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An examination of the activity of expired and mistreated commercial Australian antivenoms

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Summary Expired antivenoms may be useful in countries where snake envenoming is common and supplies are limited. This study examined the activity of expired Australasian antivenoms. Expired CSL snake antivenoms, including taipan, brown snake and polyvalent antivenoms, were used. The most current antivenom was used as the reference to compare expired antivenoms. Binding activity was assessed by enzyme immunoassay. Neutralisation of venom clotting effects was assessed by a modified clotting test using changes in optical density. Neutralisation of the in vitro neurotoxic effects of taipan venom was determined using a chick biventer cervicis nerve—muscle preparation. All antivenom batches remained active, with gradual deterioration in activity and binding over time. All batches of taipan antivenom at concentrations equivalent to the administration of one vial (including one 15 years expired) prevented clotting by taipan venom. Brown snake antivenoms also prevented clotting, except two that were 10 years old. All expired taipan/polyvalent antivenom prevented in vitro neurotoxicity at concentrations consistent with antivenom treatment. Freeze—thawing the antivenom or leaving it at room temperature for 3 days caused only small decreases in activity. CSL antivenoms are more robust than indicated on their label and maintain useful activity long past their nominated expiry dates.

1. Introduction

There is little published information on the loss of activity of antivenoms over time or when they are mistreated (e.g. left at room temperature or frozen in contradiction to the manufacturers' instructions). The first of these has implications for the potential use of antivenoms after their expiry

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date. This is an issue in Australia where there is an imbalance between the amount of antivenom available and the relatively small number of bites requiring treatment. A large proportion of antivenom purchased by hospitals in Australia is not used and is discarded once it reaches its expiration date. Expired antivenom has been used for the treatment of animal envenoming but currently cannot be used in humans except in extraordinary circumstances.

In this study, we investigated the activity of a range of expired commercial antivenoms to neutralise the clinically important procoagulant and neurotoxic effects of Australasian snake venoms as well as their ability to bind venom. In addition, we investigated the stability of antivenom and its capacity to withstand adverse conditions such as prolonged exposure at room temperature and freeze/thawing.

2. Methods and materials

2.1. Materials

The type of commercial antivenom [i.e. brown snake antivenom (BSAV), taipan antivenom (TAV) and polyvalent snake antivenom (PSAV)], the batch numbers of each vial and the expiry dates for each vial used in this study are included in Table 1. Expired antivenoms were kindly donated by Julian White, Bart Currie and the pharmacy of Calvary Mater Newcastle Hospital. CSL antivenoms were stored as unopened vials or ampoules in the refrigerator, except where indicated otherwise they were stored after opening in a closed container in the refrigerator. Fresh frozen human plasma was obtained from the Australian Red Cross; stored 10 ml aliquots were thawed at 37 °C then spun at 2500 rpm for 10 min prior to use. Calcium (60 μl of 0.2 M $CaCl_2$ per ml) was added immediately before use. In the enzyme immunoassay (EIA), the washing solution was 0.02% Tween 20 in PBS, the blocking solution was 0.5% bovine serum albumin (BSA) in PBS and the plates were Greiner high-binding Microlon (Greiner Bio-One GmbH, Frickenhausen, Germany). Tetramethylbenzidine (TMB), BSA and rabbit anti-horse IgG (whole molecule)—peroxidase conjugate were obtained from Sigma (St Louis, MO, USA). All venoms were purchased from Venom Supplies Pty Ltd. (Tanunda, Australia).

2.2. Enzyme immunoassays

EIAs were used to investigate the binding affinity of antivenom batches to brown snake and taipan venom. The method was similar to that described previously.[1] A 96-well plate was coated with venom [1 μg/ml in 0.05 M carbonate buffer (pH 9.5)] at room temperature for 1 h then overnight at 4 °C. Blocking solution (300 μl) was applied for 1 h and then samples of either BSAV, TAV or PSAV were applied at concentrations ranging from 0 mU/ml to 20 mU/ml for 1 h. After a further wash, the detecting antibody (100 μl of labelled anti-horse IgG) was applied at 0.5 μg/ml in blocking solution for 1 h. The plate was washed again and 100 μl of TMB was applied, followed by 50 μl of 1 M H_2SO_4. The plate was read at 450 nm. The standard curve (based on the most current antivenom) was fitted to a sigmoidal dose—response equation and this equation was used to calculate the concentrations of other antivenoms. Antivenom standards measured in triplicate had a coefficient of variation of <10%.

To test each of the antivenom batches, a series of dilutions of the batch was applied in duplicate to plates coated with the corresponding venom. The most current antivenom was used as the reference standard to compare the older antivenoms. For example, TAV batch #05601 (see Table 1), although opened for 9 months and past its designated expiry date, was assumed to have the stated activity of 12 000 U/vial.

Table 1 List of antivenoms used in the study.

Antivenom	Batch #	Expiry date	Volume (ml)[a]	Labelled activity (U)	Measured activity (U)
Brown snake	07301	12/98	5.1	1000	968
	07401	5/99	4.05	1000	609
	08401	3/02	6.4	1000	1020
	09601	5/05	5.2	1000	1323
	09701	7/07	8.13	1000	1319
	10201	10/07	3.11	1000	1000
Taipan	03801	5/93	43.5	12 000	6360
	04201	6/97	50	12 000	5700
	04701	6/00	36.2	12 000	5040
	05601[b]	7/05	49.1	12 000	12 000
Polyvalent[c]	08601	5/91	48.3	40 000	Br, 560; Ta, 6600
	10601	7/97	43.5	40 000	Br, 470; Ta, 7080
	10801	8/98	50	40 000	Br, 560; Ta, 5020
	11601	9/00	32.8	40 000	Br, 630; Ta, 6240
	16501[b]	7/09	35.93	40 000	Br, 1000; Ta, 12 000

[a] The volume of antivenom vials varies between batches.
[b] Opened for <1 year and stored in a closed container at 4 °C.
[c] Contains 12 000 U of taipan antivenom and 1000 U of brown snake antivenom.