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Lipoic acid is 10 times more toxic in cats than reported in humans, dogs or rats

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Summary

The antioxidant lipoic acid (LA) is administered to humans and pets. We described acute toxicity and maximum tolerated dose (MTD) of LA in cats. In progression, 10 healthy adult male cats received orally 60 (high), 30 (low), or 0 mg LA/kg (control). Serum enzyme activities and concentrations of bile acids, ammonia, amino acids (AA), LA and dihydrolipoic acid (DHLA) were measured, and tissues examined microscopically. Significant clinical toxicity with changes in ammonia and AA concentrations occurred in all high-dose cats. Oral LA produced hepatocellular toxicity and MTD was <30 mg/kg in cats.

Introduction

Over-the-counter purchase and consumption of supplements, including antioxidants, is skyrocketing. People spend billions of dollars on vitamin, supplement, and mineral products, and some, in turn, share these products with their pets. The veterinary profession is concerned with increasing 'the safety and efficacy of botanicals used in animals' (Wynn, 2002). Pharmaceutical literature contains several examples of medications that cause side-effects in different species: penicillin is fatal to guinea pigs (Fraser et al., 1991), acetaminophen causes methemoglobinaemia in cats (Sellon, 2001) and morphine can cause hyperactive hysteria in horses (Allan et al., 1998). As antioxidant variety and availability to the public are on the upswing, descriptions of their actions and side-effects are needed in target species.

Lipoic acid (LA, thioctic acid), an endogenously produced cofactor for the pyruvate dehydrogenase and α-ketoacid dehydrogenase complexes (Packer and Tritschler, 1996), was described in mammalian cells over 50 years ago (Reed et al., 1951). The antioxidant activities of LA and dihydrolipoic acid (DHLA) have been tested extensively in rats, dogs, and humans, but not in cats (Spence and McCormick, 1976; Scott et al., 1994; Bors and Michel, 1997). The establishment of safety and efficacy is critical before LA can be rationally included in the nutrition or medical care of cats.

Preliminary clinical tests administering LA to cats at a twice-daily oral dose of 30 mg/kg resulted in apparent signs of hepatotoxicity. Since that was an apparently safe dose for dogs, rats and people (Packer et al., 1995; Sandhya et al., 1995; Henriksen, 1997), we hypothesized that cats were a more sensitive species to the toxic effects of LA. Using this preliminary dose as a starting point, we evaluated the clinical, biochemical and hepatocellular effects of oral LA in cats, using a previously validated dosing progression scheme (Dixon, 1965; Bruce, 1987; Whitehead and Curnow, 1992).

Materials and methods

Ten healthy, intact adult male cats were housed in and acclimated to metabolism for 3 days before LA administration. Room temperature was maintained at 21 ± 2 °C and light was provided for 14 h per day. The cats were fed a feline adult dry maintenance diet (KalKan® Whiskas™ dry formula for adult cats, Vernon, CA, USA) and given fresh water *ad libitum*. Daily, the cats were weighed and their food intake was measured. The cats ranged from 1.5 to 6.5 years of age (mean ± SD, 2.6 ± 0.5 years) and 4.2–5.4 kg body weight (4.8 ± 0.4 kg). The protocol for this study was approved by the Animal Care and Use Committee of the University of California, Davis, in accordance with the NRC (1985) Guide for Care and Use of Lab Animals.

Using a sequential dosing procedure to minimize the number of animals needed, each cat received a gelatin capsule (Gelcap no. 0, Eli Lilly & Co., Indianapolis, IN, USA) containing LA (Chemco Industries, Los Angeles, CA, USA); the oral dose was based on cat-by-cat response. Clinical toxicity was defined as meeting at least two of three criteria after LA administration: (i) daily food intake ≤50% of the 3-day pre-dose average; (ii) ataxia, hypersensitivity, hypersalivation or vomiting; and (iii) serum ALT or AST activities at least twice the upper reference limit within 24 h. Four cats (high dose) received an oral dose of 60 mg/kg of body weight (60.3 ± 0.2 mg/kg); three (low dose) received 30 mg/kg (30.6 ± 0.4 mg/kg); and three (control) received an empty capsule. Blood was taken at 0, 2 and 24 h after dosing. Complete blood counts (Baker System 9000, ABX Diagnostics, Irvine, CA, USA), serum chemistry profiles (Hitachi 717, Roche, Indianapolis, IN, USA), and concentrations of serum ammonia (Kit 171-B; Sigma, St Louis, MO, USA), total bile acid, LA and DHLA were determined.

Urine was collected in acidified containers for 24 h on the day before and separately on the day after dosing. Total urine volume was recorded and an aliquot from each collection was stored at −80 °C for later analysis. At 24 h, each cat was euthanized by lethal intravenous dose of pentobarbital (Pentosol injection, 6 grain/ml, Med Pharmex Inc., Pomona, CA, USA) and necropsied within 30 min. Samples of liver, kidney, spleen, lung, duodenum, pancreas and skeletal muscle were fixed in 10% buffered formalin for ≥24 h before histological examination. Tissue sections were routinely processed, and 5-μm tissue slices were prepared and stained with haematoxylin and eosin for light microscopy. Samples of liver, lung, spleen, pancreas, duodenum, kidney and skeletal muscle were snap-frozen in liquid nitrogen while aliquots of bile, urine and blood were collected and stored at −80 °C for assay of LA and DHLA concentrations.

Within 10 min of euthanasia, the liver of one control and one high-dose cat were infused with glutaraldehyde (Electron Microscopy Sciences, Fort Washington, PA, USA) for electron microscopy (EM) (Zeiss 10C TEM, LEO Electron Microscopy, Inc., Thornwood, NY). The glutaraldehyde was infused via catheter into the portal vein and drained via the transected hepatic vein. Cold saline (60 ml) was flushed into the liver, followed by 60 ml of cold gluraldehyde solution. Samples of liver were minced and immersed in EM fixative for at least 24 h. Samples were routinely processed and placed on a grid (150 mesh bare copper) (Sigma) for EM examination.

Heparinized plasma samples were mixed 1 : 1 with 6% sulphasalicylic acid (SSA) (Sigma), centrifuged to remove protein, then analysed for free amino acid (AA) concentrations using an automatic analyzer with external and internal standards (Beckman 7300, Fullerton, CA, USA). Liver samples were homogenized, mixed with SSA and assayed for AA as was carried out for the plasma samples.

The LA and DHLA concentrations in the samples were measured by high-performance liquid chromatography (HPLC). Tissues were minced and mixed 1 : 3 (v : v) with 20% phosphoric acid (EM Science, Gibbstown, NJ, USA), then homogenized by sonication (Model W375, Heat Systems Ultrasonics Inc., Plain View, NJ, USA). Samples of plasma, urine, and bile were extracted and analysed according to Sen et al. (1999). Bile was

extracted with and without deproteinization to determine the fractions of LA and DHLA that were protein bound. Urine creatinine was measured using a standard kit (Sigma). Protein concentrations in bile and tissue were measured by standard kit (BSA, Bradford kit, Bio-Rad, Richmond, CA, USA).

Data were analysed by ANOVA for differences between dosage groups. $p < 0.05$ was considered significant. Maximum tolerated dose (MTD) was calculated as described by DIXON (1965) and validated by BRUCE (1987) and WHITEHEAD and CURNOW (1992). After recording outcomes in sequence, this equation was used to estimate MTD: $MTD = X_f + kd$, where X_f is the log of the final dose administered, k is the Dixon derived value, and d is the interval between the log of the doses.

Results

We observed positive toxicity responses (+) with 60 mg/kg oral LA, followed by a negative response (−) at 30 mg/kg. The remaining progression was 60 (+), 30 (−), 60 (+) and 30 (−). Additional cats received LA at each level (60, 30 and 0 mg/kg) to provide a larger sample size for laboratory tests and necropsy examinations. Based on the Dixon computational tables and the doses administered in this study, the values used for the equation $MTD = X_f + kd$ were: $X_f = 1.477$; $d = 0.699$ and $k = -0.5$. The calculated MTD for a single oral dose of LA in cats was 13 mg/kg.

Nine of the 10 cats completed the study. One high-dose cat died within 6 h of LA administration. Necropsy results revealed severe hepatic congestion and pulmonary thromboemboli. Within 2 h, those cats receiving 60 mg/kg (high-dose group, $n = 4$) showed at least a 50% decline in food intake, along with hypersalivation, hyper-irritability and ataxia. Serum ammonia concentrations at 2 h were five times above the 0 h values (from baseline of 44.0 ± 23.9 to 260.9 ± 121.3 μg/dl) and stayed above baseline for the next 24 h. In the high-dose cats, serum ALT nearly doubled at 24 h, whereas serum AST increased <25% and bile acid concentrations remained normal. Low-dose and control cats developed no signs of toxicity or significant changes in food intake, ALT, AST, bile acid, ammonia, or CBC values.

Within 2 h, the high-dose cats' plasma-free aromatic amino acid (ArAA) concentrations increased 24% above 0 h values while branched chain amino acid (BCAA) concentrations decreased 49%. These concentrations differed significantly ($p = 0.015$) from those in the other two groups of cats. The resulting mean BCAA : ArAA ratios (BCR) at 2 h for the high-dose and control cats were 1.9 and 4.0, respectively. High-dose BCR returned to baseline at 24 h (Fig. 1). The BCR in the low dose group decreased from 3.9 at 0 h to 3.1 at 2 h but returned to normal by 24 h. In the control cats, BCR did not change significantly. Most other plasma AA concentrations decreased in low- and high-dose cats at 24 h, however, there was large inter-individual variation. In liver tissue, free AA concentrations in high-dose cats were significantly higher for taurine, threonine, serine and glycine, alanine and glutamate, leucine, lysine and ornithine. In low-dose cats, only taurine increased significantly ($p = 0.01$).

There were no gross lesions in the tissues from the nine cats that completed the study. Histologically, the centrilobular regions of the livers of both high- and low-dose cats showed swollen, granular to vesicular cytoplasm, loss of distinct sinusoidal linings, and lack of lipid or glycogen stores. By EM, the liver from the high-dose cat showed expanded spaces of Disse with variable loss and disruption of hepatocyte sinusoidal microvilli (Fig. 2). There was variable dissociation of junctional complexes with separation of adjacent plasma membranes and disruption of bile canniliculi and loss of small microvilli. Hepatocellular organelles were disorganized and the smooth endoplasmic reticulum was focally dilated. Cytoplasmic blebs extruded from hepatocytes into the space of Disse, and contained mitochondria and well-demarcated, membrane-bound vacuoles with flocculent electron-dense contents. Mitochondria were no longer evenly distributed along the

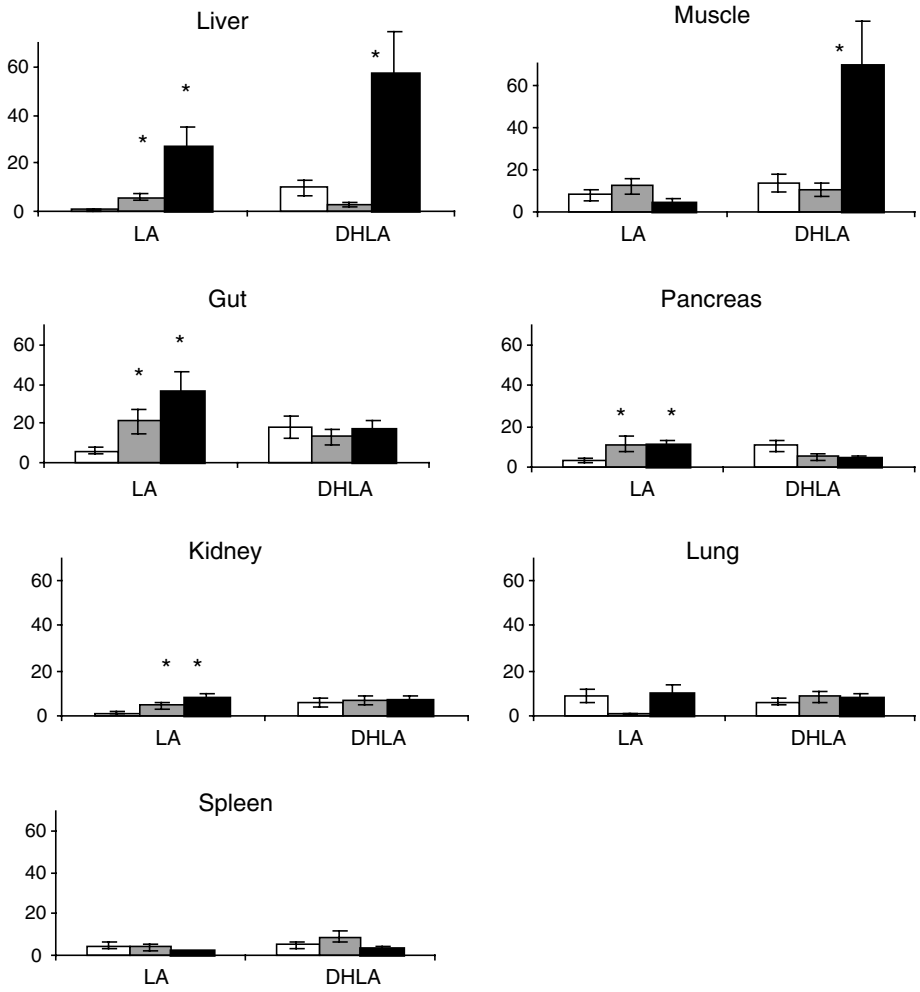

Fig. 1. Tissue concentrations (μmol/mg protein) of lipoic acid (LA) and dihydrolipoic acid (DHLA) 24 h after dosing (mean ± SEM). $n = 3$ cats per group; C, control (□); low, low-dose cats (■), 30 mg/kg and high, high-dose cats (■), 60 mg/kg oral lipoic acid. *$p < 0.05$ compared with control group (μmol/mg protein).

periphery of the hepatocyte cytoplasm, but still had an electron-dense inner matrix surrounded by a distinct membrane. Hepatocytes contained similar variably sized vacuoles that were often large and filled the cytoplasm. The abundant glycogen stores seen in hepatocytes of the control cat were missing, but occasional well-demarcated lipid-containing vacuoles were still observed. In both control and high-dose livers, sinusoidal lining cells and hepatocellular nuclei appeared normal.

The LA and DHLA tissue concentrations were significantly different among groups for liver, muscle, gut, pancreas and kidney. The LA showed a dose-dependent increase in liver, gut, and kidney, with the highest concentrations detected in liver and gut. In pancreas, LA concentration was equally increased in low- and high-dose cats. DHLA concentrations were significantly increased in liver and muscle of high-dose cats, both more than twice the LA levels in those tissues. The DHLA concentrations in the low-dose cat tissues were

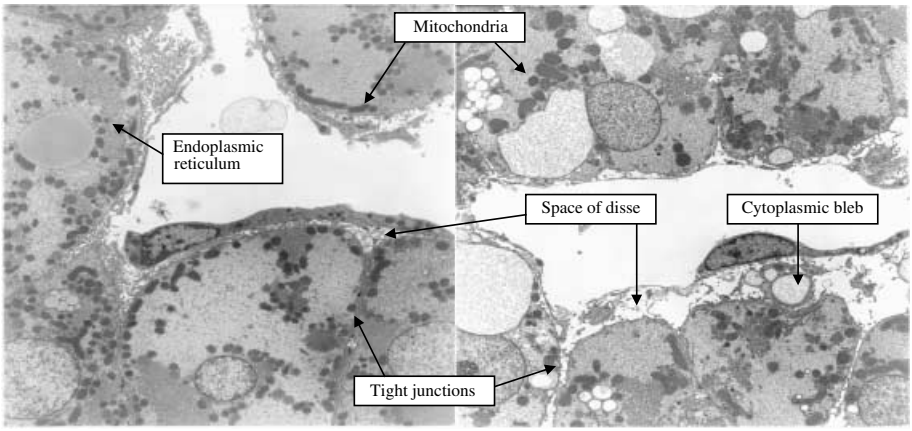

Fig. 2. Electron micrographs (5743×) of centrilobular cells from one high-dose cat (right) and one control cat (left) from liver specimens taken at 24 h after dosing. The cells are from the same liver region in both cats. Significantly different structures are indicated by text labels on the micrographs.

Table 1. **Mean concentrations of LA and DHLA in plasma, bile and urine (mean ± SD)**

		LA or DHLA concentration					
		Pre-dose		2 h post-dose		24 h post-dose	
Group	Body fluid	LA	DHLA	LA	DHLA	LA	DHLA
C	Plasma (pmol/mg protein)	0.2 ± 0.1	0	0.3 ± 0.1	0	0.2 ± 0.1	0
	Urine (pmol/mg creatinine)	0	0.4 ± 0.3	ND	ND	0	0.2 ± 0.1
	Bile (pmol/mg protein)	ND	ND	ND	ND	330 + 200	380 + 100
Low	Plasma (pmol/mg protein)	0.1 ± 0.1	0	5.1 ± 2.5	2.3 ± 2.3	0.4 ± 0.1	0
	Urine (pmol/mg creatinine)	0	0.2 ± 0.2	ND	ND	0.3 ± 0.1[a]	4.0 ± 0.5[a]
	Bile (pmol/mg protein)	ND	ND	ND	ND	1450 ± 1100	850 ± 100[a]
High	Plasma (pmol/mg protein)	0.5 ± 0.5	0	6.7 ± 1.0[a]	1.7 ± 0.4[a]	1.7 ± 1.7	2.1 ± 0.4[a]
	Urine (pmol/mg creatinine)	0	0.2 ± 0.1	ND	ND	4.4 ± 0.3[a]	2.4 ± 0.4
	Bile (pmol/mg protein)	ND	ND	ND	ND	4620 ± 1900[a]	2620 ± 2000

[a] $p < 0.05$ compared with control mean at that time point
ND, assay not done, no sample
$n = 3$ cats per group; C, control; low, low-dose cats, 30 mg/kg; high, high-dose cats, 60 mg/kg oral lipoic acid; LA, lipoic acid; DHLA, dihydrolipoic acid

similar to those in plasma. Concentrations of LA and DHLA in plasma, bile, and urine differed significantly among groups at 2 and 24 h (Table 1). In bile, results suggested that LA and DHLA were >98% protein-bound in bile in all of the cats. Compared with bile from control cats, LA concentrations were significantly increased in high-dose cats, while in low-dose cats, DHLA was significantly higher.

Discussion

In cats, administration of 60 mg/kg oral LA is associated with signs of acute toxicity clinically, biochemically and histologically. Doses of 30 mg/kg are associated with mild acute hepatocellular damage. Clearly, cats are very sensitive to the toxic effects of LA, the calculated MTD of 13 mg/kg body weight was significantly lower than the single oral dose tolerated in humans, dogs and rats: 120, 126 and 635 mg/kg, respectively (Grunert, 1960).

Hepatocellular abnormalities seen in the high- and low-dose cats were consistent with non-specific acute toxicity that causes dysregulation of ongoing hepatic processes (Gregus and Klaassen, 1996). Similar changes occur after rodent hepatocellular exposure to thiocyanate compounds, acetaminophen, or reactive oxygen species (Walker et al., 1980; Moslen, 1996).

Cellular metabolism of LA begins with reduction to DHLA by glutathione reductase, lipoamide dehydrogenase and thioredoxin reductase (Peinado et al., 1989; Arnér et al., 1996; Biewenga et al., 1996, 1997). The DHLA is excreted from cells or reoxidized or metabolized. Feline liver, kidney, and muscle appear to reduce LA or take up plasma DHLA. Increased concentrations of DHLA in pancreas, lung, gut and spleen, as well as plasma, bile, and urine, suggest widespread cellular ability to take up DHLA or reduce LA. In high-dose cats, plasma DHLA concentrations were four times greater than in control cats at 24 h, but in liver they were 20 times higher than in plasma, suggesting that feline liver both concentrates and reduces LA. The DHLA, as a potent reductant, triggers the caspase 3 cascade in mitochondria in cell culture, leading to apoptosis (Sen et al., 1999). In rat, mouse, and canine hepatocytes, metabolism and oxidation of LA can produce water-soluble metabolites including reactive dithiols which covalently bind to intracellular proteins and lead to cellular necrosis (Rosen et al., 1977; Parkinson, 1996; Schupke et al., 2001).

Increased serum ammonia concentrations, along with significantly decreased BCRs indicated hepatocyte dysfunction (Zhadkevich et al., 1989; Nijveldt et al., 2001) and here correlated well with hepatocellular changes in high-dose cats. The BCR decrease in all cats receiving LA suggests that administering even 30 mg/kg orally was toxic to cats. Changes in AA concentrations in liver and plasma suggests disruption of subcellular pathways. The pyruvate dehydrogenase complex, for example, appears to be particularly sensitive to oxidant-induced inhibition (Hong et al., 1999).

Significance

Clinical signs of LA toxicity in the high-dose cats included anorexia, hyper-irritability, hypersalivation and ataxia, signs similar to those seen in cats with arginine deficiency (Morris and Rogers, 1978). We suspect that accumulation of LA and DHLA, potentially by enterohepatic circulation or deficiency of detoxifying enzymes in cats, allows production of toxic intermediates that to the clinical, biochemical and histological signs observed. Tissue accumulation of LA could also occur with chronic dosing (Packer et al., 1995), resulting in significant liver damage at even lower doses. Before using LA as an antioxidant in cats, future studies are needed to assess chronic effects and at doses below the acute estimated MTD of 13 mg/kg, which is 1/10 to 1/40 the MTD reported in other species.

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