Repeated Independent Exposures to Domoic Acid Do Not Enhance Symptomatic Toxicity in Outbred or Seizure-Sensitive Inbred Mice

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Received December 18, 1996; accepted August 1, 1997

Domoic acid (DA) is an environmental neurotoxin to humans. This work examines whether repeated exposure to subsymptomatic or symptomatic nonlethal doses of domoic acid leads to enhanced symptomatic toxicity in ICR outbred and DBA inbred strains of laboratory mice. A multiple independent exposure paradigm was designed in which doses were administered intraperitoneally every other day for 7 days to achieve four separate exposures to domoic acid. We first examined the effect of repeated exposure on serum clearance of domoic acid. Serum domoic acid levels did not differ following a single or repeated exposure. We next examined the effect of repeated exposure on symptomatic toxicity. The mean toxicity scores did not show a significant difference between single and repeated exposures of either subsymptomatic (0.5 mg/kg) or symptomatic sublethal (2.0 mg/kg) doses of domoic acid. We then examined the effects of repeated domoic acid exposure on a second strain of mouse. DBA mice were chosen based upon their sensitivity to kainic acid-induced seizures; however, the ICR mice were more sensitive to low-dose domoic acid toxicity, particularly in terms of onset and duration of stereotypic scratching behavior. Our results indicate that both strains of mice have comparable concentration-dependent toxic responses to domoic acid; however, differences exist in the magnitude of the response and in specific symptoms. The mean toxicity scores did not show a significant difference when a single exposure (1.0 and 2.0 mg/kg domoic acid) and repeated exposure of the same dose were compared in the DBA mice. This study provides no evidence that short-term repeated exposure to domoic acid in laboratory mice alters domoic acid clearance from the serum, or leads to a more sensitive or a greater neurotoxic response.

Domoic acid, a tricarboxylic amino acid, has been identified as the toxin responsible for amnesic shellfish poisoning (Wright et al., 1989). Systemic exposure to domoic acid has been shown to elicit neuroexcitatory effects, including stereotypic behavior and seizures in rats and mice (Tasker et al., 1991; Tryphonas et al., 1990a, b; Tryphonas and Iverson, 1990). Histological analysis using c-fos and cupric silver impregnation indicated that domoic acid produces biochemical changes and neuronal death in the hippocampus and other limbic structures (Peng et al., 1997). Domoic acid exposure leads to gastrointestinal disturbances and, at higher doses, anterograde memory deficit. In the 1987 amnesic shellfish poisoning incident, only individuals aged 65 or older and individuals with preexisting illnesses were subject to severe toxic effects (Perl et al., 1990). Compromised renal clearance of the toxin was suggested to be a common predisposing factor to enhanced toxicity. Rapid clearance of domoic acid has been established to limit toxicity to laboratory animals (Suzuki and Hierlhy, 1993). More recently, neonates have been defined as a potential high-risk group, and reduced clearance of the toxin appears to be a major predisposing factor in their high susceptibility to domoic acid toxicity (Xi et al., 1997). Another potential high risk group are those individuals exposed to repeated low or subsymptomatic doses. Such groups may include populations subsistent on fishery resources. The basis for enhanced toxicity of this population could be through altered serum clearance, biochemical sensitivity, or neuronal damage.

In the present study, we evaluated existing data to establish conditions for repeated independent exposures in laboratory mice. We designed the interval between the doses to be sufficient to allow clearance of the toxin and no residual observable biochemical or behavioral effects. This design allowed the assessment of the effects of independent exposures of domoic acid. Domoic acid exposures were chosen to include the highest nonlethal dose to cause observable effects, lowest dose to cause observable effects, and highest dose not to cause observable effects. We then utilized this treatment protocol to examine whether repeated exposure leads to alterations in serum clearance of domoic acid and enhanced toxicity in ICR outbred mice using the domoic acid toxicity scoring method of Tasker et al. (1991). In addition, we examined the toxic effects of single and repeated doses of domoic acid in the DBA strain of mouse. The DBA mouse is an inbred strain, that has been used previously to assess memory impairment following domoic acid exposure.
(Sutherland et al., 1990) and a strain that is sensitive to kainic acid-induced seizures (Ferraro et al., 1995).

**EXPERIMENTAL PROCEDURES**

**Experimental animals.** All studies used female ICR (18-25 g) or DBA mice (20–30 g) obtained from Harlan Sprague Dawley, Inc. (Indianapolis, IN). Mice were housed in groups (ICR) or individually (DBA) with food and water available *ad libitum* and were maintained on a 12-hr light–dark cycle.

**Serum level of domoic acid using radioreceptor assay.** ICR mice were anesthetized with sodium pentobarbital (40 mg/kg ip) at 1 or 2 hr following intraperitoneal administration of vehicle or domoic acid, and then blood was collected by cardiac puncture. Domoic acid was administered at 0.5, 1.0, or 2.0 mg/kg, ip. Intraperitoneal administration was chosen, due to accuracy of delivery and to correspond with the existent *in vivo* data sets for domoic acid toxicity; 2.0 mg/kg is the highest nonlethal dose in mice and 0.5 mg/kg causes the lowest observable effect (Peng and Ramsdell, 1996). Domoic acid levels were determined by heterologous radioreceptor assay as previously described (Van Dolah et al., 1994). Assays were carried out in 96-well polystyrene plates in 50 nm Tris-HCl, pH 7.4, containing 1.5 mg frog brain synaptosomes, 5 nM 11H-kainic acid (New England Nuclear), and the serum sample in a total volume of 210 μl. Standard calibration solutions (National Research Council, Canada) contained 10 pm to 1 μM domoic acid. Following incubation of samples at 4°C for 1 hr, all wells were filtered onto a 96-grid glass filter mat using a 96-place filtration manifold (Millibiot, Millipore) and rinsed four times with ice-cold Tris buffer (50 mM, pH 7.4). The filter mat was dried on a slide warmer (60°C). The standard curve was derived by a four-parameter logistic fit of standard curve and unknown sample concentrations were calculated from the linear portion of this fit. Biological samples have a background activity due largely to the presence of glutamate (van Dolah et al., 1997). In serum this activity is approximately 0.4 μg/ml. All assays include a serum blank that is subtracted from experimental samples.

**Behavioral rating scale for domoic acid toxicity.** Each mouse was assigned the score shown in parentheses: hypoactivity (1), sedation (2), hyperactivity (3), scratching (4), loss of balance control (5), tremors—convulsions (6), and death (7). These domoic acid symptoms were monitored in the mice for 60 min by the same observer under blind conditions and the time of onset and duration of each symptom recorded. A score was calculated as the sum of each behavioral change quantified by the product of the duration (minutes) of the symptom and the assigned score.

**Protocol for repeated independent exposure to domoic acid.** We determined the frequency of exposures that would elicit independent effects in order to minimize interactive effects due to the presence of residual toxin. Clearance studies have indicated that domoic acid is rapidly eliminated from the body by renal filtration. Studies conducted in ICR mice indicate that within 2 hr domoic acid is eliminated by more than 93% (Peng and Ramsdell, 1996). Experiments on domoic acid induction of c-fos in ICR mice indicate that the mRNA expression returns to baseline within 2 hr; however, the Fos protein is expressed for 24 hr (Peng et al., 1994). Studies with rats indicate that the observable behavioral responses continue for about 24 hr as well (Tryphonas et al., 1990). Based upon these data, we chose to separate exposures by 48 hr. Animals were therefore given four doses separated by 48 hr. Control animals received either four injections of vehicle ( PBS) or three injections of vehicle and a single dose of domoic acid for the last treatment.

**Data analysis.** Comparisons between control and any of the treatment groups for serum domoic acid and toxicity scores were analyzed by Student’s *t* test. For multiple groups, an analysis of variance (ANOVA) was used, and *p* < 0.05 was considered to be statistically significant between groups.

**RESULTS**

**Determination of Subsymptomatic and Nonlethal Symptomatic Doses of Domoic Acid**

ICR mice were administered three different ip doses of domoic acid ranging from 0.5 to 2.0 mg/kg and toxicity was determined using the rating scale of Tasker et al. (1991). Using data for the duration and severity of observable behavioral changes, we calculated toxicity scores ranging from 50 ± 25 to 281 ± 24 with the increased doses (mean ± SE, *n* = 4). The effect at 0.5 mg/kg was not significantly different from controls and was taken as the subsymptomatic effect, and 2.0 mg/kg chosen as a strong symptomatic sublethal dose.

**Serum Levels of Domoic Acid in Single and Repeated Exposure**

Using a domoic acid radioreceptor assay, we determined the serum concentration of domoic acid at 60 and 120 min following a single and repeated dose ip injection. ICR mice were intraperitoneally administered with a subsymptomatic (0.5 mg/kg) or sublethal symptomatic (2.0 mg/kg) dose in either repeated doses (every other day ip for four times in 7 days) or a single dose on the same day of the last multiple exposure dose. Serum domoic acid levels did not differ at 60 min in a single (0.59 ± 0.04 μg/ml) and multiple (0.50 ± 0.05 μg/ml, mean ± SE, *n* = 8, *p* > 0.05) exposures, or at 120 min (single 0.124 ± 0.005 μg/ml, multiple 0.123 ± 0.004 μg/ml, respectively, *n* = 7). These results failed to indicate that previous exposures in mice to domoic acid alters the clearance of domoic acid administered in subsequent exposures.

**Observable Behavioral Responses to Single and Multiple Domoic Acid Exposures in ICR Mice**

We next examined the toxicity scores for single and repeated domoic acid exposures in ICR mice. Two different doses of domoic acid were administered (ip) in either multiple exposures (every other day ip for four times in 7 days) or a single exposure on the same day of the last multiple exposure dose to ICR mice (0.5 and 2.0 mg/kg, *n* = 4/group), and observable behavioral changes for each animal were recorded as described above. Scores of 15 ± 2 (exposure 1) and 12 ± 2 (exposure 4) were determined for 0.5 mg/kg domoic acid, and 197 ± 15 (exposure 1) and 175 ± 6 (exposure 4) were determined for 2.0 mg/kg domoic acid.
FIG. 1. Behavioral changes in ICR mice in response to multiple domoic acid exposures. Mouse behavioral changes were recorded following two different doses of domoic acid intraperitoneally (ip) administered in 100 μl 0.9% saline, pH 7.4, in either multiple exposures (every other day ip for four times over 7 days) or a single exposure on the same day of the last multiple exposure dose to ICR mice (0.5 and 2.0 mg/kg, n = 4/group). The control mice received an equal amount of saline vehicle only and had a mean score 12 ± 7. Results presented are mean ± SE per dose treatment.

FIG. 3. Behavioral changes in DBA mice in response to multiple domoic acid exposures. Mouse behavioral changes were recorded following two different doses of domoic acid intraperitoneally (ip) administered, in either multiple exposures (every other day ip for four times over 7 days) or a single exposure on the same day of the last multiple exposure dose to DBA mice (1.0 and 2.0 mg/kg, n = 9/group). The control mice received an equal amount of saline vehicle only. Results presented are mean ± SE per dose treatment.

Observable Behavioral Responses of DBA Mice to Domoic Acid

Three different ip doses of domoic acid ranging from 0.5 to 2.0 mg/kg were tested in DBA mice (n = 8/group). The same conditions were followed as outlined for ICR mice. Toxicity scores ranging from 49 ± 13 to 211 ± 38 were obtained (mean ± SE, n = 8) (Fig. 2). We found that ICR mice showed a greater magnitude of response in toxicity scores than DBA mice at a dose of 1.0 mg/kg (p < 0.001) due in part to differences in stereotypic scratching behavior. Both the onset of scratching (ICR and DBA: 17 ± 2.9 and 37 ± 10 min, respectively, p < 0.05) and the duration of scratching (ICR and DBA: 42 ± 4.8 and 20 ± 8.4 min, respectively, p < 0.05) lead to lower scores in the DBA mice. We next examined the toxic response to repeated exposures of domoic acid in DBA mice.

Observable Behavioral Responses to Single and Multiple Domoic Acid Exposures in DBA Mice

Two different doses of domoic acid were (ip) administered to DBA mice as described earlier (1.0 and 2.0 mg/kg, n = 9/group), and observable behavioral changes for each animal were recorded. For DBA mice, scores of 41 ± 13 (exposure 1) and 51 ± 5 (exposure 4) were calculated for 1.0 mg/kg domoic acid, and 146 ± 18 (exposure 1) and 126 ± 16 (exposure 4) for 2.0 mg/kg domoic acid (Fig. 3). These observations provide no indication that repeated domoic acid exposure causes further deleterious effects in the DBA strain.

DISCUSSION

Domoic acid, the causative agent of amnesic shellfish poisoning, is a potent neurotoxin that causes gastrointestinal distress and, in more severe intoxications, anterograde memory deficit. Subjects with impaired renal clearance have been defined as a high-risk group to domoic acid toxicity. In this work we have examined whether subjects receiving prior domoic acid exposures may pose a greater hazard to subsequent exposures. Our results using a laboratory mouse model fail to indicate that repeated independent exposures alter
serum clearance or enhance the sensitivity or extent of symptomatic toxicity to domoic acid. Domoic acid is cleared rapidly from the serum of primates and rodents (Truelove and Iverson, 1994). The clearance is almost complete by 2 hr and appears to result largely from passive renal filtration. Surgical or pharmacological disruption of renal clearance of domoic acid (Preston and Hynie, 1991; Robertson et al., 1992; Suzuki and Hierlihy, 1993) increases domoic acid toxicity, indicating that rapid clearance is critical in limiting the toxic effects of domoic acid. Human and neonatal rat studies indicate that reduced clearance is a major predisposing factor to high susceptibility to domoic acid toxicity (Perl et al., 1990; Xi et al., 1997). In this report, we examined whether repeated exposure to domoic acid affects the clearance of the toxin. We found that domoic acid was cleared to the same extent following one or four exposures. These results indicate that domoic acid does not alter renal clearance mechanisms.

Repeated exposure to subthreshold or symptomatic nonlethal doses of domoic acid did not cause increased sensitivity or enhanced extent of symptomatic toxicity. Although the initial action of domoic acid is at kainic acid and AMPA selective receptors (Stewart et al., 1990; Hampson et al., 1992), the neuroexcitatory effects of domoic acid likely require coordinate responses by a number of ionic mechanisms. Domoic acid increases intracellular calcium by activation of voltage-dependent calcium channels (Xi and Ramsdell, 1996) and the pronounced neuroexcitatory effect in vivo requires coincident activation of NMDA receptors (Xi et al., 1997). Studies conducted by Dakshinamurti's group indicate that both glutamic acid decarboxylase and glutamate synthase activities are reduced in domoic acid-treated mice (Dakshinamurti et al., 1991, 1993). These two enzymes are involved in maintaining homeostasis of γ-aminobutyric acid (GABA) and glutamate in the brain. Inhibition of these enzymes leads to reduced levels of the inhibitory neurotransmitter GABA and increased levels of the excitatory neurotransmitter glutamate. Persistent levels of domoic acid in brain tissue or its persistent effects on any of these enzymes or receptors would be anticipated to alter the sensitivity to subsequent exposures of domoic acid. The data from this report indicate that the symptomatic changes occurring following sublethal domoic acid exposure are fully reversible within a 48-hr period.

This study has determined toxicity scores in response to either a single or repeated exposure of domoic acid in two strains of mice. Our results indicate that repeated exposures to the highest nonlethal dose to cause observable effects, the lowest dose to cause observable effects, and the highest dose not to cause observable effects fail to enhance toxicity beyond the effects observed with a single dose. These studies used two strains of mice (ICR and DBA). Toxicity scores for a single domoic acid exposure were greater in the ICR mice, an effect due in part to an earlier onset and longer duration of stereotypic scratching behavior. This finding suggests that some strain-dependent differences exist for specific domoic acid-induced symptoms. This observation is consistent with existing data for strain differences to kainic acid in rodents (Golden et al., 1991). Subsequent studies have demonstrated that the strain difference to kainic acid-induced seizures in DBA and C57BL mice does not result from differences in the levels of kainic acid that reach the brain (Ferraro et al., 1995). Taken together, these results indicate that genetic differences in susceptibility exist for the toxic actions of domoic acid and kainic acid. Nonetheless, our results indicate that strain differences do not exist for DBA and ICR mice in terms of increased susceptibility or enhanced toxicity to repeated exposures of domoic acid. This study indicates that repeated independent exposures of subsymptomatic or sublethal symptomatic doses of domoic acid to laboratory mice fails to enhance observable behavioral toxicity. These results suggest that each exposure to low doses of domoic acid causes an independent and noninteractive effect.

ACKNOWLEDGMENT

This work was supported by the National Oceanic and Atmospheric Administration.

REFERENCES


