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Pharmacokinetics, disposition and plasma concentrations of dimethyl sulfoxide (DMSO) in the horse following topical, oral and intravenous administration.

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ABREVIATIONS

K_{ab} K_e = absorption and elimination rate constants

$t_{1/2ab}$ = absorption half-life

$t_{1/2e}$ = elimination half-life

C_1 , C_2 = central and peripheral compartments

K_{10} = elimination from the central compartment

k_{12} , k_{21} = inter-compartmental distribution rate constants

α , β = slope factor or exponents

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$t_{1/2\alpha}$, $t_{1/2\beta}$ = α , β , half-lives

V_C , V_2 = volumes of central and peripheral compartments

AUC_0^{24} , AUC_0^∞ = area under the plasma concentration curves, 24 h and infinity

Cl , Cl_F = compartment and corrected compartment clearance

ABSTRACT

Compartmental models were used to investigate the pharmacokinetics of intravenous (IV), oral (PO), and topical (TOP) administration of dimethyl sulfoxide (DMSO). The plasma concentration-time curve following a 15-min IV infusion of DMSO was described by a two-compartment model. Median and range of alpha ($t_{1/2\alpha}$) and beta ($t_{1/2\beta}$) half-lives were 0.029 (0.026-0.093) and 14.1 (6.6-16.4) h, respectively. Plasma concentration time-curves of DMSO following PO and TOP administration were best described by one-compartment absorption and elimination models. Following the PO administration, median absorption ($t_{1/2ab}$) and elimination ($t_{1/2e}$) half-lives were 0.15 (0.01-0.77) and 15.5 (8.5-25.2) h, respectively. The plasma concentrations of DMSO were 47.4 to 129.9 $\mu\text{g/ml}$, occurring between 15 min and 4 h. The fractional absorption (F) during a 24 h period was 47.4 (22.7-98.1)%. Following TOP administrations, the median $t_{1/2ab}$ and $t_{1/2e}$ were 1.2 (0.49-2.3) and 4.5 (2.1-11.0) h, respectively. Plasma concentrations were 1.2 to 8.2 $\mu\text{g/ml}$ occurring at 2 to 4 h. Fractional absorption following TOP administration was 0.48 (0.31-4.4)% of the dose administered. Clearance (Cl) of DMSO following the IV administration was 3.2 (2.2-6.7) ml/h/kg. The corrected clearances (Cl_F) for PO and TOP administrations were 2.9 (1.1-5.5) and 4.5 (0.52-18.2) ml/h/kg.

Dimethyl sulfoxide (DMSO) is a small (78.1 g/mol) aprotic molecule, which is a by-product of the wood pulp industry. Dimethyl sulfoxide has been used for a multitude of medical disorders based on a broad range of incompletely understood pharmacological properties. Its pharmacology and uses in a litany of medical disorders have been reviewed in detail; it is palliative, but not a curative preparation (David, 1972; Murdoch, 1982; Swanson, 1985; Brayton, 1986; Jacob & Herschler, 1986; Ali, 2001).

Dimethyl sulfoxide was first used in living cells in 1959 to protect them from freezing. The protective properties of DMSO are based on the physical characteristics of DMSO, low molecular weight, non-toxic, and high solubility in aqueous solutions,. (Lovelock & Bishop, 1959). Dimethyl sulfoxide's further use in biological systems was explored in 1964 (Jacob et al., 1964; Jacob et al., 1964). Initial use in patients was for the treatment of musculoskeletal injuries, inflammation, and intractable pain (Rosenbaum & Jacob, 1964; Rosenbaum et al., 1965; Rosenbaum et al., 1965). It was suggested for use in veterinary medicine in 1965 (Jacob et al., 1965). In an early symposium on the use of DMSO in veterinary and human medicine, topically applied DMSO was reported as useful for the treatment of inflammatory conditions by reducing edema and providing analgesia based on the modulation of inflammation (Brown, 1967; Goldman, 1967; Knowles, 1967; Levesque, 1967; Teigland & Saurino, 1967; Tobin, 1981). Studies have suggested that C fiber blockade may account for analgesia with DMSO (Evans et al., 1993). Direct effects of DMSO on blocking sensory neurons was also reported (Theophilidis & Kravari, 1994).

Despite a multitude of studies on the medical effects and potential benefits, DMSO has only been approved in veterinary medicine for topical administration to reduce acute swelling due to trauma (Jacob & Herschler, 1983; Hillidge, 1985). Despite its apparent ubiquitous use and lack of innocuous effects, systemic side-effects have been reported (Santos et al., 2003)

Topically applied DMSO penetrates into synovial fluid in sufficient quantities to be detected in the joint and to decrease joint inflammation produced by lipopolysaccharide (LPS)-induced synovitis (Smith et al., 1998). No detrimental results were noted following the injection of carpal joints with 2 ml of a 40% solution of 90% medical grade DMSO (Welch et al., 1989).

Studies conducted in a number of species suggested that IV administration was effective in reducing intracranial pressure and volume (Camp et al., 1981; Rucker et al., 1981; Tsuruda et al., 1983; Tung et al., 1986; Ikeda & Long, 1990; Kulah et al., 1990). Part of its therapeutic efficacy may be based on its ability to scavenge oxygen-free radicals, which have been implicated in induced tissue damages when given before, during or several hours after tissue insult (Badreldin, 2001). Dimethyl sulfoxide has been administered intravenously in the horse for the treatment of central nervous system diseases and brain edema due to trauma; a FDA non-approved use of DMSO (Beech, 1985; Blythe et al., 1986).

Dimethyl sulfoxide has a strong affinity for water, is a versatile solvent, and many agents not soluble in water are soluble in DMSO. It is considered a membrane penetration enhancer with potential for transporting other drugs into adjacent tissues ([Riviere, 2001](#)).

The specific aim of this study was to determine the pharmacokinetics and plasma concentrations after intravenous, oral and topical administration of DMSO in the horse.

MATERIALS AND METHODS

Experimental animals

The University of Pennsylvania Institutional Animal Care and Use Committee approved the study protocol. Thoroughbred geldings (5) and mares (4), 5 to 11 years old and weighing 504.3 ± 19.0 kg were used for the study. Horses were housed 2 days before the study, remained housed in stalls for the duration of the study and fed grass hay and water *ad libitum*. All horses were weighed prior to each administration and in good health as determined by physical examination, routine hematology, and clinical chemistry.

Drug administration and sample collection

Horses were assigned to a 9-horse crossover design, where each animal received on a random basis an IV, PO, or TOP drug administration. A period of at least two weeks elapsed before the next administration.

Prior to placement of a 14-Gauge catheter (Angiocath, Becton Dickinson, Sandy, UT) into the jugular veins for IV infusion of DMSO and collection of blood samples, both veins were clipped of hair, cleansed with sterile water and surgical soap (Chlorhexidine gluconate, 4%, Purdue Fredrick Co., Stamford, CT), rinsed with a bactericide (Chlorhexidine diacetate, Fort Dodge Health, Overland Park, KA), and 70% isopropyl alcohol. Only one catheter was placed for PO and TOP administrations.

Intravenous administration. Sixty ml of 90% medical grade DMSO solution (107.0 ± 4.0 mg/kg) (Domoso®, Zoetis Inc, Kalamazoo MI, USA) diluted in 1000 ml of normal saline was administered over a 15-min period with a constant infusion pump via the jugular vein. The dose selected was based on a prior administration of 0.1g/kg ([Blythe et al., 1986](#)), which is the approximate amount in the medical grade DMSO solution for administration to a horse. Blood samples were collected before the start of the infusion, at 2, 5, 10, 15 min during the infusion, 2, 5, 15, 30, 45 min, and 1, 2, 4, 6, 8, 10, 12, 16, 20, 24, 48 h post infusion. Blood samples were collected from the contralateral vein.

Oral and topical administrations. Sixty ml of 90% DMSO solution was mixed with molasses and administered orally (PO) in a dose syringe. For topical administration 60 mL of the 90% solution was rubbed on the horse's back and rump and then covered with a plastic sheet and blanket and left on for 24 hours to assure maximum exposure of DMSO over a large surface area. Blood samples were collected before drug administration and 2, 5, 15, 30, 45 min and 1, 2, 4, 6, 8, 10, 12, 16, 20, 24, 48 h post administration.

Blood samples were collected from the jugular vein via the indwelling catheter, placed in tubes with potassium oxalate (20 mg) and sodium fluoride (25 mg) as the anticoagulant (Kendall, Mansfield, MA, USA). Blood samples were stored on ice until the plasma was harvested by centrifugation (776–1318 g) at 4 °C for 15 minutes. Harvesting of plasma took place within 15 to 60 minutes of sample collection and 2 ml aliquots of plasma were immediately frozen and stored at -70°C until analyzed.

Analysis of DMSO in equine plasma

Gas and liquid chromatographic methods have been used for the determination of DMSO in various media (Garretson & Aitchison, 1982; Blythe et al., 1986; Mehta et al., 1986; Simo, 1998; Blomberg et al., 1999)

Chemicals and Reagents. Dimethyl sulfoxide was purchased from Fisher Scientific (Fair Lawn, NJ, USA) and Dimethyl sulfoxide-*d*₆ (DMSO-*d*₆) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile was purchased from Fisher Scientific (Fair Lawn, NJ, USA) and water from Honeywell Burdick & Jackson (Muskegon, MI, USA). Formic acid was obtained from EMD Chemicals Inc. (Gibbstown, NJ, USA). All solvents were high purity LC-MS/MS grade or better.

Sample Preparation. Samples were prepared by a protein precipitation method. Briefly, an aliquot of 50 µl of equine plasma was added to 1.5 ml micro-centrifuge tube (Fisher Scientific, Pittsburg PA, USA) and then 200 µl of acetonitrile (0.1% formic acid with 25 µg/ml DMSO-*d*₆, as the internal standard) was added to all micro-centrifuge tubes. The samples were vortexed on a VWR Mini Vortexer (Henry Troemner LLC, Thorofare, NJ, USA) to optimize protein precipitation. Then all tubes were centrifuged in a micro-centrifuge (Fisher Scientific Micro-centrifuge, Model 235C) for 2 to 5 minutes at 3000 rpm. Next, 25 µl of sample from the micro-centrifuge tubes was transferred to the respectively labelled auto sampler vials and 250 µl

acetonitrile (0.1% formic acid) was added. The sample vials were briefly vortexed to mix content and 10 μ l was injected in to LC-MS/MS for analysis.

Liquid chromatography and mass spectrometry. Sample analysis was performed by an LC-MS/MS system, consisting of a Surveyor MS pump with an on-line degasser, a Surveyor auto-sampler, and a Finnigan Quantum triple stage quadrupole mass spectrometer (TSQ Quantum AM) equipped with an electrospray ionization (ESI) probe (Fisher Scientific, San Jose, CA, USA). Liquid chromatography separation was performed on an Accucore HILIC column, 50 x 2.1 mm, 2.6 μ m particle size (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a guard column, (Accucore HILIC Defender Cartridge 2.6 μ m). Mobile phase A consisted of 0.1% formic acid in water, while mobile phase B comprised 0.1% formic acid in acetonitrile. Mobile phase gradient program was employed as: 0 min, 5/95 (A/B) at 0.02 ml/min; 0.2 min, 5/95 (A/B) at 0.02 mL/min; 0.22 min, 5/95 (A/B) at 0.25 ml/min; 0.5 min, 100/0 (A/B) at 0.25 ml/min; 4 min, 100/0 (A/B) at 0.25 ml/min; 4.02 min, 5/95 (A/B) at 0.45 ml/min; 5 min, 5/95 (A/B) at 0.45 ml/min; 5.02 min, 5/95 (A/B) at 0.02 ml/min; 5.08 min, 5/95 (A/B) at 0.02 ml/min. Total analysis time was about 5 minutes.

The mass spectrometer was operated in positive ion mode and the ESI source spray at 45-degree angle to the ion transfer capillary tube that guides ion beams into the mass spectrometer. The ESI source parameters were tuned by syringe infusion of 100 μ g/ml DMSO to ESI source under LC flow condition. For sample analysis, the mass spectrometer was set to data acquisition mode for selected-reaction monitoring (SRM) mode. The SRM transitions monitored in this method for DMSO were m/z 79 $\rightarrow$ m/z 64, m/z 79 $\rightarrow$ m/z 49 and m/z 79 $\rightarrow$ m/z 47. For DMSO- d_6 , m/z 85 $\rightarrow$ m/z 50 was acquired. Data acquisition and analysis were done with Xcaliber software v.1.3 (ThermoFisher Scientific, San Jose, CA, USA).

Quantification of DMSO. The SRM transitions employed for quantification analysis for DMSO and DMSO- d_6 were m/z 79 $\rightarrow$ m/z 64 and m/z 85 $\rightarrow$ m/z 50, respectively. The calibration curve was generated by plotting the ratio of peak area of DMSO to that of DMSO- d_6 (y-axis) against analyte concentration on the x-axis. The instrument lower limit of detection (LLOD) was less than 2.7 ng based on preparative method dilution factors and injection volumes applied to the 10 μ g/ml calibrator. Linear regression with 1/x weighting factor was used in describing the regression relationship. The limit of quantification (LOQ) was 1.0 μ g/ml, with the linear dynamic range of quantification being 1.0-250 μ g/ml.

Method was validated based on FDA Guidance for Bioanalytical Industry (1). This laboratory meets requirements established by International Organization for Standardization. ISO/IEC 17025:2005: General requirements for the competence of testing and calibration laboratories. <https://www.iso.org/standard/39883.html>. The laboratory is accredited by the American Association for Laboratory Accreditation (www.a2la.org).

Pharmacokinetic analysis

Compartmental analysis (Simulation, Analysis and Modeling Software, WinSaam.com) was used to describe the disposition and elimination of DMSO plasma concentrations following IV, PO, and TOP administrations. Plasma concentration time curves from each horse were analysed using conventional nonlinear least-squares regression analysis (Berman, 1968; Boston et al., 1981; Stefanovski et al., 2003). Two and three-compartment models were fitted to the plasma concentrations of DMSO following IV administrations and one-compartment model to PO and TOP administrations. The number of compartments required to best describe the DMSO plasma concentrations was based on the reduction in the sums of squares, minimization of fractional standard deviation of each compartmental parameter, and the converging of the observed and predicted plasma concentrations curves. A two-compartment model with injection into and elimination (K_{10}) from the central compartment (C_1) with inter-compartmental distribution rate constants to C_2 (k_{12} , k_{21}) was used to describe the IV DMSO administrations (**Figure 1**). A one-compartment absorption and elimination models were used to describe the PO and TOP DMSO administration. Dimethyl sulfoxide was absorbed from the cutaneous and gastro-intestinal compartments (C_0) to the central compartment C_1 (k_{01}) and eliminated (K_{10}) from C_1 (**Figure 1**).

Various schemes were used for weighting the data ($W(K)$) in the fitting process. The fractional standard deviation (FSD) was in the form of $W(K)=1/(C*QO(K)**2)$ where $QO(K)$ was the k th observed datum and C was the FSD. The standard deviation (SD) weighting scheme was of the form $W(K)=1/C**2$. The FSD weighting process favors the terminal lower concentrations of the decay curve, where the SD favors the larger and intermediate data points. The fitting process (iterations) ceased when the improvement in the sums of squares of the last iteration was $< 1\%$. The inter-compartmental micro-rate constants for the IV administration were converted to macro-constants exponents, alpha (α) and beta (β) as described (Wastney et al., 1999). The apparent volume of the central compartment (V_C), was calculated from the

amount of drug in the body at time t , divided by the plasma concentration at time t (Toutain & Bousquet-Melou, 2004).

Calculation of Secondary parameters. The A and B macro-constants coefficients ($\mu\text{g/mL}$) for the IV administration were calculated from the dose, volume of central compartment (V_C), and the relevant compartmental rate constants (Gabrielsson & Weiner, 2006). Half-lives ($t_{1/2}$) were calculated as $\text{Ln}2$ divided by the relevant rate constants. The total area under the plasma concentration time-curve from 0 h to 24 h (AUC_0^{24}) for the 3 administrations was calculated by the linear trapezoid method. The area to infinite (AUC_0^∞) was the AUC_0^{24} plus the end area correction calculated as $C_{p(t^*)}/k_e$, where $C_{p(t^*)}$, was last plasma concentration and K_e the relevant terminal elimination rate constants for the IV, PO, and TOP administrations (Riviere, 1999).

Maximum plasma concentration (C_{max}) and the time to reach the maximum plasma concentration (T_{max}) were derived directly from the plasma concentration time-curves not the fitted curves. The IV estimated volumes of compartment C_2 were calculated by the ratio of the inter-departmental rate constant k_{12}/k_{21} times volume V_C . The volume at steady state (V_{ss}) was calculated as:

$$V_{ss} = V_C \left[1 + \frac{k_{12}}{k_{21}} \right]$$

Compartmental clearance (Cl) was calculated as:

$$\text{Cl} = K_{10} * V_C$$

Bioavailability (F) absorbed from GI tract and skin was calculated as:

$$\frac{\text{AUC}_{\text{PO, TOP}}}{\text{AUC}_{\text{IV}}}$$

Where, PO, TOP, and IV were the extravascular and intravenous administrations. Clearance values for PO and TOP administrations (Cl_F) were normalized base on each horse's F.

Pharmacokinetic parameter estimates of DMSO were expressed as median and range. Plasma concentrations of DMSO were expressed as the mean and standard deviation.

RESULTS

Intravenous administration

Plasma concentration versus time-curves of DMSO following IV administration was best described by a two-compartment model (**Figure 1**). Pharmacokinetic estimates are shown in **Table 1**. Plasma concentrations following the 15-min infusion of DMSO (0.71 ± 0.026 mg/kg/min) were 268.1 ± 48.0 μ g/ml. There was a rapid initial decline in the plasma concentration and within 2 min of completing the infusion, the concentration was 224.6 ± 69.0 μ g/mL (**Figure 2a, b**). Plasma concentrations at 24 and 48 h were 52.7 ± 26.1 and 1.5 ± 1.4 μ g/ml. The $t_{1/2\alpha}$ and $t_{1/2\beta}$ phases were 1.74 min and 14.1 h, respectively. The end-area calculation of the AUC_0^∞ was $26.9 \pm 11.0\%$ of the total area.

Oral administration

Plasma concentration versus time-curves of DMSO following PO administration was best described by a one-compartment absorption and elimination model (**Figure 1**). Pharmacokinetic estimates are shown in **Table 2**. Following PO administration, the absorption was rapid and the elimination slow with a $t_{1/2ab}$ of 0.15 h and a $t_{1/2e}$ of 15.5 h (**Figure 3**). The peak plasma concentrations of DMSO occurred between 15 min to 4 h and were 47.4 to 129.9 μ g/ml. The plasma concentrations at 24 h were quantified in 7 of the 9 horses at 21.2 ± 10.6 μ g/ml and at 48 h in 6 of 9 horses at 0.68 ± 0.53 μ g/ml. The end area calculation of the AUC_0^∞ was $26.4 \pm 10.0\%$ of the total area.

Topical administration

Plasma concentration versus time-curves of DMSO following TOP administration was best described by a one-compartment absorption and elimination model (**Figure 1, 4**). Pharmacokinetic estimates are shown in **Table 2**. Following TOP administration, the $t_{1/2ab}$ was 1.2 h with a $t_{1/2e}$ of 4.5 h. In one of the 9 horses administered TOP DMSO, there was insufficient absorption to model the data. Six of the 9 horses had quantifiable concentrations of 0.36 ± 0.10 μ g/ml and 0.11 ± 0.07 μ g/ml. at 24 and 48 h. The end area calculation of the AUC_0^∞ was $14.3 \pm 10.0\%$ of the total area.

DISCUSSION

There is limited information on the distribution and elimination pharmacokinetics of DMSO in any species. In a previous study following an IV administration to the horse, the changes in the plasma concentration-time curves were also described by a 2-compartment model

with a $t_{1/2\beta}$ of 8.6 and 9.8 for doses of 1g/kg and 0.1g/kg, respectively. In this early study, plasma concentrations were measured out to 12 h, compared to our study with measurements out to 48 hours. (Blythe et al., 1986). Despite the differences in $t_{1/2\beta}$ and measurement-time scale the relationship between the distribution and elimination phases were similar

In our study and the previous horse study the distribution phase was very rapid, suggesting no plasma binding of DMSO. Earlier studies using gel filtration methods, indicated no binding to plasma proteins. This same study using autoradiographic evidence suggested that DMSO was confined to interstitial spaces, with limited penetration into cells (Malinin et al., 1969). This agrees with cryoprotective studies, where DMSO was bound to the surface of cell membranes (Greiff & Seifert, 1968).

Following IV administration to mice a biexponential pattern was observed with a rapid distribution (1.5 min) and slower elimination phase (90 min); the clearance was more rapid compared to the horse at 4.79 ml/min/kg (Kaye et al., 1983). Early studies using radio-labeled DMSO also described a rapid distribution phase which was followed by slow elimination phase (Kolb et al., 1967).

Following PO administration in our study, the absorption was rapid at 0.15 h with an elimination rate of 15.5 h. There was considerable variability in the absorption with a median bioavailability of 47.4%. There were no food restrictions before and following the PO administration and the DMSO was given by a dose syringe not a nasogastric tube, this may account for the considerable variation in the plasma concentration (**Figure 3**). The PO administration to rhesus monkeys showed a similar pattern, DMSO was absorbed rapidly, with a terminal half-life of 16 h (Layman & Jacob, 1985).

Tissue distribution in rabbits and guinea pigs was determined following the oral and topical administration using S^{35} -tagged DMSO. The tissue concentrations following topical administration were comparable to the oral administration after 4 hours and concentrations in all tissues measured were equivalent. They also reported that ~ 6% of the topically administered S^{35} -DMSO was measured in the respired air over a 24 hour period (Hucker et al., 1966). In rats 3.5% of the radioactivity was exhaled within 12 hours (Kolb et al., 1967). Following the detection of a peculiar odor in the cats exhalation, the exhaled component of DMSO was identified as the metabolite dimethyl sulfide (Distefano & Borgstedt, 1964). Metabolism of

DMSO to its metabolite dimethyl sulfide in the horse can be inferred by the odor detected on the breath.

The gel formulation of DMSO applied to elbow of man for 30 minutes, showed a 25% to 40% absorption of the dose. Application to shaved backs of miniature pigs showed a complete absorption of the dose within 4 hours (Wong et al., 1971). Earlier studies in man using radiolabeled DMSO indicated rapid absorption from both dermal and oral administration (Hucker et al., 1967) Rapid absorption was also reported in the rat where 94% of topical DMSO was absorbed within 60 minutes (McDermot et al., 1967). Similar observations were seen in dogs where 80% of the topically applied radio-labeled DMSO was absorbed after 4 hours and 90% after 24 hours (Kolb, et al., 1967).

This is contrary to our current study in horses where the fraction absorbed following topical administration was ~1%. This may have been due to preparation of the dermal surface, the location we selected, or inherit difference in the skin of various species. We did not clip the hair or prepare the skin in any manner. When applied to cutaneous portions of horse for therapeutic purposes, the hair may not be clipped for obvious reasons, winter coat, show horse, racing, etc. Our study horses did not exercise once the DMSO was applied topically and despite the covering for 24 hours, the absorption was minimal.

In a small pilot study in 7 Thoroughbred horses in training, DMSO was applied over the metacarpal area by the trainer, and the area covered with leg wraps. Blood samples were collected within an hour just prior to and ~60 min following the training run and a cool down period. The total amount applied was between 30ml and 90 ml of the 90% DMSO solution. The horses ran ~5/8 of a mile in just over 60 seconds. The mean plasma concentrations prior to exercise was 11.1 (4.0 to 15.0 µg/ml) and following exercise 34.1 (17.5 to 43.5 µg/ml). This was not a completely controlled study, but emphasizes the importance and difficulty in selecting a representative area to study topical absorption of drugs.

In this study, using the horse's back and rump we apparently selected a location of poor absorption. A number of studies have cited the location of the site as one of the criteria in determining the amount of the drug absorbed (Mills & Cross, 2006; Mills & Cross, 2006; Mills & Cross, 2006). In the field study, we studied the absorption in an area more commonly used in the racing equine and an area of apparent higher absorption; this plus exercise may account for the plasma concentration doubling following exercise. .

Dimethyl sulphone as a metabolite of DMSO was reported in 1966 as a metabolite in both man and rat and accounted for ~15% of the dose administered (Hucker et al., 1966). In our study, we did not measure the urine concentration of DMSO. The assumption is that dimethyl sulphone, a metabolite of DMSO is also excreted by the horse. In a prior study in horses ~25% of DMSO was excreted in the urine during a 12 h period (Blythe et al., 1986). Metabolism of DMSO to its highly volatile metabolite dimethyl sulfide can be inferred by the odor detected on the breath of horses.

Dimethyl sulfoxide has been used in veterinary medicine since the 1960 with approval by the FDA for topical administration only. This was the first study in the horse to compare the pharmacokinetics following the IV, TOP, and PO administrations. The pharmacokinetics following IV administration in the horse was like that described in other species, a very rapid distribution and slower elimination phase. Oral absorption was rapid and terminal elimination slow. The absorption and bioavailability following TOP administrations in the horse varies from reports in other species where absorption was more extensive. This may be due to a poor choice of location, the rump and back. In a smaller pilot study, absorption from the metacarpal area was more extensive and enhanced following exercise. This should be a warning to owners and trainers of competition horse using topical DMSO, location and exercise can produce a violation if administered too close to competition. Despite its topical, oral, and intravenous use for its apparent anti-inflammatory effects few peer review studies in the equine have verified its efficacy. This author agrees with the conclusion of others, (Schleining & Reinertson, 2007; Schleining & Reinertson, 2007) that despite the many studies in a number of species, hard evidence from well-designed studies in the equine do not exist. The approval of use of DMSO for topical use in the horse was based on clinical studies with no information of its uptake and distribution in the horse. Likewise, for its FDA non-approved intravenous use in the horse for the treatment of central nervous system diseases and brain edema due to trauma, well-designed studies to determine its efficacy in the equine do not exist.

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‘CONFLICTS OF INTEREST: the authors have no conflict of interest’.

1. FOOTNOTE - U.S. Food and Drug Administration. Guidance for industry Bioanalytical method validation.
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Table 1. Pharmacokinetic parameter estimates following a single IV administration of DMSO (107.0 mg/kg) in 9 horses.

Parameter	Median (Range)
C_p^0 (µg/ml)	811.4 (3.26-870.2)
A (µg/ml)	638.4 (196.7-739.2)
α (/h)	24.2 (7.4-27.1)
$t_{1/2\alpha}$ (h)	0.029 (0.026-0.093)
B (µg/ml)	165.9 (130.0-220.9)
β (/h)	0.049 (0.042-0.10)
$t_{1/2\beta}$ (h)	14.1 (6.6-16.4)
AUC_0^{24} (µg/ml/h)	2267.0 (1405.9-3215.4)
AUC_0^{inf} (µg/ml/h)	3186.1 (1543.8-4844.8)
V_c (ml/kg)	13.3 (11.8-33.1)
V_2 (ml/kg)	48.5 (29.1-64.7)
V_{ss} (ml/kg)	65.0 (47.0-81.4)
Cl (ml/h/kg)	3.2 (2.2-6.7)
k_{10} (/h)	0.19 (0.13-0.55)
k_{12} (/h)	17.9 (4.8-23.0)
k_{21} (/h)	4.9 (2.6-7.4)

C_p^0 = concentration at time 0; A, B = coefficients; α , β = exponents; $t_{1/2\alpha}$, $t_{1/2\beta}$ = elimination half-lives; AUC_0^{24} = area under the concentration time-curve to 24 h; AUC_0^{inf} = area under the concentration time-curve to infinity; V_c = volume of central compartment; V_2 = volume of peripheral compartment; V_{ss} = volume of distribution at steady state; Cl = clearance from the central compartment; k_{10} = elimination rate constant from the central compartment; k_{12} , k_{21} = fractional rate constants peripheral compartments

Table 2. Pharmacokinetic parameter estimates following a single topical and PO administration of DMSO (107.0 mg/kg) in 9 horses.

Parameter	Oral Median (Range)	Topical Median (Range)
K_{ab} (h)	4.6 (0.90-56.2)	0.57 (0.29-1.4)
$t_{\frac{1}{2} ab}$ (h)	0.15 (0.01-0.77)	1.2 (0.49-2.3)
K_e (h)	0.045 (0.027-0.081)	0.17 (0.06-0.33)
$t_{\frac{1}{2} e}$ (h)	15.5 (8.5-25.2)	4.5 (2.1-11.0)
AUC_0^{24} ($\mu\text{g/h/ml}$)	1189.0 (636.9-1893.9)	16.5 (10.4-66.8)
AUC_0^{∞} ($\mu\text{g/h/ml}$)	1558.7 (908.9-3436.7)	23.1 (11.7-68.6)
Cl_F (ml/h/kg)	2.9 (1.1-5.5)	4.5 (0.52-18.2)
T_{max} (h)	0.75 (0.25-4)	3 (2-4)
C_{max} ($\mu\text{g/ml}$)	91.1 (47.4-129.9)	1.8 (1.2-8.2)
F (%)	47.4 (22.7-98.1)	0.48 (0.31-4.4)

k_a , k_e = absorption and elimination rate constants; $t_{\frac{1}{2} ab}$, $t_{\frac{1}{2} e}$ = absorption, and elimination half-life; AUC_0^{24} = area under the plasma concentration-time curve; AUC_0^{∞} = area under the concentration time-curve to infinity; Cl_F (ml/h/kg) = corrected clearance; T_{max} = time to maximum concentration; C_{max} = maximum concentration; F = fractional absorption during a 24 h period.

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