Pharmacokinetics and metabolic effects of triamcinolone acetonide and their possible relationships to glucocorticoid-induced laminitis in horses

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Experiments were performed to establish the pharmacokinetics of triamcinolone acetonide and the effects of the glucocorticoid on glucose metabolism in horses. The pharmacokinetics after intravenous (i.v.) dosing was best described by a three-compartment open model. There was rapid distribution from the central compartment followed by two phases of elimination. The half-life of the rapid elimination phase was 83.5 min and of the slower phase was 12 h. The term $(V_{ss}/V_c) - 1$was 12.3 indicating extensive distribution into the tissues. Triamcinolone acetonide given i.v. or intramuscularly (i.m.) induced a prolonged period of hyperglycaemia, hyperinsulinaemia and hypertriglyceridaemia. Significant changes in plasma glucagon and serum non-esterified fatty acids were not observed. These observations suggest that the hyperglycaemia was a result of decreased glucose utilization by tissues and increased gluconeogenesis. The effects on glucose metabolism persisted for 3–4 days after triamcinolone was given i.m. at 0.05 mg/kg, the upper limit of the recommended dose range, and for 8 days when given at 0.2 mg/kg. These observations, together with recent evidence implicating inhibition of glucose metabolism in the pathogenesis of equine laminitis, indicated that triamcinolone-induced laminitis may be associated with the long duration of action of the glucocorticoid when higher than recommended doses or when repeated doses are given.

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INTRODUCTION

Triamcinolone is a potent glucocorticoid used in treating a variety of equine diseases. It is generally administered by intramuscular (i.m.) or intrasynovial injection. Glucocorticoids such as triamcinolone are known to precipitate laminitis (Lawrence *et al.*, 1985; Kelley, 1997). However, the mechanism by which they do this is poorly understood.

Recently, it has been proposed that laminitis may be a result of alterations in glucose metabolism in response to stress or infection leading to a rapid decrease in glucose utilization by the hoof tissues (Pass *et al.*, 1998). It has been suggested this causes activation of matrix metalloproteinases (MMPs) that digest the basement membrane between dermis and epidermis resulting in separation of the hoof wall from the underlying tissues (Pass *et al.*, 1998). The separation of the dermis from the epidermis is the essential lesion of laminitis (Pollitt, 1996; Pollitt & Daradka, 1998). Glucocorticoids have effects on glucose metabolism and cause insulin resistance (Wuttke, 1989; Greco & Stabenfeldt, 1997). Therefore, it is possible that these effects may contribute to the development of glucocorticoid-induced laminitis. As the extent to which triamcinolone influences glucose metabolism in horses was not known, experiments have been performed to determine this. The results are presented in this paper.

Anecdotal evidence indicates that the incidence of triamcinolone-induced laminitis is probably related to administration of multiple doses or relatively high doses of the glucocorticoid. The pharmacokinetics of triamcinolone do not appear to have been studied in horses and presumably, the recommended doses are based on studies in other species. If the pharmacokinetics of the drug in horses differed from those in other species, it could be that the recommended doses would give higher or more prolonged tissue concentrations resulting in adverse effects. To address this issue, the pharmacokinetics of triamcinolone acetonide (TMC) have been studied in horses. TMC was studied because it is the commercially available form of the steroid.

MATERIALS AND METHODS

Chemicals

TMC for intravenous (i.v.) injection and use as a standard in the high-performance liquid chromatography (HPLC) assay and aprotinin were from Sigma Chemical Co., (St Louis, MO, USA). TMC was dissolved in dimethyl sulfoxide (DMSO) (Sigma: USA) for i.v. injection into horses. TMC suspension for intramuscular injection was purchased from Jurox Pty Ltd, (Maitland, NSW, Australia). HPLC grade acetonitrile (AcN) and dichloromethane were purchased from EM Science (Gardena, CA, USA). Sodium hydroxide (NaOH) pellets were from Univar Analytical Reagents, Ajax Chemicals (Wacol, Qld, Australia) and industrial grade nitrogen gas was from BOC Gases (Rocklea, Qld, Australia).

Animals

Five horses (three mares, one gelding, one stallion; 6–14 years of age; 335–450 kg) were used. They were housed in stables and fed lucerne hay three times daily, 400 g of pony pellets twice daily (Calm Performance Pellets; Ridley Pty Ltd, Wacol, Qld, Australia) and given water *ad libitum* for the duration of the experimental period.

Experimental procedures

Three experiments were performed on each horse: TMC (0.2 mg/kg) dissolved in DMSO (0.01 mL/kg) given i.v.; DMSO (0.01 mL/kg) given i.v.; and TMC injectable suspension (0.2 mg/kg) given i.m. Blood samples were collected from a catheter (13-gauge, 25 cm length central venous catheter set using 5 cm proximal extension, Cook Veterinary Products; Brisbane, Qld, Australia) placed in the left jugular vein. A sample was collected before injection of a test substance into the right jugular vein. Subsequent samples were taken at 2, 5, 10, 15, 20, 30, 45, 60, 75, 90 and 105 min, and 2, 2.5, 3, 4, 5, 6, 8, 12, 24, 36 and 48 h after injection for analysis for TMC. Samples were also taken before dose and at 15, 30, 45, 60 and 90 min; at 2, 2.5, 3, 4, 5, 6, 8, 12, 24 and 36 h; and at 2, 3, 4, 5, 6, 8, 10, 12, 14 and 16 days after injection where necessary for analysis for glucose, insulin, glucagon, triglycerides and non-esterified fatty acids (NEFA). Blood was collected into lithium heparin tubes (Sarstedt, Mawson Lakes, SA, Australia) for plasma and was used for analysis of TMC, serum collection tubes (Becton Dickson, Vacutainer Systems, Sydney, NSW, Australia) for insulin, triglyceride and NEFA analyses, EDTA tubes containing aprotinin (100 μg/mL of blood) for glucagon analysis and fluoride oxalate tubes (Johns, Brisbane, Qld, Australia) for blood glucose analysis.

All samples were kept on ice until processed. Samples collected for plasma and serum were centrifuged in a refrigerated centrifuge at 10000 $\times g$ for 10 min. The plasma or serum was removed and aliquoted before being frozen at -20 °C for later analyses. Samples for glucose determination were stored at 4 °C and analysed within 12 h.

Three mares (9–14 years old, 335–450 kg) were given TMC i.m. at 0.05 mg/kg and samples collected for analysis of the concentrations of blood glucose.

Analytical methods

Methods for extracting and detecting TMC in horse plasma were developed and validated based on methods described by Doppenschmitt *et al.* (1996). Each plasma sample was alkalinized by adding 0.5 mL of 0.1 M NaOH to 1 mL of plasma in a stoppered glass tube. The sample was then extracted using 2 mL dichloromethane by continuously inverting and re-inverting the tube for 30 sec before centrifugation at 10000 $\times g$ for 10 min. The organic layer was removed and the plasma sample was re-extracted using a further 2 mL of dichloromethane. The combined organic extracts were dried under a stream of nitrogen at 35 °C and the residue reconstituted in 100 μL of AcN/H_2O (2:1) for injection into the HPLC system.

The HPLC system consisted of a Waters U6K injector, a 6000A pump controlled by a 680 automated gradient controller and a Waters series 441 absorbance detector set at 254 nm (Waters Australia Pty Ltd, Rydalmere, NSW, Australia). Data were recorded and analysed using a PC computer and Waters Maxima 820 Chromatography Software system. Separation was performed on a Waters Novapak C18 analytical column (3.9 × 300 mm) using an in-line pre-column filter and Novapak guard column using a mobile phase of 40% AcN in water pumped at 1 mL/min at ambient temperature. The minimum reliable detection of TMC in horse plasma was 1 ng/mL and the recovery of TMC added to horse plasma was $99.7 \pm 9.4\%$ at 10 ng/mL (mean $\pm$ SEM, $n = 6$).

Blood glucose concentrations were determined using a YSI 2300 Stat Plus Glucose and L-Lactate analyser (YSI Inc., Yellow Springs, OH, USA). Insulin and glucagon concentration were determined using radioimmunoassay (RIA) kits (Insulin Coat-A-Count and Glucagon Double Antibody) manufactured by Diagnostic Products Corporation Pty Ltd, (Los Angeles, CA, USA) which were purchased from BioMediQ, (Doncaster, Vic, Australia). Triglycerides and NEFA were determined using kits (Trace Triglyceride GPO; Trace Scientific Ltd, Melbourne, Vic, Australia and NEFA 1 C; Wako Pure Chemical Industries, Osaka City, Osaka, Japan) giving colorimetric reactions that were analysed by a Cobas Mira Colorimetric Analyser (Roche Diagnostica Pty Ltd, Castle Hill, NSW, Australia).

Data analysis

Analysis of variance and *t*-tests were used to compare the concentrations of glucose, insulin, glucagon, triglycerides and NEFA after dosing with TMC or DMSO with those before dosing.

Pharmacokinetic analyses were performed using a commercial curve-stripping program (RSTRIP version 4.12, Micromath Scientific Software, Salt Lake City, UT, USA). A weighting factor of $1/C_p^2$ was applied where C_p is the plasma concentration of TMC. A three-compartment open pharmacokinetic model (Bag-

got, 1977) best described the disposition kinetics of TMC. The model was selected on the basis of the model selection criteria generated by the RSTRIP program and visual assessment of the lines of best fit for the two- and three-compartment models. The constants describing the tri-exponential equation relating plasma concentration to time (Baggot, 1977) are given as the mean ± SEM and the parameters derived from the constants (Baggot, 1977) as the geometric mean and range (Powers, 1990).

RESULTS

Changes in glucose, insulin, glucagon, triglycerides and NEFA after TMC dose

The i.v. dose of TMC (0.2 mg/kg) had no obvious effects upon the demeanor or appetite of the horses. Appetite and thirst appeared to be reduced for 3–4 days after i.m. dosing; however, this effect was not quantitated. The horses were observed for 2–14 months after the experiments and although overt clinical laminitis did not occur, growth defects (laminar rings) developed in hooves of four of the five horses.

DMSO (0.01 mL/kg) given i.v. did not alter the concentrations of blood glucose, serum insulin, plasma glucagon, serum triglycerides or serum NEFA (Fig. 1). The pattern of the changes in the concentrations of glucose, insulin, glucagon and NEFA were similar in all five horses after i.v. and i.m. dosing with TMC. Serum triglyceride concentrations increased significantly after TMC was given i.m. (Fig. 1). The changes after i.v. dosing were not statistically significant but there was a tendency for the concentrations to increase above pre-dosing concentrations.

The concentrations of blood glucose increased significantly by 5 h after TMC (0.2 mg/kg) was given i.v. ($P < 0.05$) with a peak being reached by about 24 h ($P < 0.001$) (Fig. 1). Glucose concentrations had returned to pre-dosing concentrations by 3 days. Serum insulin concentrations increased by 12 h and peaked at about 24 h ($P < 0.001$) after i.v. dosing with TMC

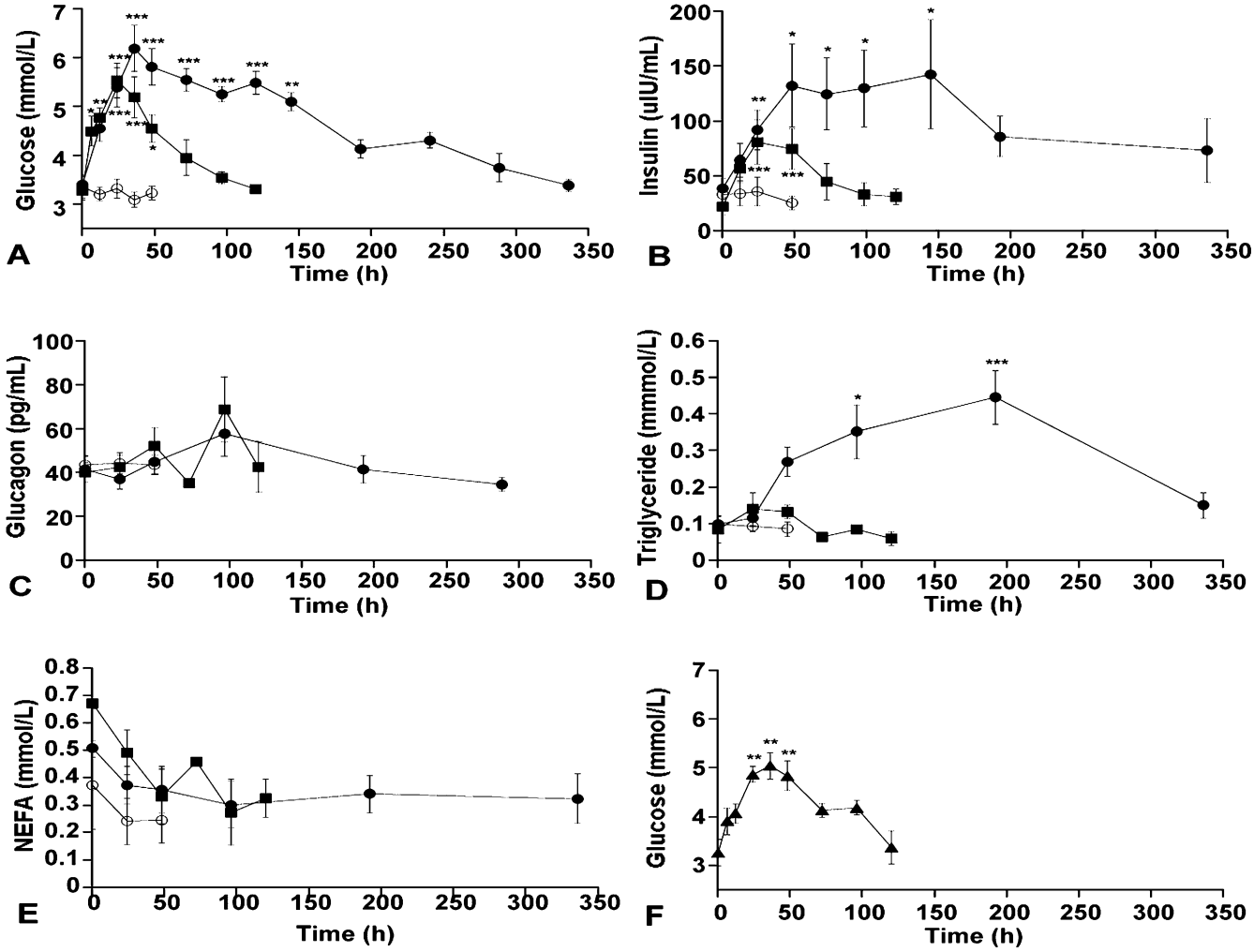

Fig. 1. Effect of TMC on blood glucose (A,F), serum insulin (B), plasma glucagon (C), serum triglycerides (D) and serum NEFA (E). (A–E) The changes after administration of DMSO given i.v. at 0.01 mL/kg (○), TMC given i.v. at 0.2 mg/kg (■) and TMC given i.m. at 0.2 mg/kg (●) (F) TMC given i.m. at 0.05 mg/kg (▲). Data are expressed as the mean ± SEM (A–E, $n = 5$; F, $n = 3$). Significant differences from pretreatment values are indicated by $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

Table 1. Pharmacokinetic analysis of the disposition of TMC (0.2 mg/kg) given i.v. to five horses

Item	Mean	SEM or Range
A_1 ng/mL	433.6	65.4
B_1 min^{-1}	0.2470	0.0664
A_2 ng/mL	81.7	9.5
B_2 min^{-1}	0.0100	0.1514
A_3 ng/mL	13.9	5.2
B_3 min^{-1}	0.0023	0.0008
$t_{1/2B1}$ min	5.2	4.5–20.3
$t_{1/2B2}$ min	83.5	31.3–170.4
$t_{1/2B3}$ h	12.0	3.0–52.0
AUC ng.min/mL	24 620	13 467–40 383
AUMC ng.min^2/mL	15 933 409	2 648 127–63 766 807
MRT min	647.2	196.6–1561.5
C_p^o ng/mL	430.4	131.8–641.6
K_{12} min^{-1}	0.1160	0.0305–0.2888
K_{21} min^{-1}	0.0496	0.0204–0.0934
K_{13} min^{-1}	0.0060	0.000017 – 0.3965
K_{31} min^{-1}	0.0016	0.0003–0.0085
K_{EL} min^{-1}	0.0208	0.0077–0.0452
V_{ss} L/kg	5.3	2.5–13.7
V_C L/kg	0.39	0.31–0.64
$V_{ss}/V_C - 1$	12.3	6.9–30.9
Cl_B mL/min.kg	8.1	4.9–14.9

Constants are expressed as the mean ± SEM and derived values as the geometric mean and range

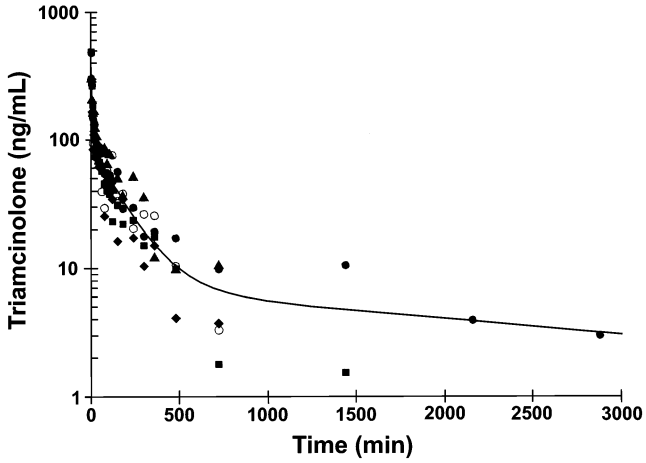

Fig. 2. Disposition curve showing the plasma concentrations of TMC given i.v. at 0.2 mg/kg to five horses and the line describing the tri-exponential equation of best fit of the average TMC concentrations at each time point. Different symbols indicate values from different horses.

(Fig. 1). They returned to pre-dosing concentrations by 3 days. No changes were observed in plasma glucagon concentrations after i.v. dosing with TMC (Fig. 1). The concentrations of triglycerides in the serum appeared to increase and reach a peak by 36 h after the i.v. dose of TMC, but the changes were not statistically significant (Fig. 1). There were no significant changes in serum NEFA concentrations after the i.v. dose of TMC (Fig. 1).

TMC (0.2 mg/kg) given i.m. caused a significant increase in the concentrations of blood glucose by 24 h ($P < 0.001$) with a maximum increase occurring at about 36 h ($P < 0.001$) after injection (Fig. 1). The glucose concentrations were not statistically different from pre-dosing concentrations by 8 days after dose of the steroid and subsequently, continued to decline. Serum insulin concentrations increased significantly by 24 h ($P < 0.01$) and peaked at about 6 days ($P < 0.01$) after injection (Fig. 1). The insulin concentrations had declined by 8 days after injection to concentrations that were not significantly different from pre-dosing concentrations. Serum triglycerides had risen significantly by 4 days ($P < 0.05$) and peaked at about 8 days ($P < 0.001$) after TMC was given i.m. (Fig. 1). Triglyceride concentrations were not statistically different from pre-dosing concentrations by 14 days after injection. There were no significant changes in plasma glucagon or serum NEFA concentrations after i.m. injection of TMC (Fig. 1).

TMC (0.05 mg/kg) given i.m. to three horses caused a significant increase in the concentrations of blood glucose ($P < 0.01$) that were sustained for about 4 days (Fig. 1).

Pharmacokinetics of TMC

The maximum concentrations of TMC detected after i.m. dosing were very low and the corticosteroid could not be detected in plasma 5 h after injection. The data were insufficient for analysis of the pharmacokinetic behaviour of TMC after i.m. injection. In contrast, TMC could still be detected for more than 12 h after i.v. injection. The pharmacokinetic parameters, describing the disposition of TMC after i.v. dose, are shown in Table 1. A three-compartment open model best described the relationship between plasma concentration and time. Figure 2 shows the plasma concentrations of TMC in the five horses and the line describing the triexponential equation of best fit of the average TMC concentrations at each time point.

The distribution half-life of TMC was rapid being 5.2 min. The elimination of TMC had two components: a rapid and a slow phase. The half-life of the rapid phase was 83.5 min and the half-life of the slow phase was 12.0 h. The term $(V_{ss} - V_c) - 1$, an indication of the distribution of TMC, was 12.3.

DISCUSSION

TMC caused a marked alteration in the glucose metabolism of horses that was manifest as hyperglycaemia. A single dose of TMC (0.05 mg/kg), given i.m., induced hyperglycaemia for at least 3 days. This dose was the maximum dose of 20 mg recommended by the Australian distributor of TMC (IVS Annual Australian Edition, 1999) if given to a 400-kg horse. The recommended dose range is 12–20 mg/horse. A higher dose, given i.m., produced a more prolonged effect on glucose metabolism.

Hyperglycaemia can be a result of increased gluconeogenesis, decreased glucose utilization or a combination of the two.

Glucagon is the major physiological regulator of gluconeogenesis (Dimitriadis *et al*., 1997; Greco & Stabenfeldt, 1997) but it did not play a role in TMC-induced hyperglycaemia. Glucocorticoids are known to decrease glucose utilization and render cells insulin resistant (Greco & Stabenfeldt, 1997). However, variable effects on gluconeogenesis from decreases to increases have been observed (Wajngot *et al*., 1990). It is probable that decreased glucose utilization made a significant contribution to TMC-induced hyperglycaemia. Increased gluconeogenesis may have contributed but the extent of this contribution may be questionable in light of conflicting reports of other experiments (Wajngot *et al*., 1990). The hyperinsulinaemia observed in the current experiments was presumably a response to hyperglycaemia in an attempt to lower blood glucose concentrations.

TMC also caused hypertriglyceridaemia, a condition facilitated by hyperinsulinaemia and hyperglycaemia (Jeffcott *et al*., 1986; Freestone *et al*., 1991). Serum NEFA concentrations did not change after the dose of TMC. A rise in serum NEFA concentrations often occurs before hypertriglyceridaemia and is the result of fatty acid mobilization. It is unclear why serum NEFA concentrations did not increase before serum triglyceride concentrations. However, glucocorticoids do alter lipid metabolism by stimulating hormone-sensitive lipase that increases mobilization of free fatty acids from adipose tissue and increases triglyceride concentrations in the blood. Glucocorticoids are also known to cause a decrease in glucose uptake and insulin resistance in the periphery (Dimitriadis *et al*., 1997). Insulin resistance is associated with both abnormal lipoprotein lipase activity and increased hepatic triglyceride formation (Jeffcott *et al*., 1986; Freestone *et al*., 1991). NEFA are rapidly extracted from the blood as a result of the large proportion of the total blood flow reaching the liver and the efficient hepatic extraction of NEFA (Herdt, 1997). This, coupled with increased endogenous triglyceride production as a result of insulin resistance, would minimize changes in NEFA in response to glucocorticoids. The fact that the animals continued to eat throughout the experimental period may also have contributed to the failure to generate a significant increase in serum NEFA concentrations. Under this condition, the horses would have been utilizing volatile fatty acids generated in the lower intestine rather than triglyceride hydrolysis for energy generation. If this were the case, NEFA generation would have been minimized despite the fact that serum triglyceride concentrations would have increased as a result of the increased rate of triglyceride formation in the liver caused by insulin resistance.

Investigation of the pharmacokinetics of TMC indicated that the prolonged effects of TMC on metabolism are probably related to the persistence of the drug. The disposition of TMC after i.v. dosing was best described by a three-compartment open pharmacokinetic model. After the i.v. dose, TMC was relatively, rapidly distributed outside of the central compartment. V_{ss} represents the proportionality constant giving the amount of drug in the body at steady-state and is independent of drug elimination. It reflects the anatomical space occupied by the drug and the relative degree of drug binding in the blood and extravascular tissues (Gibaldi, 1991). The quantity $(V_{ss}/V_c) - 1$ allows estimation of the proportions of the drug in the central and peripheral compartments. A high $(V_{ss}/V_c) - 1$, as was seen with TMC in horses, indicates that a larger proportion of TMC distributed to the peripheral compartments than remained in the central compartment (Ritschel, 1992). The elimination of TMC was characterized by a rapid phase with a mean half-life of approximately 1.5 h and a slow phase with a mean half-life of 12 h. As a result of the slow elimination phase, TMC persisted, at least in low concentrations, for a relatively long period. This accounts, in part, for its duration of action as estimated by the effects on blood glucose and plasma insulin concentrations.

Other publications of the pharmacokinetics of TMC administered i.v. to humans and rabbits, reported that the pharmacokinetics of the drug were best described by a two-compartment open model (Abraham *et al*., 1983; Derendorf *et al*., 1995). This is in contrast to the three-compartment model that was appropriate for the behaviour of the steroid in horses. However, blood samples were collected for only 7 h in rabbits and 24 h in humans and the slow elimination phase may not have been detectable within these times. The elimination half-lives in humans and rabbits were similar to the rapid elimination half-life in horses. Closer inspection of the data from studies in humans, indicates that a second elimination phase may in fact be present (Derendorf *et al*., 1995).

The present observations may have relevance to the problem of laminitis associated with administration of TMC. Experiments utilizing cultured hoof explants demonstrated that an acute reduction in glucose utilization in the tissue resulted in separation of the epidermal and dermal lamellae as occurs in laminitis (Pass *et al*., 1998). Furthermore, because many of the causes of laminitis are likely to reduce glucose utilization in peripheral tissues, it has been proposed that this may contribute to the pathogenesis of laminitis (Pass *et al*., 1998). The hyperglycaemia caused by TMC appears to be a result of, at least in part, a reduction in glucose utilization. Therefore, it is possible that this contributes to the development of TMC-induced laminitis.

The observations presented in this paper have implications for the use of TMC in horses. The recommended doses of TMC for horses are 12–20 mg/horse i.m. Repeat doses can be given at 48 h. The maximum recommended dose is 0.05 mg/kg for a 400 kg horse and this would induce hyperglycaemia for about 3 days after a single dose. Repeated doses would maintain the effect throughout the period of treatment. Anecdotal reports indicate that TMC is sometimes given at doses up to 100 mg/horse and by daily dosing at various dosages. These large or frequent doses would produce prolonged hyperglycaemia and glucose starvation of the tissues.

Given that the dosing regimens that induce laminitis have not been confirmed experimentally, it seems prudent that the manufacturer's recommendations for the use of TMC be observed until further information is available.

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