1 g dose and 271.7 (mcg/mL) after the 3 g dose. This suggests an initial volume of the central compartment of about 11-12 L or approximately 220-242 mL/kg, using the relationship V(d) = dose/Co. Volume of distribution (area estimate) after equilibration for the 3 g dose IV was equivalent to about 65% of the body volume and after the 1 g dose about 112% of the body volume.

The pharmacokinetic parameters for the IM dosages are shown in Table II. After IM administration, time to reach maximum concentration was more rapid after 1 g (0.6 h) than after the 3 g dose (1.4 h); Cmax (maximum concentration) after the small dose (19.9 mcg/mL) was approximately 1/3 the higher dose (58.9 mcg/mL). After IM administration AUC parameters were much higher than after the IV injections. The time that AA levels were ≥ 5 mcg/mL after IM injection were not significantly different between the 1 and 3 g dosages however time that levels were ≥ 10 mcg/mL was significantly longer for the 3 g dose. AUC after 1 g IM dose was approximately 70% the AUC of the larger dose. The decay slope from 24 h onward of the low IM dose was 0.0204 ± 0.0039 h^{-1} for 1 g and 0.0317 ± 0.0157 h^{-1} for 3 g. Availability of AA by the IM route was estimated using the relationship AUC_{IM} = AUC_{IV} (22).

The low dose was 608.6% and the high dose was 295.8%.

Ascorbic acid (AA) concentrations in sheep plasma prior to administration of drug were 1.41 ± 0.2 mcg/mL (range 0.4 to 3.6 mcg/mL).

DISCUSSION

Ascorbic acid is a highly labile chemical that is oxidized by the enzyme dehydroascorbate reductase (6). The transformation of AA to metabolite, and the reverse, occurs readily in blood and plasma and can also occur in plasma outside the animal’s body. The addition of metaphosphoric acid to the plasma prevents this breakdown prior to analysis and therefore should give a more accurate assessment of actual AA

TABLE I. Pharmacokinetics of intravenously administered ascorbic acid in sheep

<table>
<thead>
<tr>
<th>Parameters</th>
<th>1 g IV n = 5</th>
<th>3 g IV n = 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (mcg/mL)</td>
<td>67.2 ± 19.8</td>
<td>206.3 ± 24.0*</td>
</tr>
<tr>
<td>B (mcg/mL)</td>
<td>15.5 ± 1.8</td>
<td>65.4 ± 6.66*</td>
</tr>
<tr>
<td>Co (mcg/mL)</td>
<td>82.7 ± 20.6</td>
<td>271.7 ± 28.9*</td>
</tr>
<tr>
<td>alpha (h^{-1})</td>
<td>6.02 ± 1.02</td>
<td>6.35 ± 1.72</td>
</tr>
<tr>
<td>beta (h^{-1})</td>
<td>0.4134 ± 0.0434</td>
<td>0.6830 ± 0.0203*</td>
</tr>
<tr>
<td>t_{1/2} alpha (h)</td>
<td>0.115</td>
<td>0.109</td>
</tr>
<tr>
<td>t_{1/2} beta (h)</td>
<td>1.681</td>
<td>1.0141*</td>
</tr>
<tr>
<td>V(d) (mL/kg) estimated from AUC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC (mcg*h/mL)</td>
<td>1115.1 ± 63.3</td>
<td>652.6 ± 89.3</td>
</tr>
<tr>
<td>AUC (mg*h/mL)</td>
<td>47.8 ± 3.40</td>
<td>146.2 ± 21.4*</td>
</tr>
<tr>
<td>trapezoidal method</td>
<td></td>
<td></td>
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<tr>
<td>(to infinity)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clearance (mL/min/kg)</td>
<td>47.7 ± 3.35</td>
<td>139.3 ± 19.1*</td>
</tr>
<tr>
<td></td>
<td>412.2 ± 23.5</td>
<td>448.0 ± 66.0*</td>
</tr>
</tbody>
</table>

* A, B = are the Y intercepts of distribution, elimination respectively
* Co = zero time intercept of the plasma drug concentration/time curve extrapolated to zero
* alpha and beta = the rates of distribution and elimination, respectively
* harmonic mean of half-lives of distribution (alpha) and elimination (beta)
* V(d) = volume of distribution
* AUC = area under the curve
* significantly different from 1 g dose using Student’s t test at 0.05 level
levels in the blood. This step appears to have been left out of many of the reports of AA levels in animals (8,10,15,26,27,29).

While it was not possible to obtain information from other reports on AA levels in sheep in using similar HPLC methods, MacPherson (26) reported 4–8 mcg/mL in the plasma of clinically normal sheep and MacPherson and Moon (27) 5.79 ± 0.60 mcg/mL in cobalt supplemented sheep. Richetti (29) compared AA levels in the milk of native Italian sheep, goats, and cattle, and reported sheep and cattle were similar but goats were higher. These levels are much higher than detected in pretreatment samples in the present study and may reflect the inclusion of AA metabolites in the AA levels due to the lack of specificity of the methods used.

The animals in the present study were given standard dosages, either 1 or 3 g, rather than a dose/kg BW; the distribution parameters calculated as V(d)area = Dose/(AUC*beta) are therefore listed as estimates only. However, since interanimal weight variation was small, the 20 mg/kg and 60 mg/kg used in calculations of Vd(area) will be close estimates of the dosages that AA animals received on a per/weight basis. The terminal elimination rate constants (beta) were significantly different between the high dose IV and the low (Table I). The reason for the apparent effect of dose on increasing the rate of removal of AA may be found in the report by Blanchard (3). Urinary excretion, the main route of AA elimination, can be dramatically reduced when an animal is deficient. Blanchard reported "an inverse relationship between the half-life of vita-

Table II. Pharmacokinetics of intramuscularly administered ascorbic acid in sheep

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatment groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 g IM n = 5</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>0.6 ± 0.01</td>
</tr>
<tr>
<td>Cmax (mcg/mL)</td>
<td>19.9 ± 1.99</td>
</tr>
<tr>
<td>Time (h) ≥ 5 (mcg/mL)</td>
<td>14.6 ± 4.05</td>
</tr>
<tr>
<td>Time (h) ≥ 10 (mcg/mL)</td>
<td>4.72 ± 0.82</td>
</tr>
<tr>
<td>Terminal Decay (h⁻¹)</td>
<td>0.0204 ± 0.0039</td>
</tr>
<tr>
<td>AUC (mcg*h/mL)</td>
<td>290.3 ± 46.1</td>
</tr>
</tbody>
</table>

* Tmax = time to maximum concentration  
* Time ≥ 5 time plasma levels are equal to or above 5 or 10 mcg/mL  
* Terminal Decay = decay rate from 24 h onward  
* AUC = area under the curve extrapolated to infinity  
* significantly different from 1 g dose using Student's t test at 0.05 level

Figure 3. Plasma ascorbic acid (AA) in sheep following 1 g and 3 g IM dosages. The points for AA are mean ± SEM. n = 5

The elimination rates we calculated after a single IV bolus, 0.4134 and 0.6830, of AA appears to be more rapid than reported for man, i.e. 0.265 h⁻¹ (3). The fact that the dose given to man was by a 90 min IV infusion could have influenced the outcome since the levels in the blood would not have reached the very high levels encountered when a bolus is administered. If indeed high levels reduce the kidney's tendency to conserve AA (3) this could account for the differences in elimination. A species effect could also have accounted for this difference.

The volume of the central compartment for AA after IV administration (200 mL/kg) is greater than 3 times the estimated blood volume of sheep (30). This indicates that AA moves quickly and easily into the extravascular fluid even as it makes its first circuit through the circulatory system. The higher volume of distribution at the low IV dose may indicate a concentration limited partitioning or binding of AA in tissues that become less important as the dose increases. The formula used to calculate Vd(area) suggests that the difference is due to the lower value of the hybrid elimination constant beta, since AUC was proportional to dosage (Table I).

The AUC parameter can be used as a measure of total exposure of an individual to a drug. Two methods were used to calculate AUC; the standard equation [A/alpha + B/beta] after IV administration, requiring parameters for distribution and elimination, and the method of trapezoids with an estimate of AUC to infinity (Ct/beta; where Ct is the last observed AA concentration) using beta the terminal decay slope (21). While small differences were observed between these 2 methods of AUC calculation at the 1 g IV dosage, they were virtually the same at the high IV dose (Table 1). Since AUC data from the IM study was obtained using the trapezoidal method, this was the method of calculation used in determining the bioavailability by the IM route. The AUC for the 1 g IV dose, 39.7 mcg*h/mL, was approximately 1/3 the AUC for the high dose, 127.9 mcg*h/mL, confirming a close relationship between dosage and total exposure by this IV route. After IM administration, on the other hand, the AUC parameters were much larger (Table I and II) but the dose relationship was less defined. The AUC after the low dose IM 290.3 ± 46.1 mcg*h/mL was more than half the AUC after the high 421.1 ± 39.6 mcg*h/mL. Bioavailability after IM administration appeared to be much higher than after IV exposure and a route of administration effect is therefore apparent. The reason for the greater AUC after IM administration and thus greater exposure to AA, may be due to the much slower disappearance of AA from the plasma.
The reasons for this slower decay are discussed below.

Detailed absorption and elimination parameters were not available after IM administration, in this study. There were not adequate points in the period after administration, when absorption was the dominant factor to estimate absorption parameters. As is shown in Figure 3, the decay rates appeared to change several times during the 48 h after IM administration. At least 3 factors could account for this observation. One possibility is that Flip-Flop kinetics could be occurring during part of the decay, with rate of absorption rather than rate of elimination determining the terminal decay rates. For this to occur, the rate of absorption has to be slower than the rate of elimination. This may account for the relatively slow decline in AA levels shortly after peak levels were observed. However, this does not seem like a strong possibility for the later stages of the decay curve because while the terminal elimination slope, after the low IM dose was slower than after the high dose, the rate of absorption, if we use time to Tmax as an indicator, was more rapid for the low dose. A second possibility is that a “conservation” mechanism such as reported in other species (3) is operating. This is a possibility and the fact that the AUC is disproportionately large after the low dose would support this concept of conservation of the vitamin. If a conservation mechanism is affecting the loss from the body, probably not constant over the range of plasma levels, then first order decay kinetics would not prevail. The third possibility is that endogenous synthesis of AA by the sheep has a greater impact on plasma AA levels and decay when they are low vs when they are high. This could account for the slower decay in the low dose experiment, and deviation from first order kinetics. The correlation coefficients of the terminal elimination slopes calculated from 24 h to 48 h were very high, 0.9991 and 0.9649 for low and high doses respectively, using the mean data points. The high correlation coefficients calculated for the decay components in the 2 compartment open model used to describe AA plasma level changes after IV administration support our assumptions of first order kinetics following this route of administration.

Although absorption rates from the IM sites could not be calculated, based on the Tmax parameters, uptake for the most part appeared to be quite rapid. The mean time ≥ 5 mcg/mL was greater after 1 g IM than after the 3 g dose although the difference was not significant. The time for the low dose group was increased by one animal that maintained these concentrations for nearly 24 h. This individual also had very high pretreatment AA levels in plasma and since these observations were not corrected for background AA the concentration detected could have been maintained at this high level by the endogenous AA levels. The actual times that AA levels were above 5 mcg/mL after 3 g IV ranged from 2 to 4 h whereas after the 1 g IM the range was between 7 and 24 h. At the higher concentration (≥ 10 mcg/mL) the length of time for the high dose group was significantly greater than the low. The authors were not able to obtain from the literature anything resembling a therapeutic or desirable level of AA in the plasma for ruminants. Levels in the 5–10 range were reported naturally in ruminants (24).

The above findings indicate that the administration of AA to sheep by injection, to prevent scurvy or to supplement anti-scorbutic levels during periods of stress or low AA availability, will cause an increase in plasma AA concentrations (Figure 1–3). The AUC parameters suggest that if AA supplementation is justified, the use of the IM route could be of greater benefit than the IV route and would thus appear to be preferred.

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REFERENCES


