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Effect of Chlorpromazine on the Toxicity in Mice of the Venoms and Neurotoxins from Various Snakes

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Running Title: **Chlorpromazine & Snake Venoms**

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Abbreviation: PBS, phosphate-buffered saline.

ABSTRACT

I determined the efficacy of chlorpromazine with respect to reducing the toxicity in mice of *Bungarus caeruleus* venom, *Bungarus multicinctus* venom and its neurotoxic components α-bungarotoxin and β-bungarotoxin; *Crotalus durissus terrificus* venom and its neurotoxic component crotoxin; and *Oxyuranus scutellatus* venom and its neurotoxic component taipoxin. Venom or toxin was administered *i.p.*, followed immediately by an *i.p.* injection of chlorpromazine (1 mg/kg). The effect of chlorpromazine on lethality caused by the venom or toxin was recorded 24 hr later. Chlorpromazine proved to be an effective antagonist of the toxicity of *B. caeruleus* venom, *B. multicinctus* venom, and β-bungarotoxin without itself being overtly toxic. It increased the LD_{50} of *B. caeruleus* venom, *B. multicinctus* venom, and β-bungarotoxin by 8.7-, 2.6-, and 3.8-fold, respectively, while having no effect on the other venoms or toxins. Chlorpromazine was also injected at different times both before and after the injection of venom or toxin. Protection from lethality was maximal when chlorpromazine was administered immediately after injection of venom or toxin.

Keywords: snake venom; toxins and antitoxins; ( )

Antivenoms are the pharmacological agents currently used for treatment of intoxication due to snake venoms. Several factors, however, limit their usefulness. A given antivenom is effective against the venom from only a small number of species of snakes. In addition, some people are hypersensitive to antivenoms. Finally, antivenoms require refrigeration and are expensive --- two factors that limit their availability, especially in less affluent regions of the world. Treatment of snake venom intoxication would be greatly enhanced if a drug could be found which would overcome these deficiencies of antivenoms.

Several potent snake venoms contain neurotoxins that constitute the most lethal components of the venom. The best studied of these venoms is that of the Formosan krait *Bungarus multicinctus*, which is the most poisonous snake in Taiwan, and whose bites have resulted in a 23% mortality rate (Kuo and Wu, 1972). The venom of *B. multicinctus* contains two neurotoxins that contribute to its toxicity. α-Bungarotoxin is a postsynaptic neurotoxin which binds to the nicotinic acetylcholine receptor at the neuromuscular junction and blocks muscle contraction stimulated by acetylcholine. β-Bungarotoxin is a presynaptic neurotoxin which inhibits the release of acetylcholine from neurons, also blocking muscle contraction (Chang, 1985). The two toxins work in concert to cause respiratory failure, which is the ultimate cause of death due to the venom of *B. multicinctus* . β-Bungarotoxin is the most toxic component of *B. multicinctus* venom and the most investigated of the snake presynaptic neurotoxins, making it useful to study in conjunction with the venom. Other snakes such as *Bungarus caeruleus* (Indian krait), *Crotalus durissus terrificus* (South American rattlesnake), and *Oxyuranus scutellatus* (taipan) have venoms with presynaptic neurotoxins that also block the release of acetylcholine from neurons (Chang, 1985).

Although the mechanism of action of the presynaptic neurotoxins of snake venoms is unknown, it is possible that they act through a phosphatidate 2-acylhydrolase (EC 3.1.1.4) (trivial name: phospholipase A_2) activity (Chang, 1985). Chlorpromazine has been shown to be an effective inhibitor of mammalian phospholipase A_2 activity (Franson *et al.*, 1983; Jain *et al.*, 1984; Jain and Jahagirdar, 1985; Taniguchi *et al.*, 1988) and therefore may be an effective antagonist of the toxicity of snake presynaptic neurotoxins and of the venoms of which they are a part.

Materials and Methods

Materials. *Bungarus caeruleus*, *B. multicinctus*, *C. durissus terrificus*, and *O. scutellatus* venoms and α-bungarotoxin, β-bungarotoxin, and crotoxin were purchased from Miami Serpentarium Laboratories, Salt Lake City, UT. Taipoxin was the gift of Dr. John Middlebrook of this institution. Lyophillized venoms and toxins (except crotoxin) were dissolved (1 mg/ml) in deionized water. Lyophillized crotoxin was dissolved (1 mg/ml) in 10 mM sodium phosphate, 10 mM ammonium acetate, pH 4.0; centrifuged at 3000 x g for 5 min; and the pellet discarded. Aliquots of venom and toxin solutions were stored at -20 °C and were not refrozen after thawing. Further dilution of all venoms and toxins was performed using gel-phosphate buffer (0.2% gelatin, 0.4% sodium phosphate, pH 6.2). Chlorpromazine hydrochloride was purchased from Sigma Chemical Co., St. Louis, MO. All references to chlorpromazine imply the hydrochloride salt. Chlorpromazine was dissolved in 150 mM sodium chloride, 6 mM sodium phosphate (pH 7.2) (PBS).

Methods. Female ICR mice (20-30 g; Harlan Sprague-Dawley, Inc., Frederick, MD) were housed 5 per cage, maintained on a 12 hr light-dark (1800 - 0600) cycle, and allowed free access to food and water. The mice were injected *i.p.* with the venom or toxin of interest in gel-phosphate, followed by an *i.p.* injection of either PBS (control) or chlorpromazine in PBS. All doses, expressed per kg mouse, were adjusted for the weight of the animal, and were administered in a volume of 10 ml/kg. Almost all of the animals that survived 24 hr after the injection of venom or toxin recovered fully, and the number of animals that died within 24 hr was used as the measure of toxicity. Each experiment was repeated (sometimes with different, but overlapping doses), and the data from the repeated experiments were combined. Each data point represents at least 5 mice. A p value associated with a change in LD_{50} due to chlorpromazine treatment refers to the significance of the chlorpromazine effect on the dose-response curves as calculated by logit analysis and not to the significance of the difference between LD_{50}s. Other tests of significance (two-tailed) were calculated by contingency analysis (overall and adjusted multiple *post hoc* comparisons). Statistical tests were considered significant when $p < .05$.

Results

Dose-response of chlorpromazine. Mice were injected with venoms or toxins at a dose level that was targeted to be the minimal 100% lethal dose (usually about 2 times the respective LD_{50}). The injection of venom or toxin was immediately followed by a separate injection of chlorpromazine. If no dose of chlorpromazine resulted in at least 90% survival in the presence of a venom or toxin, then no further investigation of that particular chlorpromazine-venom (toxin) combination was pursued. Chlorpromazine protected mice from the toxicity of *B. caeruleus* venom, *B. multicinctus* venom, and β-bungarotoxin (Figure 1a) while providing little protection from *C. durissus terrificus* venom, crotoxin, *O. scutellatus* venom, or taipoxin (Figure 1b). When chlorpromazine did enhance survival, protection increased with increasing amounts of chlorpromazine up to 0.5 mg/kg in the case of β-bungarotoxin and 1 mg/kg in the cases of *B. caeruleus* venom and *B. multicinctus* venom. Higher doses of chlorpromazine resulted in a decline in effectiveness. The ED_{50}s of chlorpromazine (calculated using the rising phase of the appropriate dose-response curve) were 0.28, 0.10, and 0.27 mg/kg with respect to *B. caeruleus* venom, *B. multicinctus* venom, and β-bungarotoxin. Combining these values with LD_{50}s (calculated using the falling phase of the appropriate dose-response curve) of 13, 7.2, and 13 mg/kg resulted in therapeutic indices of 46, 72, and 48 for *B. caeruleus* venom, *B. multicinctus* venom, and β-bungarotoxin, respectively.

Effect of chlorpromazine on dose-response of venoms and toxins. I tested chlorpromazine for its ability to increase the LD_{50}s of *B. caeruleus* venom, *B. multicinctus* venom, α-bungarotoxin, and β-bungarotoxin. Mice were injected with various doses of venom or toxin immediately followed by administration of 1 mg/kg of chlorpromazine. Chlorpromazine increased the LD_{50} of *B. caeruleus* venom 8.6-fold from 32 μg/kg to 275 μg/kg ($p < .0005$), of *B. multicinctus* venom 2.6-fold from 47 μg/kg to 120 μg/kg ($p = .012$), and of β-bungarotoxin 3.7-fold from 11 μg/kg to 41 μg/kg ($p = .004$) (Figure 2). In the case of β-bungarotoxin, chlorpromazine completely protected mice from approximately twice a minimal 100% lethal dose of the toxin. In contrast, chlorpromazine had no effect on the LD_{50} of α-bungarotoxin, decreasing it from 240 μg/kg to 210 μg/kg ($p = .38$) (data not shown). An injection of gel-phosphate buffer

followed immediately by an injection of 1 mg/kg of chlorpromazine had no overt effect on 20 mice observed for 48 hr.

Effect of time of injection of chlorpromazine. I investigated the effect of injecting chlorpromazine at different time intervals both before and after the injection of amounts of *B. caeruleus* venom, *B. multicinctus* venom, or β-bungarotoxin targeted to be minimal 100% lethal doses. Chlorpromazine provided maximal protection in all 3 cases when it was injected immediately following (+0 min) the injection of venom or toxin (Figure 3). Only in the case of *B. caeruleus* venom, however, did chlorpromazine offer significant protection at other than at +0 min. With this venom chlorpromazine afforded a 70% survival rate when it was administered at +15 min. At 30 min its protection fell to a statistically non-significant 30%.

Discussion

I have shown chlorpromazine to be an effective antagonist in mice of the toxicity of *B. caeruleus* venom, *B. multicinctus* venom, and β-bungarotoxin. Another drug reported to be effective against the toxicity of *B. multicinctus* venom and β-bungarotoxin is uranyl ion (UO_2^{2+}). Lin-Shiau (1983) and Lin-Shiau *et al.* (1983) demonstrated that UO_2^{2+} antagonized the toxicity of *B. multicinctus* venom and β-bungarotoxin in chicks and mice. Uranyl ion increased the LD_{50} of the venom 5.7-fold and the LD_{50} of β-bungarotoxin 5-fold in mice (all compunds administered *i.p.*). By comparison, chlorpromazine increased the LD_{50} of *B. multicinctus* venom 2.6-fold and the LD_{50} of β-bungarotoxin 3.8-fold. In addition, chlorpromazine caused an 8.7-fold increase in the LD_{50} of the venom of the closely related snake *B. caeruleus* . The effect of the 2 compounds on α-bungarotoxin was very different. Chlorpromazine had no effect on the LD_{50} of α-bungarotoxin while UO_2^{2+} actually caused a 20-fold decrease in the LD_{50}. The time course of protection provided by the 2 compounds was similar. Lin-Shiau *et al.* (1983) found that uranyl ion began to lose its effectiveness when it was administered more than 10 min after the injection of β-bungarotoxin and it was completely ineffective when administered 30 min afterwards. Chlorpromazine was effective only when it was administered immediately after *B. multicinctus* venom or β-bungarotoxin, although in the case of *B. caeruleus* venom the drug was still effective when adminstered 15 min after intoxication. The toxicity of UO_2^{2+} in mice was reported to be "low" with 1 death among the 15 mice tested. An effective dose of chlorpromazine was overtly nontoxic. As a potential antidote in humans for poisoning due to *B. caeruleus* or *B. multicinctus* venom, chlorpromazine has the advantage of having a long history of usage in humans (Barnhart, 1987).

Phospholipase A_2 activity is a common feature of presynaptic neurotoxins from snakes and may be the primary cause of their toxicity (Chang, 1985). Lin-Shiau and Fu (1986) found that 250 μM UO_2^{2+} inhibited the phospholipase A_2 activity of β-bungarotoxin by 65% and also approximately doubled the time to β-bungarotoxin-induced neuromuscular blockade at the mouse phrenic nerve-diaphragm. Although not necessarily an indication of chlorpromazine's effect on the phospholipase A_2 activity of β-bungarotoxin, chlorpromazine was found to have K_Is of 27 μM and

42 μM against pig pancreatic phospholipase A_2 (Jain *et al.*, 1984; Jain and Jahagirdar, 1985), a K_I of 25 μM against rabbit myocardial phospholipase A_2 (Franson *et al.*, 1983), and a K_I of less than 100 μM against rabbit leukocyte phospholipase A_2 (Taniguchi *et al.*, 1988). In addition, a single dose of chlorpromazine (2.5 mg/kg) increased the duration of respiratory activity in mice subjected to acute hypobaric hypoxia, possibly through the drug's anti-phospholipase A_2 activity (Nikolov, 1984). It is therefore possible that chlorpromazine is also acting through its anti-phospholipase A_2 activity to antagonize the toxicity of β-bungarotoxin and thus the toxicity of the venom from *B. multicinctus* . This mode of action could also account for the the protective effect of chlorpromazine toward *B. caeruleus* venom. In this regard, Lee *et al.* (1976) isolated components of *B. caeruleus* venom with β-bungarotoxin-like activity. Chlorpromazine could be antagonizing the toxicity of these components in a manner similar to its action on β-bungarotoxin, thus providing protection from the venom.

In contrast to its effect on the venoms from the 2 snakes of the genus *Bungarus*, chlorpromazine did not protect mice from *C. durissus terrificus* venom or its presynaptic neurotoxin crotoxin, or *O. scutellatus* venom or its presynaptic neurotoxin taipoxin. β-Bungarotoxin, crotoxin, and taipoxin have similar effects on neuromuscular transmission, have phospholipase A_2 activity, and are thought to act through similar mechanisms (Chang, 1985). With respect to the action of chlorpromazine, however, β-bungarotoxin is quite distinct from crotoxin and taipoxin. There is evidence that β-bungarotoxin, crotoxin, and taipoxin bind at different sites on the presynaptic membrane (Chang and Su, 1980). Chlorpromazine may act to inhibit the binding of the toxins differentially, thus providing differential protection from their respective venoms. Alternatively, chlorpromazine may be less effective against the postbinding activity of crotoxin and taipoxin than that of β-bungarotoxin, resulting in less protection from *C. durissus terrificus* venom and *O. scutellatus* venom.

Whatever its mode of action, chlorpromazine has been shown to be an effective antagonist *in vivo* of the toxicity of *B. caeruleus* venom, *B. multicinctus* venom, and the latter's most toxic component, β-bungarotoxin. Research into its mechanism of action, however, may lead to even more effective agents for the treatment of envenomation by snakes.

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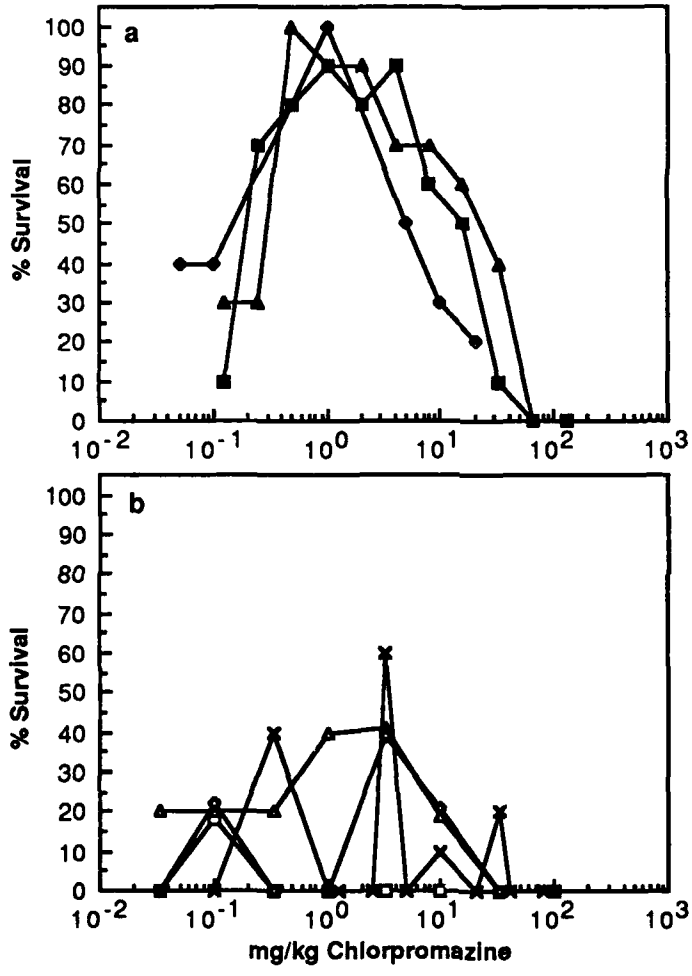
Figure 1. Dose-response of chlorpromazine. (**a**) Mice were injected with 80 μg/kg of *B. caeruleus* venom (■), 75 μg/kg of *B. multicinctus* venom (◆), or 25 μg/kg of β-bungarotoxin (▲), followed immediately by a separate injection of various doses of chlorpromazine. In most experiments no mice that received a postinjection of PBS alone (controls) survived 24 hr (not shown on graph). The results of overall significance tests and the significance tests of the optimal dose of chlorpromazine were: *B. caeruleus* venom, $p_{overall} = .0001$, $p_{1\ mg/kg\ vs.\ control} = .0011$; *B. multicinctus* venom , $p_{overall} = .0056$, $p_{1\ mg/kg\ vs.\ control} = .024$; β-bungarotoxin, $p_{overall} = .0001$, $p_{0.5\ mg/kg\ vs.\ control} = .0011$. (**b**) Mice were injected with 200 μg/kg of *C. durissus terrificus* venom (□), 100 μg/kg of crotoxin (△), 20 μg/kg of *O. scutellatus* venom (◇), or 2 μg/kg of taipoxin (✕), followed immediately by a separate injection of various doses of chlorpromazine. In most experiments no mice that received a postinjection of PBS alone (controls) survived 24 hr (not shown on graph).

Figure 2. Effect of chlorpromazine on the LD_{50} of venoms and toxins. Mice were injected with various amounts of venoms or toxins, followed immediately by a separate injection of either PBS (open symbols) or 1 mg/kg chlorpromazine (closed symbols). *B. caeruleus* venom (□,■); *B. multicinctus* venom (△,▲); β-bungarotoxin (◇,◆).

Figure 3. Effect of time of injection of chlorpromazine relative to time of injection of venom or toxin. Mice were injected with 50 μg/kg of *B. caeruleus* venom (■), 80 μg/kg of *B. multicinctus* venom (▲), or 25 μg/kg of β-bungarotoxin (◆), each preceeded (negative times) or followed (0 and positive times) by an injection of 1 mg/kg of chlorpromazine. Control animals received an injection of venom or toxin that was either preceeded (-45 min) by an injection of PBS (one-half of controls) or followed (+45 min) by an injection of PBS (one-half of controls). Tests of significance: *B. caeruleus* venom, $p_{overall} = .0001$, $p_{0\ min,\ +15\ min\ vs.\ control} = .0007$; *B. multicinctus* venom, $p_{overall} = .0001$; $p_{0\ min\ vs.\ control} = .0007$; β-bungarotoxin, $p_{overall} = .0001$; $p_{0\ min\ vs.\ control} = .0007$. The remaining comparisons were not significant.

Index terms: chlorpromazine, β-bungarotoxin, *in vivo*, presynaptic neurotoxin, snake venom, toxicity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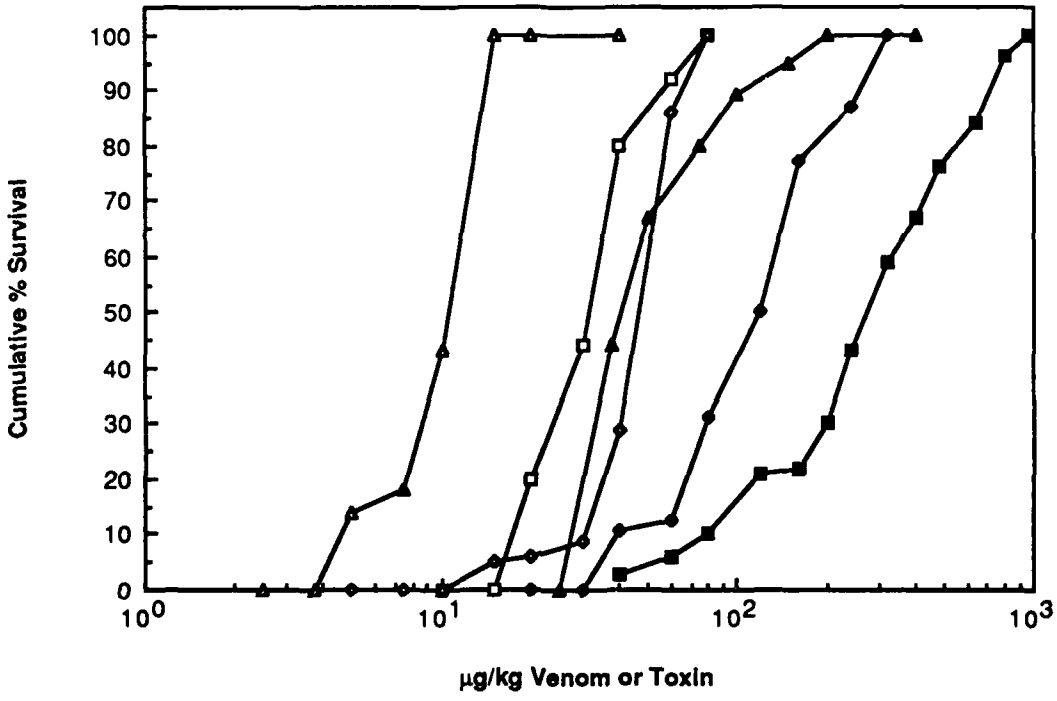
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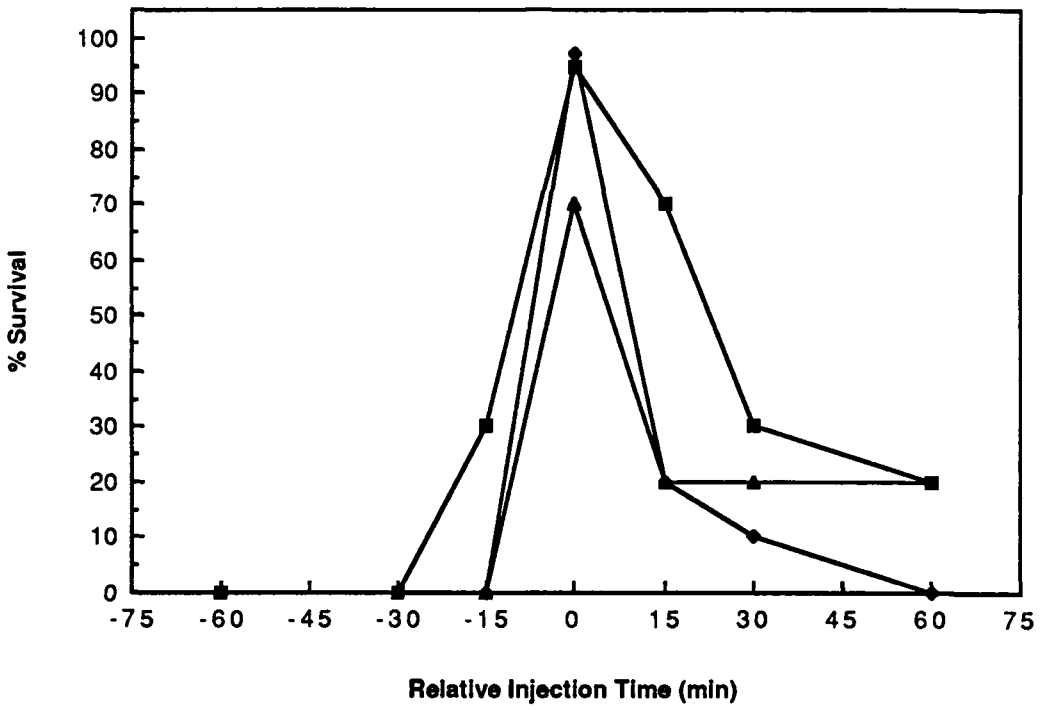
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