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Brief communication

Pharmacokinetics of melamine in pigs following intravenous administration

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Abstract

Melamine-contaminated pet food was recently added as a supplement to livestock feed. There is little or no information concerning the pharmacokinetics of melamine in livestock, and the aim of this study was to obtain pharmacokinetic parameters for this contaminant in pigs. Melamine was administered intravenously to five weanling pigs at a dose of 6.13 mg/kg and plasma samples were collected over 24 h, extracted for melamine, and then analyzed by HPLC–UV. The data was shown to best fit a one-compartment model with melamine's half-life of 4.04 (±0.37) h, clearance of 0.11 (±0.01) L/h/kg, and volume of distribution of 0.61 (±0.04) L/kg. These data are comparable to the only mammalian study in rats and suggests that melamine is readily cleared by the kidney and there is unlikely to be significant tissue binding. Further tissue residue studies are required to assess the depletion kinetics of this contaminant in the pig which will determine whether residue levels in the kidney should be of public health concern if pigs were exposed to a similar dose.

Keywords: Melamine; Pigs; Pharmacokinetics

1. Introduction

Melamine, (2,4,6-triamino-*s*-triazine), is a chemical intermediate used to manufacture amino resins and plastics. Melamine has a pK_b of 9.0 because of the several amino groups that confer basic properties. In the spring of 2007 there was a major pet food recall in the United States following complaints that pet foods contaminated with melamine and several of its analogues were probably responsible for renal disease and/or deaths in dogs and cats exposed to contaminated pet food (Burns, 2007a,b). Unfortunately, livestock feed was supplemented with some of this contaminated pet food to a level of 30–120 ppm in swine feed (US FDA, 2007). These levels of feed contamination were estimated by USDA to unlikely place human health at risk (USDA, 2007), yet the public is still concerned about consuming meat from hogs and poultry exposed to melamine and/or its analogues.

Previous toxicology studies determined that melamine toxicity in mammals is generally very low, and this is supported by the large oral LD50 of 3161 mg/kg in rats (OECD, 2002). Furthermore, melamine does not undergo significant metabolism and should for the most part be readily cleared via the kidney. The diuretic effects of this chemical has been reported in rats and dogs (Lipschitz and Stokey, 1945). However, the risk assessment of melamine is complicated by the fact that differential toxicity has been reported across various animal species; and it is reasonable to assume that species-specific toxicokinetics may contribute to reported species differences. Male Fischer 344 rats exposed to melamine in their diets for 103 weeks developed transitional-cell

Abbreviations: AUC, area under the curve; Cl, clearance; HPLC, high performance liquid chromatography; IV, intravenous; Kel, elimination rate constant; LD, lethal dose; LOQ, limit of quantification; MRT, mean residence time; SPE, solid-phase extraction; $T_{1/2}$, half-life.

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carcinomas of the urinary bladder in statistically significant proportions compared to controls (NTP, 1983; Melnick et al., 1984; Ogasawara et al., 1995). The carcinogenic effects were strongly correlated with the presence of urinary bladder stones, and there is no evidence of direct molecular interactions attributed solely to melamine. Genotoxicity studies have also demonstrated that melamine is very unlikely to be a mutagen (Shelby et al., 1993). There is however sufficient evidence to suggest that the mechanism of renal toxicity in dogs and cats is associated with crystallization in the kidney followed by renal failure as observed with the recent adverse effects recently reported in pets in the United States (FDA, 2007; Burns, 2007a,b).

Previous toxicokinetic studies in male Fischer rats demonstrated that more than 90% of the oral dose was eliminated in the urine within 24 h and there was more melamine recovered in the kidney and bladder than in other tissues (Mast et al., 1983). These toxicokinetic findings provide some support for the toxicity reported in rats and can be attributed to the adverse effects observed in pets exposed to melamine-contaminated feed.

The purpose of this study was to characterize the disposition of melamine in pigs as its pharmacokinetics in this species has not been described in the literature. Data from this intravenous study can be used to accurately assess such kinetic parameters as half-life, volume of distribution, and clearance of this contaminant in pig which is required for accurately predicting plasma concentration in pigs exposed to similar or larger doses in swine production systems.

2. Materials and methods

2.1. Animals

This study was approved by North Carolina State University Institutional Animal Care and Use Committee. Five healthy weanling (8–10 weeks) Landrace–Yorkshire pigs (*Sus scofa domestica*) of 15.35 (±0.47) kg were acclimated for one week prior to surgery (Table 1). The pigs were housed in an AALAC accredited facility on an elevated floor and provided water (*ad libitum*) and 15% protein pellets. Animals were held off feed and water the night before surgery.

2.2. Intravenous catheter surgical implantation

On the day of surgery, anesthesia was induced with an intramuscular injection of ketamine, tiletamine, and xylazine followed by mask induction with 5% isoflurane (Minrad Inc., Bethlehem, PA) in 100% O_2. Pigs were orotracheally intubated, positioned in dorsal recumbency, maintained at a surgical plane of anesthesia (1.5–2.5% isoflurane in 100% O_2 in a semi-closed circuit), and allowed to spontaneously ventilate. The left jugular was identified by surgical cut-down using an aseptic technique. A 12 gauge polyethylene catheter was advanced through jugular vein approximately 6–10 inc. The incision was closed and the catheter was secured around the neck of the animal. A 22 gauge catheter was also placed in the auricular (ear) vein for melamine administration. Pigs were then returned to their stalls and allowed to recover fully from anesthesia.

2.3. Animal dose and sampling

A 6.13 mg/kg dose of melamine (1,3,5-triazine-2,4,6-triamine, CAS 108-78-1, EU# 203-615-4, 99+% purity) (Sigma–Aldrich; St. Louis, MS)

Table 1
Compartmental (A) and non-compartmental (B) pharmacokinetic parameters of melamine in pigs after IV dosing with 6.13 mg/kg melamine

Pig ID	$T_{1/2}$ (h)	Cl (L/h/kg)	V_{ss} (L/kg)	AUC (h μg/mL)	Kel (1/h)	MRT (h)
(A)						
Pig 1	2.63	0.16	0.59	39.10	0.26	3.79
Pig 2	3.99	0.10	0.57	62.26	0.17	5.76
Pig 3	4.78	0.11	0.78	54.43	0.14	6.89
Pig 4	4.65	0.08	0.56	72.90	0.15	6.71
Pig 5	4.31	0.09	0.56	67.63	0.16	6.22
Mean	4.07	0.11	0.61	59.26	0.18	5.87
SE	0.39	0.01	0.04	5.89	0.02	0.56
(B)						
Pig 1	2.67	0.18	0.68	34.90	0.26	4.23
Pig 2	3.29	0.11	0.51	57.40	0.21	5.43
Pig 3	5.45	0.11	0.90	54.06	0.13	8.37
Pig 4	4.74	0.09	0.61	68.85	0.15	7.22
Pig 5	3.56	0.09	0.48	65.84	0.19	6.10
Mean	3.94	0.12	0.63	56.21	0.19	6.27
SE	0.50	0.02	0.07	5.97	0.02	0.72

$T_{1/2}$ = half-life; Cl = clearance; V_{ss} = volume of distribution; AUC = area under the curve extrapolated to infinity; Kel = elimination rate constant; MRT = mean residence time.

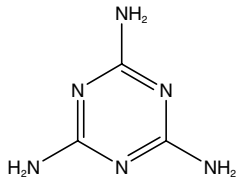

Fig. 1a. Chemical structure of melamine (1,3,5-triazine-2,4,6-triamine; C_3H6N6) with a pK_a of 5.0 and molecular weight of 126.12.

Fig. 1a was calculated according to the weight of each animal and prepared in sterile water. The day following surgery, the pigs were dosed via the ear vein catheter, which was then removed. A 5 mL blood sample was drawn at 0 (pre-dose), 1, 2, 4, 8, 12, 24, 36, 48 h post dose from the jugular catheter and replaced with lactated ringers solution. Blood was drawn into heparinized tubes, spun immediately and plasma was decanted. The plasma was frozen at 0 °C until processed. All samples were analyzed within 1 week of the study.

2.4. Sample extraction

Plasma samples were thawed; vortexed extracted using Water's Oasis® MCX 1 cc, 30 mg (Milford, MA) solid-phase extraction cartridges. One milliliter of plasma sample or standards were loaded into SPE cartridge conditioned and equilibrated with 1 mL methanol and 1 mL ultra pure water. Cartridges were than washed with 1 mL of 2% formic acid in water and then followed by 1 mL methanol. Samples were eluted with 1 mL of 5% ammonium hydroxide in water and placed in a 60 °C TurboVap® LV evaporator (Zymark Corp., Hopkinton, MA) to dryness under a 20 psi stream of nitrogen. Samples were then reconstituted in 250 μL of 0.05 M sodium phosphate buffer at pH 7.5. All reagents were of HPLC grade and provided as follows: Methanol (Sigma–Aldrich; St. Louis, MS), 88% Formic Acid and Sodium Phosphate (Fisher; Fairlawn, NJ), Ammonium Hydroxide (Mallinckrodt; Paris, KY), and ultra pure water (Pure Water Solution; Hillsborough, NC). Plasma standards were prepared using porcine plasma obtained from non exposed animals in heparinized tubes, centrifuged, and frozen until needed. Standards from 0.01 μg/mL to 10 μg/