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The acute toxicity of tri-n-butyltin glycocholate

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The oral acute toxicity for tri-n-butyltin glycocholate (TBT-GA), a newly synthesized organotin steroid, was determined using Long Evans rats. The compound was suspended in corn oil and adminstered by gavage using standard techniques. Unlike tri-n-butyltin taurocholate, which exhibited two different toxicities, one for the tri-n-butyltin moiety and one for the taurocholic acid moiety, the TBT-GA exhibited a single toxicity, that of the whole molecule. The LD_{50} value was 213 mg kg^{-1} (0.274 mmol kg), which on a millimolar basis is similar to that observed for other tri-n-butyltin compounds. The dead rats exhibited distended stomachs, enlarged ceca, and lesions in the intestinal tract.

The actual cause of death could not be positively identified. Animals that survived more than three days also exhibited time- and dose-related atrophy of the thymus gland. With 36% more male than female rats succumbing to TBT-GA, the chemical appears to be more toxic towards male than female rats.

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

INTRODUCTION

Except for the methyl- and ethyl-tin compounds, most organotin compounds (OTC) have low mammalian toxicities.[1,2] At low dosage many dialkyl and trialkyltin compounds cause premature atrophy of the thymus gland[3–4] and can cause impairment of T cells, resulting in immunosuppression.[3] At high dosage, trialkyltin compounds cause severe damage to the gastrointestinal tract.[6] The lesions in the stomach and intestines impair gastric function, reduce normal food and waste absorption and may lead to starvation or dehydration as well as other toxic effects.[2,7] The metabolism of organotin compounds by the liver and intestines has been studied by several investigators.[8,9] Although there is some debate about the validity and usefulness of the standard LD_{50} test,[10] at the present time there is no other acceptable procedure for the initial toxicological evaluation.

Alkyl and aryl tin-steroid compounds have recently been prepared[11–13] by simple syntheses (reactions of either the organotin oxide, hydroxide or methoxide with the steroid). *In vitro* testing of a number of these compounds on an epidermoid carcinoma cell line (KB) and mouse lymphocytic leukemia (P388) has shown antitumor activity;[13,14] these results warrant further investigation to determine the merits of these compounds as anticancer agents. The acute toxicity of tri-n-butyltin taurocholate (TBT-TA) has been investigated.[15] TBT-TA, which is an ionic compound, exhibited a double toxicity caused by the taurocholate and tributyltin moieties, respectively. In order to understand further the biochemistry of the new organotin steroids, the acute toxicity of tri-n-butyltin glycocholate (TBT-GA, $C_{38}H_{69}NO_6Sn$), a covalent compound,[13] was studied. The results are presented in this paper.

EXPERIMENTAL

Chemicals

Tri-n-butyltin glycocholate (TBT-GA) was prepared as described in the literature.[13] The molecule is a covalent compound and appears to have a tetracoordinated tin atom.[13] It was administered to Long Evans rats by intragastric gavage using standard procedures.[16]

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Animals

Five male and five female 3–6-month-old Long Evans rats (weight range 92–344 g) were used for each trial. The animals were obtained from either Pocono Rabbit Farm, Canadensis, PA 18325, USA, or Charles River Laboratories, Wilmington, MA 01887, USA, and housed five male or five female rats to a cage. They were maintained in metabolic cages 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 intragastric gavage (100–600 mg kg^{-1} of TBT-GA dispersed in approximately 1.0 cm^3 of corn oil).

The general health of the animals was observed a minimum of twice daily pre- and post-administration of the TBT-GA. During the 550 mg kg^{-1} data collection, one male and one female rat were administered ~1.0 cm^3 of corn oil; during another trial (600 mg kg^{-1} data set) one male and one female rat were administered stoichiometric quantities of glycocholic acid (GA) (Chem Registry No. 863-57-0) in ~1.0 cm^3 of corn oil; a third pair of animals was administered stoichiometric quantities of tributyltin oxide (TBTO) (350 mg kg^{-1} data set) in ~1.0 cm^3 of corn oil; a fourth pair of untreated animals was administered no chemicals at all but maintained on the same diet as the other animals. All animals were observed at least twice daily for eight days after administering the chemical or until death. At the end of eight days, all surviving animals were sacrificed using chloroform. All treated and untreated animals were autopsied upon mortality. Pathologic changes in the stomach, intestinal tract, lungs, and thymus were noted.

RESULTS AND DISCUSSION

The toxicity of TBT-GA was dose-related (Table 1 and Fig. 1). The LD_{50} (determined by standard methods using a probit plot of the data) for TBT-GA is 213 mg kg^{-1} or 0.274 mmol kg^{-1} (Fig. 1). On a molar basis, this is in the same range as previously published for similar tributyltin compounds.[15,17]

The untreated animals exhibited no toxic effects, returned to a normal feeding pattern within 24 h and showed no abnormalities when sacrificed on day 8. One TBTO-treated animal died on day 3 (158 mg of TBTO kg^{-1} of rat) and exhibited lesions in the gastrointestinal track. The toxicity was very similar to that reported in the literature for similar TBT compounds.[6,15,17–19]

All of the rats administered the TBT-GA developed abdominal distension and tenderness. All test animals, including those treated with only GA, suffered from diarrhea and dehydration for about three days; this may have been caused by the bile acid moiety on the molecule. These conditions were not permanent and disappeared in those rats that survived more than three days. The animals ate litter, and those animals that died within three days had small quantities of litter in their stomachs. Similar gastrointestinal effects have been observed in connection with organolead poisoning[6] and with TBT-TA.[15]

Table 1 LD_{50} data for Long Evans rats administered tri-n-butyltin glycocholate (single dose)

Dose (mg kg^{-1})	Number dead			Survived 8 days	Total number of subjects
	At 24 h	At 1–3 days	At 4–8 days[a]		
100	1	1	0	8	10
250	2	4	1(4)	1	9
350	2	4	0	3	10
400	5	4	0	2	11
450	5	2	0	3	10
500	3	1	1(4)	5	10
525	1	7	0	4	12
550	4	1	2(5)	3	10
600	8	1	0	1	10

[a] Number in parentheses is the day of morbidity.

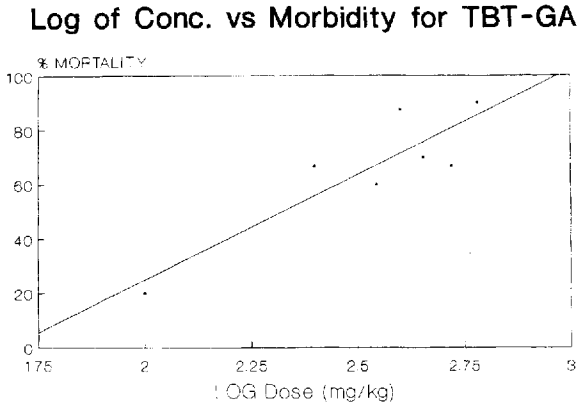

Figure 1. Mortality of Long Evans rats versus log of dose of tri-n-butyltin glycocholate (mg kg^{-1}) for three days.

Gross analyses of dead rats

Those animals that died within eight days exhibited distended stomachs and some had lung lesions. The lung lesions are similar to those observed with heavy metal intoxication.[6] The data were inconsistent; the lung lesions were not observed in all the animals and may have been caused by mechanical compression of the lung tissue from the dilated stomach. No lung lesions were observed in the control rats, including those administered the TBTO. All animals that succumbed within eight days exhibited a loss of weight (see Table 2); however, these animals, in general, did not eat or drink and the loss of weight was probably due to lack of nutrition, an indirect effect of the compound, i.e. they were disinclined to eat.[2,6] Most animals lost about 10% of their weight during the 24-h fast prior to administration of the chemicals. Those animals that survived eight days had approximately the same weight as at the beginning of the experiment (Table 2). However, the animals administered the higher TBT-GA doses did not recover the lost weight as rapidly as those administered lower doses.

Table 2 Average percentage of initial weight[a] at time of death

Dose (mg kg^{-1})	Dead at 24 h	Dead at 1–8 days	Survivors
100	90.5	91.5	99.0[b]
250	93.5	90	103
350	92	85	99.9
400	89	85	76[c]
450	81	77	95
500	—	86	95
525	86	82	103
550	95	88	89[d]
600	92	87	78

[a] Mass prior to fast. [b] One low. [c] Only two rats survived. [d] One rat continued to lose weight.

The average thymus/body weight ratios for the animals that succumbed at each concentration and time were compiled and are presented in Table 3. The animals in the 525 mg kg^{-1} test were placed in formaldehyde and used for other microscopic studies. The data are presented as two ratios, (A) mass of thymus/mass of animal prior to the pretreatment fast and (B) mass of thymus/mass of animal at time of mortality. The GA-treated animals had a ratio of 0.00161 and were used as a reference since the ratio was within the normal range observed for adult rats.[5] Except for the value calculated for the 100 and 500 mg kg^{-1} data points, the atrophy of the thymus appeared to be time- and concentration-dependent. The thymus/body weight ratio for all animals, except those which were sacrificed on day 8 for the 100 and 500 mg kg^{-1} point, was less than for those which succumbed at 24 h. Whereas most animals that survived until day 8 regained their lost body mass, the thymus involution was still evident. These observations were consistent with those reported for other TBT compounds.[1–5,7,15,17,18]

As with the TBT-TA[15] and other tin compounds,[19] the size of the cecum at the time of morbidity was four to five times greater than normal and the enlargement appeared to be dose-related. The lungs and liver were congested and the stomach dilated. The small intestines showed focal areas of narrowing. The only long-term toxic effect observed in the animals which survived the test was the thymic atrophy, which is common for many alkyltin compounds.[3–5]

Table 4 summarizes for each time-frame, the percentage loss of animals in regard to gender. The mortality of male rats was greater than females at 24 h, three days and eight days, indicating a gender-related toxicity for the compound. A similar observation was observed with dioctyltin dichloride with Lewis rats[20] and a detailed appraisal of the TBT-GA and TBT-TA gender-related toxicity has been accepted for publication.[21]

Microscopic description

Microscopic investigation of selected rat tissue showed diffuse congestion of the heart, lungs, liver, spleen and

Table 3 Average ratio of thymus mass to body mass[a]

Dose (mg kg^{-1})	Dead at 24 h		Dead at 1–8 days		Survivors	
	Initial,[b] A	Final,[c] B	Initial,[b] A	Final,[c] B	Initial,[b] A	Final,[c] B
100	1.18	1.25	0.50	0.55	1.20	1.21
250	1.11	1.23	0.74	0.79	0.68	0.66
350	1.06	1.17	0.61[d]	0.64[d]	0.66	0.66
400	1.27	1.42	1.21	1.42	0.60	0.83
450	0.98	1.21	0.59	0.96	0.93	0.98
500	1.82	—	1.53	1.80	1.85	1.96
525	No data collected					
550	1.28	1.25	—	—	Small, broken up	
600	1.50	1.63	—	—	0.255	0.326
GA(e)	—	—	—	—	1.61	1.60
TBTO(e)	0.74	0.86	—	—	0.57	0.60

[a] All data × 10^3.
[b] Ratio (A) based upon mass of animal prior to food and water fast.
[c] Ratio (B) based upon mass of animal at time of mortality.
[d] Older animals whose thymus might have atrophied before testing.
[e] Control.

Table 4 Gender-related mortality during acute toxicity study of TBT-GA

Time	No. affected (%)	
	Male	Female
24 h	19/48 (40)	12/48 (25)
3 days	32/48 (67)	25/48 (52)
8 days	33/48 (69)	27/48 (56)

kidneys. There was no evidence of pneumonia in the animals studied. The stomachs were partially lined with squamous epithelium, a normal histologic feature in the rat. The small intestine showed focal and diffuse necrosis, mostly full thickness but occasionally confined to mucosa. The absence of inflammation in the adjacent tissue indicated that these changes were autolytic (*post mortem*) but the possibility of toxic vasospasm and ischemic necrosis cannot be entirely excluded. The diffuse congestion in the other organs represented a terminal, shock-related phenomenon.

Microscopic evaluation of one set of animals (525 mg kg^{-1}) indicated some *post mortem* autolysis of the organs. The small intestines exhibited no gross abnormalities. The livers were congested and showed some hydropic degeneration. Those animals that survived eight days exhibited some glycogen deposition. However, these changes were not consistent. The exact cause of death could not be determined from the histologic studies.

Thymic damage in the 525 mg kg^{-1} set of animals was more consistent and easier to identify. Only two rats exhibited completely normal thymi (one died on day 2 and the other survived the study). The other thymi exhibited mild degeneration, manifested as large cells with clear cytoplasm, resulting in a 'starry sky' appearance. This phenomenon was also observed in the rat that died after 24 h. Clusters of degenerating lymphocytes were evident and some animals showed significant medullary expansion and cortical depletion.

CONCLUSION

Since there is little difference in the acute toxicity of TBT-GA and TBT-TA, although the compounds are quite different in their physical–chemical properties,[9] it would appear that the toxicological properties are based upon either the hydrolysis of the compound in the stomach or on the partitioning of the aqueous/lipid fractions in the rat intestinal tract.[22] Since bile acids can form ion-pairs, which are soluble in the lipid portion of a rat ileum,[22] it appears that ionization of the TBT bile acid derivatives is unimportant. In the case of ionic compounds, the cation would form an ion-pair

in the duodenum and be absorbed, whereas a covalent compound, if it survived the stomach, would be directly absorbed when it enters the duodenum. Because there is little difference in the toxicity of TBT-GA and other TBT compounds, the major toxicity probably depends upon the TBT moiety of the molecule.

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