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The acute toxicity of tri-n-butyltin taurocholate

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The acute toxicity for tri-n-butyltin taurocholate (TBT-TA), a newly synthesized organotin steroid, was determined using Long Evans rats. The organotin compound was suspended in corn oil and administered by gavage using standard techniques. The TBT-TA exhibited a taurocholic acid toxicity at 24 h and a tributyltin toxicity at three days. The LD_{50} values were 611 and 384 mg kg^{-1} respectively. The dead rats exhibited distended stomachs, enlarged cecums, and lesions in the gastrointestinal tract. The toxicity is similar to that observed with other trialkyltin compounds..

Keywords: LD_{50}, tri-n-butyltin taurocholate, Long Evans rats, acute toxicity

INTRODUCTION

Except for the triethyltin compounds, most organotin compounds have low toxicities.[1] Many dialkyl and trialkyltin compounds cause premature atrophy of the thymus gland[2–4] and impairment of B and T cells which causes immunosuppression,[2] but little else has been observed at low dosage (<25 mg kg^{-1}). At high dosage (150 mg kg^{-1}), some organotin compounds cause severe damage to the stomach.[5] The stomach lesions would impair gastric function, reduce normal food and water consumption, and may lead to starvation or dehydration.[1]

Organotin-steroid compounds are relatively new. They have been prepared[6,7] by simple syntheses (reactions of either the organotin oxide, chloride or methoxide with the steroid). *In vitro* testing of epidermoid carcinoma cell line (KB) and mouse lymphocytic leukemia (P388) on a number of these compounds have shown some degree of antitumor activity.[8]

The detoxification of organotin compounds by the liver and intestines has been studied by several investigators;[9,10] however, there is little or no information on the toxicity of organotin steroids.

Although there is some debate about the validity and usefulness of the standard LD_{50} test,[11] at the present time there is no other acceptable procedure for the initial toxicity evaluation of a new drug, food additive or chemical. This work was initiated to help in the initial evaluation of tri-n-butyltin taurocholate (TBT-TA) as a potential new drug.

EXPERIMENTAL

Chemicals

Tri-n-butyltin taurocholate (TBT-TA) was prepared by our group as described in the literature. The TBT-TA was prepared by refluxing stoichiometric quantities of tributyltin oxide and taurocholic acid in benzene. The water produced in the reaction was removed by azeotropic distillation with benzene. The unreacted tributyltin oxide was removed on an alumina column.[12] The molecule is a weakly ionizing compound and contains a hydrated tributyltin cation and taurocholate anion.[12] It was administered to Long Evans rats by gavage using standard procedures.[13]

Animals

Five male and five female 5–6-month old Long Evans rats (approximate weight 200 g) were used for each data point. The animals were obtained from the Pocono Rabbit Farm, Canadensis, PA, USA and housed five male or five female rats to a cage. They were maintained on Bedacobs™ (ground corn cobs) and fed Purina Certified Lab Chow™ and distilled water *ad libitum* for five days prior to each acute toxicology trial. Food and water were withheld from the animals for 24 h prior to administration by gavage of 350–600 mg kg^{-1} of TBT-TA dispersed in approximately 1.0 cm^3 of corn oil. In a single

trial (450 mg kg^{-1}) one male control rat and one female control rat were administered 1.0 cm^3 of corn oil; another male and another female control were administered stoichiometric quantities of the sodium salt of taurocholic acid (Chem Registry No. 145-42-6) in 1.0 cm^3 of corn oil. The animals were observed for 24 h and one-half of the control rats were sacrificed at two days. After death, all test animals and controls were autopsied for gross organ failure. At the end of 14 days all remaining animals were sacrificed and autopsied. Pathological changes in the stomach, intestinal tract, liver, kidneys, heart, lungs, spleen and thymus were noted. The general health of the animals was observed pre- and post-mortem. Three animals had intestinal tumors that were identified as melanocarcinoma.

RESULTS

The LD_{50} data are presented in Table 1. The animals administered corn oil or taurocholic acid exhibited no toxic effects at a mole equivalent of 450 mg kg^{-1} of TBT-TA. They returned to a normal feeding pattern within 30 h and showed no abnormalities when sacrificed on day 14.

All of the rats administered the TBT-TA developed bloating in the stomach and gas formed throughout the small intestines. The animals squealed upon palpation of the stomach area, indicating a great deal of discomfort. The condition was not permanent and disappeared in those animals which survived more than three days. The animals ate litter and those which died within three days had small quantities of litter in their stomachs. Similar gastrointestinal effects were observed with organolead poisoning.[5]

Those animals which died within 24 h showed a distended stomach and some lesions in their lungs. The lung lesions are similar to those observed with heavy metal intoxication. The data were inconsistent; the lung lesions were not observed in all the animals which died in the first three days. No lesions were observed in those rats which lived for 14 days. The animals which succumbed within three days exhibited a loss of weight; however, these animals, in general, did not eat or drink and the loss of weight was probably due to lack of nutrients and not to the toxicity of the compound.[5] Those animals which survived 14 days had the same or greater weight than at the beginning of the experiment.

Some thymus atrophy was evident and the cecum was four to five times larger than normal. Some rats showed lesions in their small intestines. Except for the thymus atrophy, common for many alkyltin compounds,[2–4] all organs returned to normal by day 14 and indicated no long-term toxic effects from the single chemical dose.

The toxicity was dose-related (Table 1). The data points for 500 mg kg^{-1} for the 24 h and three-day periods appeared to be erroneous and they have not been used in the plot. The LD_{50} for TBT-TA is 611 mg kg^{-1} or 0.72 mmoles kg^{-1} for 24 h (Fig. 1). At all dosages above 384 mg/kg or 0.45 mmoles/kg 50% of the animals died within three days (Fig. 1).

DISCUSSION

The LD_{50} toxicity of TBT-TA for 24 h appears to be 611 mg/kg or 0.72 mmol kg^{-1}. The TBT-TA is a weakly ionizing compound[12] and presents two toxic moieties as it passes through the gastro-

Table 1 LD_{50} data for Long Evans rats administered tri-n-butyltin taurocholate

Dose (mg kg^{-1})	Number dead			
	At 24 h	At 3 days	At 4–13 days[a]	Survived 14 days
350	2	1	1 (6)	6
400	2	4	0	4
450	3	5	1 (8)	1
500	3	2	1 (7)	4
550	5	4	0	1
600	5	5	0	0

[a]Number in parentheses is day of mortality.

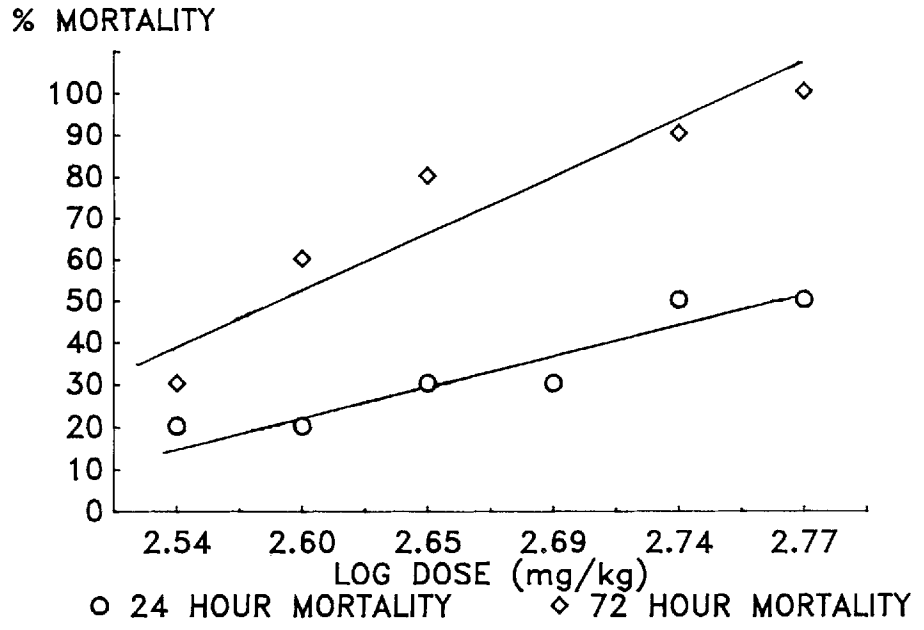

Figure 1 Mortality of Long Evans rats versus log of dose of tri-n-butyltin taurocholate ($mg\,kg^{-1}$) for 24 h and three days.

intestinal tract. The initial toxicity at 24 h is that of the taurocholate anion and the value is close to that reported for molar toxicity for taurocholic acid.[14] The TBT moiety has a delayed toxicity in animals[15] and LD_{50} after two or three days is $384\,mg\,kg^{-1}$ or $0.45\,mmol\,kg^{-1}$, about the same as observed for tributyltin compounds which exhibit some degree of ionization in polar solvents.[16] The distended stomach has been observed with acute toxicity of tripropyl, tributyl and triphenyltin compounds; with organolead compounds it was caused by gastric fluid accumulation.[5] The distended stomach was probably caused by the tributyltin moiety, but this has not been universally observed with all TBT compounds.[15] The TBT-TA exhibited *in vitro* anticancer properties [ED_{50} of 0.2–0.4 ppm ($mg\,kg^{-1}$)];[12] however, due to the presence of the tri-n-butyltin moiety, the compound will probably exhibit cytotoxicity toward the thymus gland and fail the *in vivo* tests.[17] The toxicity study will help understanding of the oral limits for ingesting organotin steroids and is to our knowledge unique in that there are two toxicity curves.

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