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Kinetics and Tolerance of Salinomycin in Camels

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Abstract: The pharmacokinetics of salinomycin following oral administration of 0.1 μg kg^{-1} body weight was studied in camels. Peak plasma concentration occurred at 40 min and decline after 2 h to fall below the limit of assay detection at 16 h post-dosing, with terminal half-life of 150 min. Animals given salinomycin at doses of 0.4-0.8 μg kg^{-1} orally developed neurological signs with some serobiochemical alterations. It is concluded that camels may be one of the most sensitive species to salinomycin toxicity.

Key words: Camel, pharmacokinetics, tolerance, salinomycin, serobiochemical, peak plasma

INTRODUCTION

A large scale epidemic of neuromuscular disease involved camels occurred last year in Saudi Arabia. One of the possible suspected agents that was identified by ministry of agriculture to cause these deaths was salinomycin. The others were aflatoxin and alamenium, contaminating camel feed.

Salinomycin is relatively new polyether antibiotic produced by *Streptomyces albus* (Kinashi *et al*., 1973). It is monovalent carboxylic ionophore that forms lipid soluble complexes primarily with potassium ions (Novilla, 1992). It has a cationic selectivity in order of $K^+ > Na^+ > Cs^+ >> Ca^+$ (Westley, 1982). Like other ionophores salinomycin is highly effective against gram-positive bacteria, including *Mycobacterium* sp., but it is ineffective against gram-negative bacteria and fungi (Westley, 1982). Although, not officially approved as a feed additive for cattle, salinomycin has been shown to enhance feed efficiency and daily weight gains in cattle and sheep Its potency is about 3 times higher than that of lasalocid or monensin (Merchen and Berger, 1985). Other effects of salinomycin include growth promotion in weaning and finishing pigs (Leeson *et al*., 1981), control of coccidiosis in poultry and steers (Merchen and Berger, 1985) and improved performance of broilers (Merchen and Berger, 1985).

Much information has accumulated on the effects of lasalocid and monensin on cattle performance (Schelling, 1984) and alteration in ruminal fermentation patterns (Stuart, 1982; Schelling, 1984). Salinomycin was approved as a coccidiostat for chicken by the federal drug administration in 1983. Over-dosage or use in non target animal species can result I n toxicosis. Therefore, toxicity has been reported since then in Turkeys (Yong, 1990), pigs (Konstanz *et al*., 19 95) and cats (Van der Lide-Sipman *et al*., 1999). This study was conducted to investigate the pharmacokinetics and tolerance of salinomycin in camels.

MATERIALS AND METHODS

Pharmacokinetics studies

Animals and preparations: Eight camels (*Camelus dromedarius*) aged 3-4 years and weighing 200-300 kg body weight were used for pharmacokinetics studies of salinomycin. The animals had free access to food and drinking water. Each animal was weighed before the start of each experiment. Animals were cannulated under strict aseptic conditions with plastic canula No. 90 (Portex Ltd., England) for administration of the drug and collection of blood samples.

Drug administration: A single dose 0.1 μg kg^{-1} body weight of salinomycin (Hoffman, La Roche, Nutley, NJ) was mixed with wheat bran syrup placed in a 60-cc plastic syringe without needle hub and given orally to camels.

Collection of blood samples: Blood samples (5 mL) were collected in heparinized tubes at 0, 5, 2, 4, 6, 9, 12 and 16 h post-treatment. Blood samples were centrifuged at 2000 g for 10 min and plasma separated and stored at -20°C until analysis.

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Assay of salinomycin: The salinomycin in plasma samples was estimated by Thin Layer Chromatographic (TLC) assay (Martinez and Shimoda, 1983; Heil *et al*., 1984). A solvent system described by Dimenna *et al*. (1986) was used to develop salinomycin spots on TLC plates. Salinomycin spots on the silica gel plates were scraped into centrifuge to precipitate the silica gel. Samples were taken from the supernatant and measured calorimetrically at 518 nm in a spectrophotometer (Spectronic 70, Bausch and Lomb, USA). Salinomycin concentrations were obtained by extrapolation from a standard curve of the drug. The present study revealed that the lower sensitivity limit and Rf-value of salinomycin were 0.1 and 0.7 μg mL^{-1}, respectively.

Pharmacokinetics analysis: Determination of the appropriate pharmacokinetics model was based on examination of individual concentration-time curves and on estimation of goodness of fit parameters. Areas Under the Curves (AUC) were determined by trapezoidal approximations. Time taken to achieve maximal plasma concentration following administration (t_{max}) was calculated using differential calculus (George and Ross, 1995). Data were further analyzed using non-compartmental methods (Gibaldi and Perrier, 1982). Mean oral Transit Time (MTT_{oral}) was calculated as the ratio of the Areas Under the first Moment Curve (AUMC) and concentration-time curves (AUC).

Tolerance studies: Sixteen camels aged 3-4 years and weighing 200-300 kg body weight were used. They were fed wheat bran with hay and water. Animals were divided into 4 groups of 4 animals each. Animals in group 1 were kept as untreated controls. Animals in groups 2-4 were given salinomycin mixed in wheat bran at a concentration of 0.2, 0.4 and 0.8 μg kg^{-1} body weight, respectively for 2 days. All animals were observed for clinical signs. Blood samples were collected on 2nd days post dosing into plain tubes to obtain serum for biochemical measurements.

Analytical methods: Serum proteins, glucose, aspartate aminotransferase, lactic dehydrogenase and creatine kinase were determined by clinical chemistry analyzer (Roche products, Herts, UK) using specific kits. The heparinized samples were analyzed for hematology variables including total leukocyte, RBC, Hb and PCV using veterinary automated hematology (Roche products, Herts, UK).

Statistical calculations: The pharmacokinetics data were calculated according to the method of Baggot (1978). Student t-test was used to compare the difference between the mean values.

RESULTS

Following oral administration of salinomycin to camels rapid absorption was observed resulting in peak plasma concentrations in the time interval from 0.5-2 h (Fig. 1). Mean C_{max} was 2.28 μg mL^{-1} at 40 min. Plasma concentration declined after 2 h post-dosing to fall below the limit of quantification at 16 h post administration. The pharmacokinetics values of salinomycin calculated by non-compartmental methods are shown in Table 1. The terminal half-life and MTT were 150.3 and 220 min, respectively.

Results of tolerance studies are shown in Table 2. Within 24 h, the camels in group 3 (receiving 0.4 μg kg^{-1}) and group 4 (receiving 0.8 μg kg^{-1}) became dull and developed generalized weakness. Some camels have abnormal gait with still neck and limbs and developed

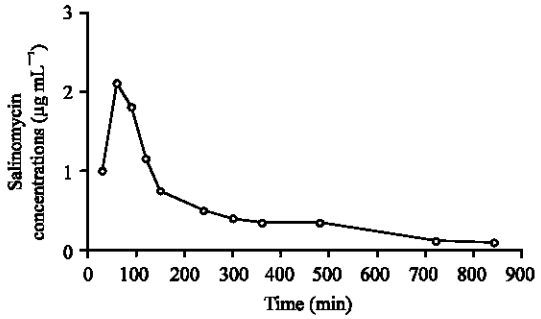

Fig. 1: Salinomycin concentration in serum after a single oral administration of 0.1 μg kg^{-1} body weight (mean±SEM of 6 camels)

Table 1: Pharmacokinetics parameters (mean±SD) of salinomycin preparation after oral administration of single dose 0.1 μg kg^{-1} body weight (n = 8)

Kinetic parameters	Values
AUC (μg/mL/min)	243.00±81.0
C_{max} (μg mL^{-1})	2.28±0.31
T_{max} (min)	40.00±8.10
$t_{1/2}$ (β) (min)	150.30±21.0
MTT_{oral} (min)	220.00±32.0

AUC: Area Under the Curve; C_{max}: Maximal plasma concentration; T_{max}: Time to achieve maximal plasma concentration; $t_{1/2}$ (β): Elimination half-life and MTT_{oral}: Mean Transit Time

Table 2: Serum biochemical values of camels treated with salinomycin on second day post-dosing

	Camels			
Parameters	Group 1	Group 2	Group 3	Group 4
TP (mg mL^{-1})	5.3±0.2	5.4±0.2	4.8±0.2	4.3±0.2
Glu (mg mL^{-1})	110.0±120	112.0±10	88.0±9.0*	84.0±8.0*
AST (IU L^{-1})	6.3±0.3	6.4±0.3	130.0±15*	190.0±18*
LDH (IU L^{-1})	130.0±150	133.0±14	460.0±40*	620.0±55*
CK (IU L^{-1})	63.0±500	62.0±4	320.0±22*	460.0±28*

TP: Total Protein; Glu: Glucose; AST: Asparlate Aminotranseferase; LDH: Lactic Dehydrogenase; CK: Creatine Kinase; *Significant difference ($p<0.05$)

ataxia and some were recumbened. Three camels in group 4 died on 4th day post dosing. Serum biochemical values on the 2nd day of post dosing showed decreased ($p<0.05$) glucose, protein, Aspartate Aminotransferase (AST), Lactic Dehydrogenase (LDH) and Creatine Kinase (CK) compared to controls (Table 2).

DISCUSSION

The early occurrence of salinomycin after 40 min of peak concentrations reflects rapid absorption may be in the upper parts of the gastro-intestinal tract. The terminal half-life of 2.5 h and mean transit time of 3.4 h indicate a moderately fast elimination (Lindsay and Blagburn, 2001). Ionophores are rapidly metabolized by the liver (Aleman *et al*., 2007) and may be the liver is the primary site of ionophore storage, but the compound may also be present in other tissues such as the heart, skeletal muscle and stomach.

Salinomycin given at doses of 0.4-0.8 μg kg^{-1} body weight produced toxic neurological effects such as weakness, abnormal gait, ataxia, stiffness and recumbence. Toxicosis of ionophores such as salinomycin was reported in horses (Van Amstel and Guthrie, 1985; Aleman *et al*., 2007), cattle (Novilla and Folkerts, 1992), sheep (Confer *et al*., 1983), camels (Wernery *et al*., 1998) and humans (Story and Doube, 2004). Ionophores, when bound with appropriate cations, disrupt trans membrane on-gradients and electrical potentials that are required for normal cell function. Such as for excitable nervous tissue (Doebler, 2000). Ionophores like salinomycin that binds K^+ can result in inhibition of ATP hydrolysis in the mitochondria with subsequent decreased cell energy and death (Amend *et al*., 1980; Novilla, 1992). The drug produced in camels decreased glucose and protein and increased aspartate Aminotransferase (AST), Lactic Dehydrogenase (LDH) and Creatine Kinase (CK), which could be a consequence of cell structural damage of many organs in the body (Sako *et al*., 2007). High CK and other enzymes were detected in human after a single exposure of inhaled salinomycin (Story and Doube, 2004) and in camels and horses given feed contained salinomycin (Wernery *et al*., 1998; Aleman *et al*., 2007). The reported LD_{50} of salinomycin in horses is 0.6 μg kg^{-1} (Hansen *et al*., 1981; Kronfeld, 2002). In camels, pelleted feed contained 0.7-1.4 μg kg^{-1} salinomycin have produced toxicity.

CONCLUSION

In this study, only 0.4 μg kg^{-1} was proved toxic to camels, suggesting that camels may be one of the most sensitive species to salinomycin.

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