Evaluation of Dimethyl Sulfoxide Effects on Endotoxin Induced Delayed Gastric Emptying in Horses

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ABSTRACT

Endotoxaemia is one of the most severe and ubiquitous disease processes in horses; and delayed gastric emptying is one of the most clinically significant manifestations of endotoxaemia. Current information suggests that oxidative damage plays a key role in the systemic effects induced by endotoxin and this raises the possibility that antioxidants, such as dimethyl sulfoxide (DMSO), might have a protective effect. No study has yet evaluated the efficacy of DMSO in ameliorating delayed gastric emptying caused by endotoxaemia in horses. We hypothesized that DMSO would ameliorate delayed gastric emptying caused by intravenous lipopolysaccharide (LPS) administration. Horses were randomly assigned to one of 4 groups; of 6 horses each: Normosol (Abbott Laboratories, Abbott Park, Illinois, USA) + LPS (0.2μg/kg bwt, i.v.); DMSO (1g/kg bwt, i.v.) + Saline; High-dose DMSO (1g/kg bwt, i.v.) + LPS; Low-dose DMSO (20 mg/kg bwt, i.v.) + LPS. All horses in all groups received acetaminophen (20 mg/kg reconstituted in one liter of warm tap water) by nasogastric intubation. Data for acetaminophen concentrations in the blood as a measure of gastric emptying were examined for the effect of treatment using a repeated-measures mixed-model ANOVA. A value of $P < 0.05$ was considered significant. Delayed gastric emptying occurred in all horses receiving LPS, as indicated by the lower concentrations of acetaminophen in these groups along the study period. DMSO did not ameliorate the effect of LPS on gastric emptying. Low-dose DMSO actually exacerbated the LPS-induced delay in gastric emptying. In this study, DMSO did not have any protective effect against LPS-induced delayed gastric emptying in horses.

Key words: Horse; Gastric Emptying; DMSO; Endotoxaemia; Lipopolysaccharides.

INTRODUCTION

Endotoxaemia is a common occurrence in the horse following conditions such as intestinal strangulation, colitis, metritis and pleuritis, and is the leading cause of morbidity, mortality and economic loss to the equine industry (1). Lipopolysaccharide (LPS), a component of the outer cell membrane of Gram-negative bacteria, is responsible for the adverse effects which includes, cardiovascular depression, pulmonary hypertension, arterial hypoxemia, and small intestinal ileus (2). Lipopolysaccharide promotes inflammation

by stimulating the release of prostaglandins, histamine, serotonin, kinins, platelet-activating factors and other mediators (2). These changes, in turn, cause decreased tissue perfusion and oxygenation and eventually lead to organ failure and death (1). Because current information suggests that oxidative damage plays a key role in the systemic effects induced by LPS (Fort Dodge Animal Health, Fort Dodge, Iowa, USA), this raises the possibility that antioxidants, such as dimethyl sulfoxide (DMSO), can protect against LPS deleterious effects, including delay in gastric emptying.

Dimethyl sulfoxide, an oxygen free radical scavenger and anti-inflammatory agent, is used clinically for the treatment of endotoxaemia in horses (4), however, its efficacy remains unproven (5). In human blood 1% DMSO inhibits IL-8 production (6) and in rats DMSO at 140 mM concentration; prevents adhesion of neutrophils to endothelium (7). At 0.5-3% DMSO effectively reduces platelet aggregation by scavaging radical oxygen species, in human's *in vitro* platelet concentrates (8) and the same effect was demonstrated in a study in mice using intra-peritoneal DMSO at 0.5 g/kg (9). In mice intra-peritoneal DMSO at 7 g/kg inhibits LPS-induced TNF-alpha production and intercellular adhesion molecule (ICAM-1) formation, reduces activation of the nuclear transcription factor kappa B (NF-kappa B) and markedly reduces LPS-induced liver damage (10). In a study using rats, intra-peritoneal DMSO (6.5 g/kg) proved highly effective in preventing gastrointestinal lesions caused by induced endotoxaemia (11). The dose range for intravenous DMSO use in horses is wide, we elected to use 20 mg/kg since this dose was found to have positive effects in small intestinal ischemia models both in adults (12) and in foals (13) and we elected the 1 g/kg dose since it is a safe; and is a commonly used regimen by equine clinicians (14, 15).

Gastric emptying can be assessed in several ways, one of the most simple and reliable methods; is the acetaminophen absorption test (16). Acetaminophen passes unchanged in the stomach and gets absorbed mainly in the small intestine. Measuring acetaminophen in the blood indicates the rate of absorption; and from that, the rate of gastric emptying can be extrapolated, with fast absorption indicating faster gastric emptying. Acetaminophen absorption test has proved to have comparable results to the gold standard of scintigraphy assessed liquid gastric emptying (17).

The present study was designed to evaluate the protective effect of two doses of DMSO on the delayed gastric emptying induced by LPS in horses. Acetaminophen absorption rate was used in order to assess gastric emptying. The hypothesis for the study was that DMSO would ameliorate the delay in gastric emptying; caused by intravenously administered LPS in horses.

MATERIAL AND METHODS

The study was approved by the University of Tennessee Institutional Animal Care and Use Committee.

Horses

Eighteen healthy, adult horses (15 American Quarter Horses and 3 Thoroughbreds) from the University teaching herd participated in the study. Horses ranged in age from 3 to 14 years old (median 7 years) and weighed 440 to 590 kg (mean 468 kg). Three were geldings and 15 were mares. Horses were withheld food for 24 hours; and water for 2 hours, prior to participation in the study.

Horses were randomly assigned to three groups (n=6 horses/group). Horses participating in the DMSO-Saline group were later randomly assigned to one of the LPS groups after a two-week washout period. Each horse received LPS treatment on one occasion only, so that tolerance to LPS was avoided. Each experiment was initiated between 08:00 and 08:30 hours; to avoid the effects of diurnal variation on measured parameters. Treatment groups were as follows:

Normosol-LPS: Normosol-R (balanced electrolyte solution) pre-treatment + LPS (Sigma, St. Louis, Missouri, USA) + (*E. coli* 055:B5, 0.2μg/kg bwt, i.v.)

High-DMSO-LPS: DMSO (1g/kg bwt, i.v.) pre-treatment + LPS (0.2μg/kg bwt, i.v.)

Low-DMSO-LPS: DMSO (20 mg/kg bwt, i.v.) pre-treatment + LPS (0.2μg/kg bwt, i.v.)

DMSO-Saline: DMSO (DOMOSO (Fort Dodge Animal Health, Fort Dodge, Iowa, USA) 1g/kg bwt, i.v.) pre-treatment + Saline.

EXPERIMENTAL DESIGN

Treatments

For each horse, a 14G, 12 cm catheter, was introduced into the jugular vein and maintained only for the delivery of the intravenous treatment.

Horses were pre-treated with either 5L Normosol, Low-dose DMSO (20 mg/kg bwt) or High-dose DMSO (1 g/kg

Table 1: Pharmacokinetic variables of acentaminophen, as measured in the blood of horses after oral administration; in order to examine the effect of LPS and DMSO on gastric emptying.

	Cmax (µg/mL)	Tmax (min)	AUC_{0-120} (µg·h/mL)	AUC_{0-240} (µg·h/mL)	$T_{1/2}$ (min)
LPS	2.81*	77.5	202.4	357.65	111.17
DMSO	4.87*	53.33*	354*	579.66*	154.72
LPS + High DMSO	3.76	75.83	192.4	374.66	109.06
LPS + Low DMSO	2.25*	157.5*	105.81	274.53	90.75

Cmax= Maximal concentration of acetaminophen measured in the blood

Tmax= Time it took acetaminophen to reach its maximal concentration in the blood

AUC0-120= Area Under the Curve of acetaminophen concentration over time (from time zero to 120 minutes)

AUC0-240= Area Under the Curve of acetaminophen concentration over time (from time zero to 240 minutes)

T1/2 = Half life of acetaminophen concentration in the blood

LPS= Lipopolysaccharide (endotoxin)

DMSO= Dimethyl sulfoxide

* Significant difference

bwt) made up to 5 L in Normosol. Pre-treatments were administered over 1 hour.

Endotoxin administration: Immediately after completion of the pre-treatment, horses were given an infusion of either LPS (0.2µg/kg bwt diluted in 1L 0.9% NaCl) or 1L 0.9% NaCl administered over 30 minutes.

Acetaminophen administration: Immediately after completion of the LPS or Saline infusion, horses were given acetaminophen (20 mg/kg bwt dissolved in 1L of warm tap water) by nasogastric intubation.

Blood samples were collected from the jugular vein using a vacutainer with a 20 gauge; 2.5 cm long needle, at 90 minutes prior to and at 0, 5, 10, 15, 20, 25, 30 ,35, 40, 45, 60, 75, 90, 120, 150, 180, 210, 240, 300 and 360 minutes after completion of the LPS or saline infusion. Blood (2 ml) were placed in a serum tube (2 ml) for acetaminophen analysis. Pharmacokinetic values obtained and compared between the groups included: T_{max} (Time taken for acetaminophen to reach its maximal concentration in the blood), C_{max} (Maximal concentration of acetaminophen measured in the blood), AUC_{60} (Area Under the Curve of acetaminophen concentration over time {from time zero to 60 minutes}), AUC_{120} (Area Under the Curve of acetaminophen concentration over time {from time zero to 120 minutes}), AUC_{240} (Area Under the Curve of acetaminophen concentration over time {from time zero to 240 minutes}), and $T_{1/2}$ (Half life of acetaminophen concentration in the blood).

Serum acetaminophen concentrations were determined by a quantitative colorimetric method (18). The lower sensitivity level of the assay was 0.25 mg/dl. Three ml of 3% trichloroacetic acid were added to 0.3 ml serum and centrifuged at 1000 G for 10 minutes until a clear supernatant was obtained. Two ml of this supernatant were mixed with 0.5 of sodium nitrite (0.07 mol/l) solution and incubated at 37°C for 10 minutes. Sodium hydroxide (0.1 ml, 8 mol/l) was added as a coloring agent. The absorbance of the test solution was read at 430 nm, using water as the blank medium. The concentration of acetaminophen in each of the serum samples was calculated as the mean of 3 replicates. Standard curves were developed from known amount of acetaminophen in pooled horse sera. The maximal inter- and intra-assay coefficient of variation for all acetaminophen concentration tested were 5 and 6.5 %, respectively. The pharmacokinetic analysis of the obtained concentration–time data was performed using a standard, non-compartmental analysis approach with the software package WinNonlin 6.2 (Pharsight Corp., St Louis, MO, USA).

Statistical analysis

Absorption data were analyzed with mixed model analysis of variance using a completely randomized design with repeated measures over time (SAS 9.1, Cary, NC). DMSO treatment was the between horse factor, with horse as the experimental unit. Least squares means were calculated, and compared with Fisher's protected LSD at the 5% significance level. In addition, a linear polynomial contrast was used to test

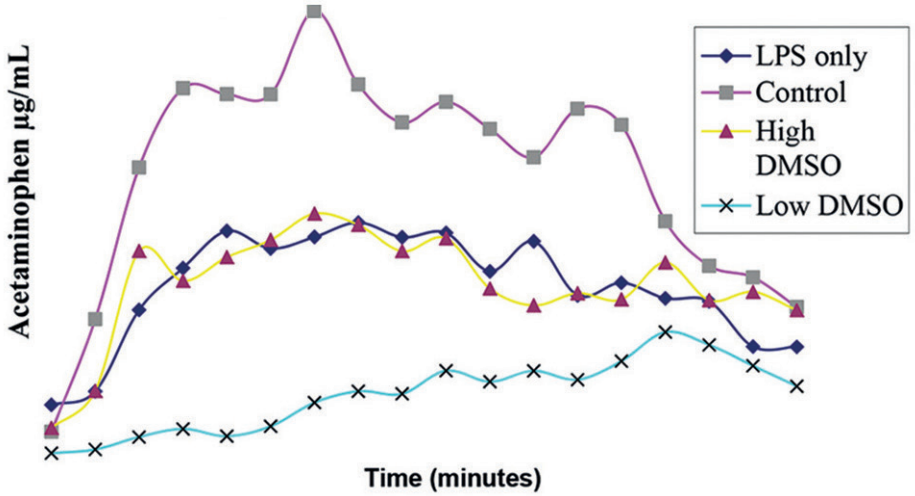

Figure 1: Average concentration of acetaminophen over time, as measured in the blood of horses in all four groups of treatments.

for response trends across DMSO concentrations. Kinetic variables, with one measure per horse, was analyzed similarly, but without the repeated measures component.

A value of $P<0.05$ was considered significant.

RESULTS

The half-life of acetaminophen was not significantly different between the groups (Table 1). For horses in the DMSO-Saline group, AUC_{120} and AUC_{240} were significantly greater ($P<0.0001$), when compared to all LPS-treated groups (Figure 1). The maximal concentration of acetaminophen in the blood (C_{max}) was higher in the DMSO-Saline group compared to the LPS and the LPS-Low DMSO group ($P=0.05$). The time it took to acetaminophen to reach C_{max} in the blood (T_{max}), was shorter in the DMSO group than in the LPS-Low DMSO group ($P=0.04$).

DISCUSSION

Delayed gastric emptying occurred in all horses receiving LPS, as indicated by decreased acetaminophen absorption compared to horses receiving no LPS. None of the horses in the DMSO-Saline treatment group exhibited any adverse effects. Overall, pre-treatment with high-dose DMSO, did not change the negative effects of LPS on acetaminophen absorption and thus did not have any protective effect on LPS-induced delayed gastric emptying.

We expected a more positive effect of DMSO against LPS-induced delay in gastric emptying based on its reported protective outcome in endotoxemic rats and its widespread use and anecdotal reports of efficacy in endotoxaemic horses. Several studies support a dramatically effective role for DMSO in endotoxaemic rats. In a lethal model of endotoxaemia, 100% of rats receiving DMSO at 4.2 g/kg intra-peritoneal survived as opposed to fewer than 60% of control rats (19). In another study, 6.5 g/kg intra-peritoneal DMSO completely prevented LPS-induced hemorrhagic enteritis in rats and attenuated LPS-induced blood lactate increases (11). One possible explanation for the lack of efficacy of DMSO in the present study is the species-specific sensitivity to LPS as it is well accepted that horses are among the species most sensitive to LPS (20). In a previous study, we found very limited effect of DMSO on the clinical parameters that were altered by induced endotoxaemia in horses (21). However, small intestinal ileus is a major complication after colic surgery, and endotoxaemia is considered to play an important role in this complication. Thus, investigating ways to ameliorate this devastating complication, is a worthy study goal.

According to some of our pharmacokinetic data, such as the C_{max} and the T_{max}, administration of low-dose DMSO not only did it not have a protective effect against LPS, but actually, resulted in even a further delay in gastric emptying. This can also be noticed in Figure 1, in which the LPS+ Low-dose DMSO group, is represented by the graph, showing the least acetaminophen absorption over time. This is obviously,

contradictory to our expectation that DMSO will result in increased gastric emptying by ameliorating the deleterious effects of LPS on gastric motility. High-dose DMSO did not significantly prevent the delay in gastric emptying induced by the LPS, however, as opposed to the low-dose DMSO, it did not cause any further delay in gastric emptying. Thus, the effect of DMSO on LPS induced delay in gastric emptying, is not a simple dose dependent relationship. It is difficult to rationalize the fact that the low-dose DMSO exacerbated the effect of LPS on gastric motility. Further studies, using intermediate doses of DMSO; and using alternative methods to evaluate gastric emptying; may help elucidate this unexpected result.

One limitation of the present study is the small number of horses, which when combined with the high variability in response to LPS, reduced the power of the study to detect significant difference among treatments.

Another potential limitation of the current study is the use of an anti-inflammatory drug (Acetaminophen) as a marker of gastric emptying. A study in mice found that acetaminophen can abolish the effect of LPS on gastric emptying (22) and another study showed that acetaminophen can prevent NO (Nitric Oxide) production (23). However, several studies have already established acetaminophen as an efficient and accurate marker of gastric emptying, when endotoxin was used as a model for delayed gastric emptying in horses (24-26).

Acetaminophen undergoes enzymatic metabolism, and some of its metabolites, such as *N*-Acetyl-p-benzoquinonimine (NAPQI) can be hepatotoxic. DMSO can decrease this acetaminophen hepatotoxicity by inhibiting the enzymes that convert acetaminophen into its toxic metabolites (27). On the other hand, multiple studies in human and horses used acetaminophen absorption test successfully to assess liquid phase gastric emptying; with no noticeable detrimental effect to the participants (17, 26, 28, 29).

In addition, a control group receiving only isotonic fluids would be ideal in order to isolate the effect of DMSO on gastric emptying. However, DMSO is commonly used as a carrier in evaluating gastric emptying, and nonetheless according to some data, at the concentration used in the current study, does not affect gastric emptying (30).

There was no apparent protective effect of DMSO in this study and therefore there is no support for its use as a treatment for endotoxaemia. Support for the clinical use of DMSO comes from its intestinal anti-ischemic and anti-adhesive effects (12, 13). However, based on the finding in this study, DMSO does not appear to have a role in decreasing endotoxaemia induced delay in gastric emptying in horses.

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REFERENCES

1. Sykes, B.W. and Furr, M.O.: Equine endotoxaemia--a state-of-the-art review of therapy. Aust.Vet. J. 83:45-50, 2005.
2. Werners, A.H., Bull, S. and Fink-Gremmels, J.: Endotoxaemia: a review with implications for the horse. Equine Vet. J. 37:371-383, 2005.
3. Hammond, R.A., Hannon, R., Frean, S.P., Armstrong, S.J., Flower, R.J. and Bryant, C.E.: Endotoxin induction of nitric oxide synthase and cyclooxygenase-2 in equine alveolar macrophages. Am. J. Vet. Res. 60:426-431, 1999.
4. Shuster, R., Traub-Dargatz, J. and Baxter, G.: Survey of diplomates of the American College of Veterinary Internal Medicine and the American College of Veterinary Surgeons regarding clinical aspects and treatment of endotoxemia in horses. J. Am. Vet. Med. Assoc. 210:87-92, 1997.
5. Schleining, J.A. and Reinertson, E.L.: Evidence for dimethyl sulphoxide (DMSO) use in horses. Part 2: DMSO as a parenteral anti-inflammatory agent and as a pharmacological carrier. Equine Vet. Educ.19:598-599, 2007.
6. DeForge, L.E., Fantone, J.C., Kenney, J.S. and Remick, D.G.: Oxygen radical scavengers selectively inhibit interleukin 8 production in human whole blood. J. Clin. Invest. 90:2123-2129, 1992.
7. Sekizuka, E., Benoit, J.N., Grisham, M.B. and Granger DN.: Dimethylsulfoxide prevents chemoattractant-induced leukocyte adherence. Am. J. Physiol. 256:H594-597, 1989.
8. Cetin, M., Eser, B., Er, O., Unal, A., Kilic, E., Patiroglu, T., Coskun, H.S., Altinbas, M., Arslan, D. and Ilhan, O.: Effects of DMSO on platelet functions and P-selectin expression during storage. Transfus Apher. Sci. 24:261-267, 2001.
9. Rosenblum, W.: Dimethyl sulfoxide effects on platelet aggregation and vascular reactivity in pial microcirculation. Ann. N. Y. Acad. Sci. 411:110-119, 1983.
10. Essani, N.A., Fisher, M.A. and Jaeschke, H.: Inhibition of NF-kappa B activation by dimethyl sulfoxide correlates with suppression of TNF-alpha formation, reduced ICAM-1 gene transcription, and protection against endotoxin-induced liver injury. Shock. 7:90-96, 1997.
11. Brackett, D.J., Lerner, M.R. and Wilson, M.F.: Dimethyl sulfoxide antagonizes hypotensive, metabolic, and pathologic responses induced by endotoxin. Circ. Shock 33:156-163, 1991.
12. Dabareiner, R.M.,White, N.A., Snyder, J.R., Feldman, B.F. and-Donaldson, L.L.: Effects of Carolina rinse solution, dimethyl

sulfoxide, and the 21-aminosteroid, U-74389G, on microvascular permeability and morphology of the equine jejunum after low-flow ischemia and reperfusion. Am. J. Vet. Res. 66:525-536, 2005.
13. Sullins, K.E., White, N.A., Lundin, C.S., Dabareiner, R. and Gaulin, G.: Prevention of ischaemia-induced small intestinal adhesions in foals. Equine Vet. J. 36:370-375, 2004.
14. Ainsworth, D.: Treatment of endotoxemia In: Mair, T., Divers, T. and Ducharme, N., eds.: Equine gastroenterology. London: W.B. Saunders, 191-196, 2002.
15. Blythe, L.L., Craig, A.M., Christensen, J.M., Appell, L.H. and Slizeski, M.L.: Pharmacokinetic disposition of dimethyl sulfoxide administered intravenously to horses. Am. J. Vet. Res. 47:1739-1743, 1986.
16. Sanaka, M., Kuyama, Y. and Yamanaka, M.: Guide for judicious use of the paracetamol absorption technique in a study of gastric emptying rate of liquids. J. Gastroenterol. 33:785-791, 1998.
17. Glerup, H., Bluhme, H., Villadsen, G.E., Rasmussen, K., Ejskjaer, N. and Dahlerup, J.F.: Gastric emptying: a comparison of three methods. Scand. J. Gastroenterol. 42:1182-1186, 2007.
18. Hale, P.W. Jr. and Poklis, A.: Evaluation of a modified colorimetric assay for the determination of acetaminophen in serum. J. Anal. Toxicol. 7:249-251, 1983.
19. Breen, R., Connell, R. and Harrison, M.: The effects of dimethyl sulfoxide on endotoxin-induced pulonary disfunction: A biochemical and electron microscope study. Ann. N.Y. Acad, Sci. 411:324-327, 1983.
20. Opal, S.M. and Yu, R.L. Jr.: Antiendotoxin strategies for the prevention and treatment of septic shock. New approaches and future directions. Drugs. 55:497-508, 1998.
21. Kelmer, G., Doherty, T.J., Elliott, S., Saxton, A., Fry, M.M. and Andrews, F.M.: Evaluation of dimethyl sulphoxide effects on initial response to endotoxin in the horse. Equine Vet. J. 40:358-363, 2008.
22. De Winter, B.Y., Bredenoord, A.J., De Man, J.G., Moreels, T.G., Herman, A.G. and Pelckmans, P.A.: Effect of inhibition of inducible nitric oxide synthase and guanylyl cyclase on endotoxin-induced delay in gastric emptying and intestinal transit in mice. Shock. 18:125-131, 2002.
23. Inaba, H., Yoshigai, E., Okuyama, T., Murakoshi, M., Sugiyama, K., Nishino, H. and Nishizawa, M.: Antipyretic analgesic drugs have different mechanisms for regulation of the expression of inducible nitric oxide synthase in hepatocytes and macrophages. Nitric Oxide. 44:61-70, 2015.
24. Doherty, T.J., Andrews, F.M., Abraha, T.W., Osborne, D. and Frazier, D.L.: Metoclopramide ameliorates the effects of endotoxin on gastric emptying of acetaminophen in horses. Can. J. Vet. Res. 63:37-40, 1999.
25. Meisler, S.D., Doherty, T.J., Andrews, F.M., Osborne, D., Frazier, D.L.: Yohimbine ameliorates the effects of endotoxin on gastric emptying of the liquid marker acetaminophen in horses. Can. J. Vet. Res. 64:208-211, 2000.
26. Valk, N., Doherty, T.J., Blackford, J.T., Abraha, T.W. and Frazier, D.L.: Phenylbutazone prevents the endotoxin-induced delay in gastric emptying in horses. Can. J. Vet. Res. 62:214-217, 1998.
27. Yoon, M.Y., Kim, S.J., Lee, B.H., Chung, J.H. and Kim, Y.C.: Effects of dimethylsulfoxide on metabolism and toxicity of acetaminophen in mice. Biol. Pharm. Bull. 29:1618-1624, 2006.
28. Doherty, T.J., Andrews, F.M., Blackford, J.T., Rohrbach, B.W., Sandin, A. and Saxton, A.M.: Effects of lipopolysaccharide and phenylbutazone on gastric contents in the horse. Equine Vet. J. 35:472-475, 2003.
29. Doherty, T.J., Andrews, F.M., Provenza, M.K. and Frazier, D.L.: Acetaminophen as a marker of gastric emptying in ponies. Equine Vet. J. 30:349-351, 1998.
30. Pestel, S., Martin, H.J., Maier, G.M. and Guth, B.: Effect of commonly used vehicles on gastrointestinal, renal, and liver function in rats. J. Pharmacol. Toxicol. Methods. 54:200-214, 2006.