Bull. Environ. Contam. Toxicol. (1994) 52:479–486

Environmental Contamination and Toxicology

Serum Domoic Acid Clearance and Clinical Observations in the Cynomolgus Monkey and Sprague-Dawley Rat Following a Single IV Dose

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Received: 13 April 1993/Accepted: 1 August 1993

An outbreak of food poisoning in Canada in 1987 originated from the consumption of cultured blue mussels that contained high concentrations (960-1280 ug/g) of the potent neuroexcitatory amino acid, domoic acid (Iverson et al. 1989; Quilliam and Wright 1989). Surveillance mechanisms now established in Canada prevent the marketing of mussels containing more than 20 ug/g of domoic acid and there have been no reports of intoxication since the original episode. However, recent reports have shown that domoic acid is now present in razor clams and crabs taken from the West Coast of the United States. In addition severe signs of neurotoxicity and death were reported in brown pelicans after consuming domoic acid contaminated anchovies (Work et al. 1991). Although estimates of the total ingested dose of domoic acid among affected Canadian patients ranged from 60 to 290 mg, domoic acid was not detected in samples of blood, serum or cerebrospinal fluid acquired at least 2 days after the onset of symptoms (Perl et al. 1990). Since all of the most severely affected patients were aged and some had preexisting medical complications (eg. renal disease, hypertension) it seems likely that their condition exacerbated the toxic effect of domoic acid. Preston and Hynie (1991) reported that domoic acid concentrations were elevated in the plasma and brain of nephrectomized rats suggesting that compromised excretion can be an important factor in domoic acid poisoning.

Previous studies have shown that the monkey is considerably more sensitive to the overt effects of orally administered domoic acid than is the rodent (Iverson et al. 1990) and more closely mimics the human response. While dose-response characteristics and some excretion data for both animal models are available (Iverson et al. 1990; Tasker et al. 1991; Tryphonas et al. 1990), little comparative data exists with respect to pharmacokinetic parameters.

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The present studies were therefore undertaken to determine several basic pharmacokinetic parameters at doses that produce overt clinical signs in both rats and monkeys.

MATERIALS AND METHODS

The jugular vein and carotid artery of six female Sprague-Dawley rats (mean weight ± standard deviation, 240.9 ± 10.3 g) were chronically cannulated and exteriorized through the dorsal scapular region. Rats were allowed to recover from the surgery for at least two days prior to testing during which time cannula patency was maintained with heparinized saline (10 IU/mL). Domoic acid (Diagnostic Chemicals Ltd.) dissolved in saline was administered as a bolus dose through the jugular cannula at doses of 500 ug/kg body weight (2 rats) and 1000 ug/kg body weight (4 rats). Seven blood samples of approximately 0.3 mL were collected from the carotid cannula at 2 to 100 min following the dose and the serum was separated and frozen until analyzed for domoic acid (preliminary studies carried out in this laboratory established that domoic acid was present entirely in the serum or plasma fraction and that plasma protein binding was negligible). Urine samples were not collected. Rats were observed for signs of domoic acid toxicity for 24 hr following dosing.

Four female cynomolgus monkeys (mean weight ± standard deviation, 3.70 ± 0.6 kg and estimated ages 14, 7, 12 and 14 years for monkey numbers 117, 332, 20, 191 respectively) were dosed intravenously using the radial vein with 50 ug/kg body weight domoic acid dissolved in saline. The dose was delivered as a bolus in a total volume of 1 mL. Blood samples of approximately 2 mL were acquired by femoral venipuncture at various time periods up to 240 min post-dosing. One monkey had four samples taken while at least six samples were acquired from the other three monkeys. Serum was separated from the blood samples and stored frozen until analyzed for domoic acid. Urine was collected when available and stored as for blood. The monkeys were observed for 24 hr following dosing for overt signs of domoic acid toxicity.

Serum samples were analyzed for domoic acid in triplicate by radioimmunoassay (Newsome et al. 1991). The limit of detection for this method was approximately 50 ng/mL for serum and 150 ng/mL for urine.

The pharmacokinetic characteristics of domoic acid were determined for each animal using a two-compartment model and by model independent methods. Coefficients of the biexponential serum concentration-time curve were determined by linear regression of the log residual

concentration-time curve (α phase) and linear regression of the terminal portion of the log concentration-time curve (β phase). Area under the concentration-time curve (AUC) and area under the first moment of concentration-time curve (AUMC) were calculated by the trapezoidal rule and corrected by extrapolation to infinite time. Parameters determined were half-life ($t_{½}\alpha$,$t_{½}\beta$), apparent volume of distribution (Vd), clearance (Cl), apparent volume of distribution at steady state (Vss) and mean residence time (MRT).

RESULTS AND DISCUSSION

Elimination of domoic acid from serum was biphasic in both species and was adequately described by the equation for a two compartment model. The serum concentration-time curves are shown in fig.1 (rats) and fig.2 (monkeys). The pharmacokinetic parameters determined are shown for rats in table 1 and monkeys in table 2. The domoic acid concentrations found in the monkey urine samples expressed as percent of dose are shown in fig. 3. Domoic acid was not detectable in the 24 hr sample from monkey 332 and no urine sample was available from monkey 117. After 24 hr, the portion of the dose accounted for was 90, 67 and 63 percent for monkeys 332, 191 and 20 respectively.

The effect of domoic acid observed in the 1000 ug/kg rats included scratching with the ipsilateral hind foot alternating between the left and right sides (rats 3,4,6), chewing (rat 6), and an epileptiform seizure (rat 5) which involved salivation, head jerking, rearing up on the hind legs and tremors. Scratching in all 3 cases occurred at approximately 50 min following dosing and lasted for 80, 49 and 91 minutes respectively for rats 3, 4 and 6. No other signs were observed after scratching ceased. The severe seizure activity exhibited by rat 5 occurred at 18 min following dosing and lasted for approximately 5 min after which no further signs of overt toxicity were observed. One of the rats (rat 2) dosed with 500 ug/kg showed no response while the other (rat 1) had a slight seizure at 20 min following dosing.

All monkeys exhibited a period of gagging and vomiting with onset times and durations as indicated in table 2. No other overt signs of toxicity were observed.

The dose administered to the monkeys in the present experiment was selected so that clear signs of domoic acid poisoning (vomiting) would be observed. It has previously been shown that domoic acid is capable of damaging the area postrema which includes the vomit center (Tryphonas et al. 1990) and that vomiting is the

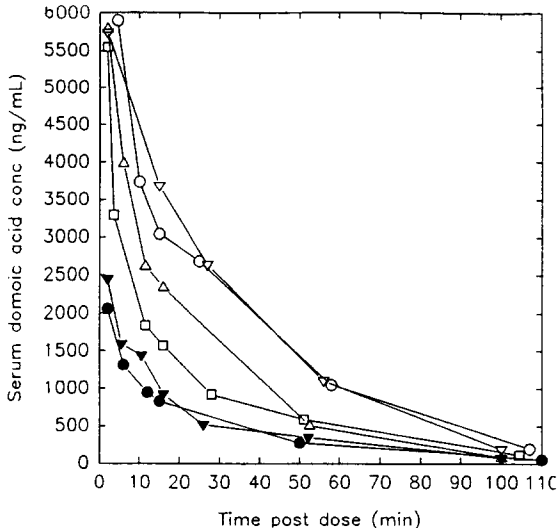

Figure 1. Serum domoic acid concentrations in Sprague-Dawley rats following a single intravenous dose of domoic acid (filled symbols, 500 ug/kg; hollow symbols, 1000 ug/kg). Rat# 1 (●), 2 (▼), 3 (○), 4 (▽), 5 (□), 6 (△).

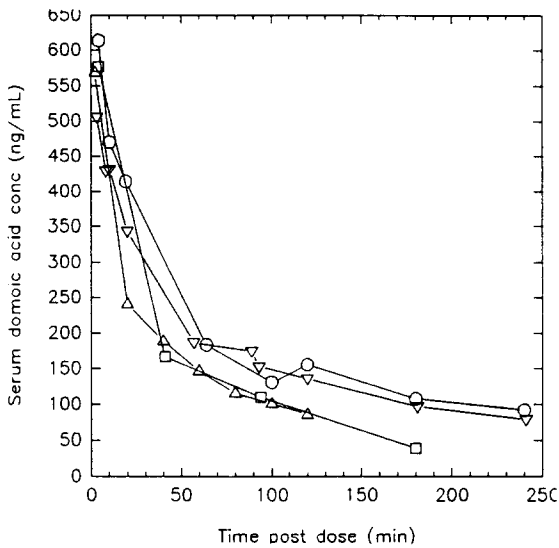

Figure 2. Serum domoic acid concentrations in cynomolgus monkeys following a single intravenous dose of domoic acid (50 ug/kg). Monkey# 117 (▽), 332 (△), 20 (○), 191 (□).

most sensitive clinical sign in the monkey (Iverson et al. 1990). Iverson et al. (1990) demonstrated that a dose of 50 ug/kg caused an episode of gagging and vomiting

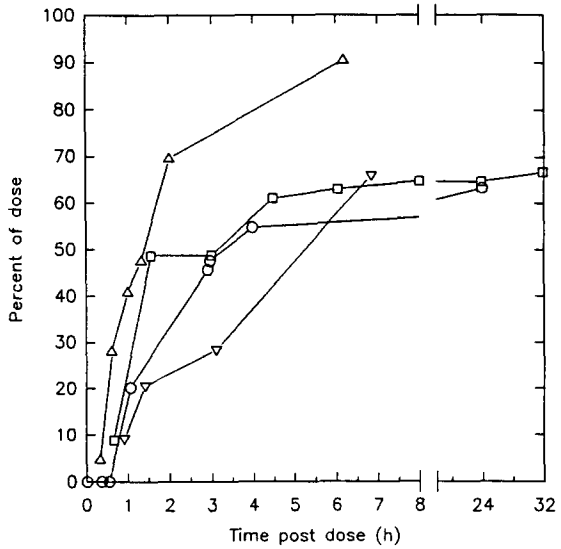

Figure 3. Urine domoic acid concentrations expressed as cumulative percent of dose from cynomolgus monkeys following a single intravenous dose of domoic acid (50 ug/kg). Monkey# 117 (∇), 332 (Δ), 20 (○), 191 (□).

that lasted approximately 40 min whereas 12.5 ug/kg caused only a slight gag response and 6.25 ug/kg produced no overt effect. In the present experiment the duration of response ranged from 9 to 62 min and corresponded with serum domoic acid concentrations greater than approximately 180 ng/mL.

Although pharmacokinetic parameters varied between monkeys, it is apparent that domoic acid was excreted more rapidly in the urine of monkeys that exhibited the shortest duration of effect. At 90 min following the dose, monkeys 191 and 332 excreted approximately 45 and 52 % of the dose respectively, whereas monkeys 117 and 20 excreted only approximately 20 and 25 % of the dose. The between-monkey variation remains unexplained but may well be typical for this species.

Preliminary studies in the rat, using a dose equivalent to that given to the monkeys (50 ug/kg), elicited no observable response and resulted in serum concentrations below the detection limit of the assay with the exception of the initial sample. With the higher doses used in the present experiment the response was variable. At a dose of 500 ug/kg rat 1 exhibited seizure activity whereas rat 2 was unaffected. When dosed with 1000 ug/kg, rat 5 developed severe seizures whereas the other three rats developed only the domoic acid scratch response. The seizures in the most severely affected rats (1 and 5) occurred within 20 min of dosing during which time domoic

Table 1. Pharmacokinetic parameters for domoic acid in rats following a single IV bolus dose.

Rat#	Dose ug/kg	β	$t_{1/2}\beta$ min	AUC ug/mL/min	Vd mL/kg	Cl mL/min/kg	Vss mL/kg	MRT min
1	500	0.029	23.6	46.8	363.1	10.7	321.0	30.0
2	500	0.031	22.1	51.6	309.5	9.7	301.7	31.1
Mean		0.030	22.9	49.2	336.3	10.2	311.4	30.6
3	1000	0.030	23.0	170.3	195.0	5.9	205.9	34.9
4	1000	0.035	19.6	186.7	151.7	5.4	160.4	29.7
5	1000	0.020	24.1	89.6	388.7	11.2	357.3	31.9
6	1000	0.041	17.1	116.2	212.0	8.6	191.8	22.3
Mean		0.032	21.0	140.7	236.9	7.8	228.9	29.7
SD		0.009	3.2	45.5	104.4	2.7	87.7	5.4

Table 2. Pharmacokinetic parameters and clinical data for vomiting in monkeys following a single IV bolus dose of domoic acid.

Monkey#	Dose ug/kg	α	$t_{1/2}\alpha$ min	β	$t_{1/2}\beta$ min	AUC ug/mL/min	Vd mL/kg	Cl mL/min/kg	Vss mL/kg	MRT min	Vomiting Onset min	Vomiting Duration min
117	55	0.046	15.2	0.0049	140.5	55.6	201.8	1.0	189.3	189.3	14	23
332	50	-	-	0.0104	66.7	29.2	160.8	1.7	154.2	90.7	9	9
20	50	0.041	16.7	0.0037	185.6	67.8	199.4	0.7	167.2	238.8	2	62
191	50	-	-	0.0106	65.3	28.3	166.6	1.8	152.3	84.6	8	11
Mean		0.044	16.0	0.0074	114.5	46.1	178.3	1.25	159.3	148.8		
SD				0.0036	59.0	18.7	26.0	0.48	28.8	78.3		

acid serum levels had declined to approx 700 and 1300 ng/mL respectively. The scratch response in rats 3,4 and 6 did not occur until 50 min after dosing at which time serum levels of domoic acid had declined to approximately 1400, 1400 and 600 ng/mL respectively.

The most severely affected animals (rats 1 and 5 and monkeys 117 and 20) had a greater Vss than the others, suggesting that domoic acid was more widely distributed in these particular animals. However, in contrast to the rats the MRT was much longer in the most severely affected monkeys(117 and 20).

Despite the between-animal variability, the relative lack of sensitivity of the rat is consistent with the short MRT and rapid clearance of the toxin in this species compared to that seen in the monkey. It is important to note however, that the overt clinical signs presented by the two species originate from different areas of the brain. The acute behavioral effects noted in the rat (scratching, seizures) originate from areas of the brain that lie within the blood brain barrier (ie. hippocampus) while the primate vomit center exists within the area postrema which is external to the blood brain barrier. The rodent area postrema may be effected as well but because rats cannot vomit, no response is observed using our methods. These differences (MRT, target organ) probably account for the reduced sensitivity of the rat.

It is impossible to rule out the potential for crossreactivity of the domoic acid antibody used in the radioimmunoassay with compounds other than domoic acid, in particular, possible domoic acid metabolites. However, present data indicates that there is no significant domoic acid metabolism in rats (Suzuki 1993). In addition, unpublished data from this laboratory indicated that the HPLC determination of the domoic acid concentration in the urine from a treated monkey remained identical with that of an acid or base hydrolysed sample, providing evidence that no domoic acid conjugates were present in the urine. The radioimmunoassay used in the present experiment provided the necessary sensitivity that could not be achieved by HPLC.

In view of the short half life of domoic acid in both rats and monkeys, it is not surprising that domoic acid was not detected in the blood of humans two days after the ingestion of highly contaminated mussels during the 1987 Canadian domoic acid poisoning incident. At that time the available methodology was capable of detecting only about 1 ug/mL as a lower limit, while the RIA method used in the present study had a lower limit of 50 ng/mL of serum. Even with the increased sensitivity, serum levels of domoic acid are below the detection limit in

primates by about four hours after dose administration. Further research in the areas of tissue distribution, absorption and factors affecting individual variability are required to explain the human situation.

Acknowledgments. The authors appreciate the assistance of Mr. Leigh Martin, Mrs. Cheryl Armstrong and the staff of the Animal Resources Division.

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