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Domoic Acid: A Teratology and Homeostatic Study in Rats

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In 1987, about 175 victims developed moderate to serious toxicologic symptoms after eating freshly harvested blue mussels (Mytilus edulis L.) cultivated in the eastern estuaries of Prince Edward Island. The symptoms consisted of nausea, vomiting and disorientation, with 3 fatalaties (Iverson et al 1989; Tryphonas et al 1990a). This novel outbreak was attributed to the domoic acid present in the mussels as well as to the presence of the diatom Nitzschia pungens in waters where the mussels were cultivated. The diatom Nitzschia pungens f. multiseries, is a staple of blue mussels and is capable of synthesizing domoic acid (Bird et al 1988; Bates et al 1989). Previous to the 1987 outbreak, domoic acid appears not to have been detected in mussels.

Studies concerning the biologic effects of domoic acid revealed its excitotoxic properties in a number of experimental species in our laboratories (Tryphonas et al 1990 a & b; Tryphonas and Iverson, 1990). This report presents the results of a teratology and a homeostatic study with rats.

MATERIALS AND METHODS

A preliminary teratology study was conducted with toxic mussel extracts containing domoic acid prepared by the method suggested for the bioassay of paralytic shellfish poison (Iverson et al 1989). In a subsequent study, domoic acid was obtained from R.F. Addison (National Research Council, Canada). It was extracted from cooked mussel "meat" which had been contaminated during the fall of 1987, and stored frozen until the spring of 1988. The domoic acid was then "isolated" using a combination of ion-exchange and reverse phase chromatography followed by crystallisation. This product was recrystallised twice from water, and probably contains at least one water molecule of crystallisation. Molar absorbance (assuming one

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H_2O/molecule) at 242 nm was 2.42 x 10^4 AU/mol (Literature value for anhydrous domoic acid is 2.63 x 10^4). Maximum absorbance for the recrystallised domoic acid was at 242 - 243 nm. The melting point was not determined on this sample as the hydrated form rapidly decomposes. The isomer distribution was not determined. Since the results from the two teratology studies were essentially similar, only the latter study is reported. Different concentrations of the twice recrystallised domoic acid were dissolved in bacteriologically sterile distilled water such that a volume of 10 ml/kg body weight of the solution was administered for the different dosages.

Female Sprague-Dawley rats, 175-225 g in body weight, were obtained from Charles River Breeding Laboratories, St-Constant, Québec. They were paired overnight with proven males, and the morning that a positive vaginal smear was observed, was counted as day 1 of gestation. After mating, all females were individually caged with ad libitum access to feed and water.

In the teratology study, 9 to 15 randomly selected mated females, were assigned to the control and each domoic acid-treated group. Domoic acid at dosages of 0.25, 0.5, 1.0, 1.25, 1.75 or 2.0 mg/kg body weight was injected intraperitoneally (ip) on a daily basis from the 7th through the 16th day of pregnancy. The limited quanitity of domoic acid precluded the use of oral route, which otherwise would have been the route of choice. Females were weighed on days 1, 6 through 16 and 22 of gestation. On the 22nd day of gestation, all dams were killed by carbon dioxide anesthesia and the implants were evaluated following routine teratologic procedures. The body weight of the dam was determined without the uterus and conceptuses. About two-thirds of the live fetuses from each litter were examined for fetal development after alizarin red staining. The remainder were fixed in Bouin's fluid and sectioned to study visceral anomalies.

For the homeostatic study, dams had their carotid artery cannulated on day 9 of pregnancy to facilitate blood sampling. On day 11 of pregnancy, groups of 3-5 dams received dosages of 0, 0.5, 1.0 or 1.5 mg of domoic acid (in water)/kg body weight i.p. (10 mL/kg bw). They were then placed in individual metabolic cages for the next 24 hours during which time complete urine samples were collected. In addition, 1 mL blood samples were obtained at 1, 3, 6 and 9 h post dosing. Following the previous methods (Khera, 1991), the blood or plasma samples were analyzed for (i) pH, PO_2, PCO_2 and HCO_3^-, (ii) total hemoglobin and its constituents, oxy-, carboxy-, met- and reduced-hemoglobin content, (iii) osmolality, and (iv) Na^+ and K^+ concentrations. Kidney function was evaluated by determining the

Table 1. Maternal and fetal effects of domoic acid administered intraperitoneally to rats on days 7-16 of pregnancy

	Dose-group (mg/kg bw)					
	Control	0.25	0.50	1.0	1.25	1.75
Number of dams: at term/initiated	12/15	13/14	14/15	14/14	12/13	6/12
Number of live fetuses/litter (Mean ± SE)	14.3 ± 0.7	12.9 ± 0.9	10.6 ± 1.2[a]	11.1 ± 1.2[a]	12.7 ± 0.9	14.2 ± 1.9
Percent: ((Resorptions + dead fetuses ÷ total implants) X 100)	0.6	4.0	2.0	2.5	2.6	8.6
Mean (± SE) fetal weight,	4.9 ± 0.03	4.9 ± 0.04	4.9 ± 0.03	4.8 ± 0.04	4.8 ± 0.04	5.1 ± 0.06
Number: runted fetuses	0	0	2	0	0	1
Number studied for visceral (and skeletal) defects	54 (118)	51 (117)	47(101)	48(108)	46(106)	26(59)
Percent: anomalous/total fetuses	26	31	38	38	46	47
Percent anomalies[b]						
Hydroureter	0	2	2	8	7	0

Table 1 (Cont'd)

	Dose-group (mg/kg bw)					
	Control	0.25	0.50	1.0	1.25	1.75
Kidneys without papilla	0	0	0	4	2	0
Cervical Centra: missing or retarded in ossification	30	35	43	43	50	61
Ribs						
rudimentary on 7th cervical	1	0	1	1	0	2
rudimentary: 13th	2	1	3	0	5	2
14th ribs	0	3	1	0	6	2
wavy ribs	1	1	3	0	1	0
sternebrae: retarded ossification	1	2	4	3	11[a]	0
metacarpals and metatarsals: retarded ossification	9	21	12	15	20	17

[a] $p \leq 0.05$
[b] (Number of fetuses with visceral or skeletal anomalies ÷ total number of fetuses examined) X 100.

volume of urine excreted and analyzing the urine for pH, osmolality, Na^+ and K^+ concentrations.

The arithmetical mean (M) and standard error ($\pm$ SE) were calculated for all monitored parameters. The t-test was used for comparing the treated group with the concurrent control. Fetal values were analyzed using the litter as the basic unit. The proportion ($\underline{p}$) of a litter found to have a particular attribute was calculated and transformed to a normally distributed variable arc sin $\sqrt{\underline{p}}$ value. The M $\pm$ SE of these values or for the test group were then derived. All data were analyzed using a commercial programme prepared for the F and unpaired tests for unequal variances. Only treated and control group differences at $P \leq 0.05$ are reported.

RESULTS AND DISCUSSION

The 2 mg/kg daily dose of domoic acid administered to 9 dams caused death in 6 dams after 2 doses and abortion in the remaining three after 3 doses. An abortifacient effect was also attributed to the 1.75 mg/kg/day dose since six of the 12 test rats were pregnant and aborted prior to the cesarean section. The incidence of non-pregnancy was 3 in control and zero or 1 in each of the remaining test groups (Table 1). No signs of maternal toxicity were observed at doses of up to the 1.25 mg/kg/day. The maternal body weight was measured on day 1, daily on days 6-16, and on day 22 (with or without the uterus and its conceptuses) and there was no significant reduction in body weight gains ($p \leq 0.05$) for any of the dose groups (data not shown).

A reduction in the number of live fetuses per litter was found in the 0.5 and 1.0 mg/kg dose groups (Table 1). However, the reduction was neither dose-dependent, since it was not observed at the 1.25 and 1.75 mg/kg doses, nor associated with any increase in the incidence of resorptions plus dead fetuses. The only effect that was statistically significant ($p \leq 0.05$) was an increased incidence of retarded ossification of the sternebrae in the 1.25 mg/kg group; however, this anomaly, was not considered to be dose-related since there was a zero incidence of this anomaly in the 1.75 mg/kg group. A 6% incidence of a 14th rib in the 1.25 mg/kg dose group (Table 1) was comparable with its 4% incidence in our historical control data. The 0.25 mg/kg dose did not show any statistically significant ($p \leq 0.05$) maternal or fetal toxicity.

The following changes were observed for the homeostatic parameters analysed (data not shown): (i) hyperosmolality of plasma at all test intervals (1, 3, 6 and 9 hr) and increased plasma levels of Na^+ at 1 and 3 h in all treated groups, (ii) high K^+ levels in the plasma at 9 h interval

in all treated groups, (iii) increased PCO_2 values at 3 h in all treated groups and at 9 h in the 1.5 mg/kg group. The immediate response in embryonic development of these alterations was not investigated. However, these changes failed to cause any long-term effect upon fetal development as observed in term fetuses. The remaining parameters, pH, PO_2, HCO_3^- and the total hemoglobin were not significantly altered ($P \leq 0.05$). The values of oxy-, carboxy-, met- and reduced-hemoglobin for all the domoic acid groups were comparable to the control values. Domoic acid caused a dose-related increase in urinary Na^+ concentration, which was statistically significant for the 1.0 and 1.5 mg/kg ($P \leq 0.001$) groups. The urine values for the quantity excreted, pH, osmolality and K^+ concentration were within the control range (data not shown).

In the present reproduction study, a 0.25 mg/kg dose could be considered a no effect level in the mouse, since this dose failed to exhibit a significant ($P \leq 0.05$) effect on the maternal or fetal organism. However, our study alone in a single species is insufficient for extrapolating reproduction effects of domoic acid to humans. Studies in other mammalian species will be required for an adequate estimation of human safety.

REFERENCES

Bates SS, Bird CJ, de Freitas ASW, Foxall R, Gilgan M, Hanic LA, Johnson GA, McCulloch AW, Odense P, Pocklington R, Quilliam MA, Sim PG, Smith JC, Subba Rao DV, Todd ECD, Walter JA and Wright JLC (1989) The pennate diatom *Nitzschia pungens* as the primary source of domoic acid, a toxin in shellfish from Eastern Prince Edward Island, Canada. Can J Fish Aquat Sci 46:1203-1215

Bird CJ, Boyd RK, Brewer D, Craft CA, de Freitas ASW, Dyer EW, Embree DJ, Falk M, Flack MG, Foxall RA, Gillis C, Greenwell M, Hardstaff WR, Jamieson WD, Laicock MV, Leblanc P, Lewis NI, McCulloch AW, McCully GK, McInterney-Northcott M, McInnes AG, McLachlan JL, Odense P, O'Neil D, Pathak V, Quilliam MA, Ragan MA, Seto PF, Slim PG, Tappen D, Thibault P, Water JA, and Wright JLC (1988) Identification of domoic acid as the toxic agent responsible for the P.E.I. contaminated mussel incident: A summary of work conducted at the Atlantic Research Laboratory of the National Research Council, Halifax, between 13 December 1987 and 11 January 1988. Atlantic Research Laboratory Technical Report No. 56, NRCC No 29083.

Iverson F, Truelove J, Nera E, Tryphonas L, Campbell J and Lock E (1989) Domoic acid poisoning and mussel associated intoxication. Preliminary investigation into the response of mice

and rats to toxic mussel extracts. Food Chem Toxicol 27:377-384

Khera KS (1991) Chemically induced alterations in maternal homeostasis and histology of conceptus: their etiologic significance in rat fetal anomalies. Teratology 44:259-297

Tryphonas L and Iverson F (1990) Neuropathology of excitatory neurotoxins: the domoic acid model. Toxicol Pathol 18:165-169

Tryphonas L, Truelove J, and Iverson F (1990b) Acute parenteral neurotoxicity of domoic acid in cynomolgus monkeys (M. fascicularis). Toxicol Pathol 18:297-303

Tryphonas L, Truelove J, Nera E, and Iverson F (1990a) Acute neurotoxicity of domoic acid in the rat. Toxicol Pathol 18:1-9