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Short Communication

Commercial monovalent antivenoms in Australia are polyvalent

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ABSTRACT

Monovalent antivenoms have a lower volume of specific antibodies that may reduce reactions but require accurate snake identification to be used. Polyvalent antivenoms are larger volume and may have a higher reaction rate. However, they avoid the problem of snake identification and may be more cost-effective to manufacture. We have previously shown cross-neutralisation of two Australian elapid venoms, tiger snake (*Notechis scutatus*) and brown snake (*Pseudonaja textilis*) venoms, by their respective monovalent antivenoms. In this study enzyme immunoassays were used to quantify the amount of monovalent antivenom (quantity of monovalent antibodies to a specific snake venom) in vials of commercially produced antivenom in Australia. All antivenoms tested appeared to be polyvalent and contain varying amounts of all five terrestrial snake monovalent antibodies based on their binding to the five representative venoms. Redback spider antivenom did not have any measurable binding affinity for any of the five snake venoms, showing that the observed binding is not due to non-specific interactions with equine protein. The antivenoms had expiry dates over a 15 year period, suggesting that the antivenoms have been mixtures for at least this time. This study cannot be used to rationalise hospital stocks of antivenom in Australia because there is no guarantee that the antivenoms will remain as mixtures. However, it would be possible for the manufacturer to reduce the number of types of snake antivenoms available in Australia to two polyvalent antivenoms which would simplify treatment of snakebite.

There is ongoing debate about the advantages and disadvantages of monovalent versus polyvalent antivenoms, particularly for use in the rural tropics to treat snake envenoming. Polyvalent antivenoms are easier to manufacture and more cost-effective, and avoid the problem of the incorrect antivenom being given due to diagnostic error. However, they are often larger in volume with a potential increased risk of adverse reactions. In contrast, monovalent antivenoms appear to be a better option in an individual patient due to their containing a low volume of specific antibodies for the snake species/genus involved. The downside is that they require that the correct snake is known otherwise the treatment may be ineffective if the snake identification is incorrect.

In Australia monovalent snake antivenoms have been manufactured for decades and a snake venom detection kit (SVDK) is available to assist in determining which monovalent antivenom should be administered. A large volume polyvalent antivenom is available that includes the equivalent of at least one vial of each of the five monovalent snake antivenoms. The availability of five terrestrial snake monovalent antivenoms and a polyvalent

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antivenom is costly because hospitals have to maintain a number of antivenoms, and there still exists the possibility of the incorrect monovalent being given. All of this assumes that the antivenoms are in fact monovalent.

We have recently demonstrated cross-neutralisation of two Australian elapid venoms, tiger snake (*Notechis scutatus*) and brown snake (*Pseudonaja textilis*) venoms, by their respective monovalent antivenoms (O'Leary et al., 2007). This appears to be due to the antivenoms labelled as monovalent not being monovalent and containing antibodies against the other four groups of snakes. Estimates from this study suggested that each brown snake vial contains 60–70% of a tiger snake vial, and each tiger snake vial contains about 1.5 times a brown snake vial. The reason this has occurred is probably because it is more cost-effective to immunise animals (horses) against all snake types and then simply guarantee in the vials that there is a minimum amount of the appropriate monovalent antivenom, while allowing other monovalent antivenoms to be present in the vial. This means that many Australian monovalent snake antivenoms may be polyvalent or a mixture of some or all monovalent snake antivenoms.

We aimed to determine the contents of all monovalent and the polyvalent CSL Ltd snake antivenoms by measuring their ability to bind the five major medical groups of Australasian snake venoms.

All venoms were purchased from Venom Supplies Pty Ltd (Tanunda, South Australia). Tetramethylbenzidine (TMB), bovine serum albumin (BSA) and rabbit anti-horse IgG (whole molecule) peroxidase conjugate were obtained from Sigma. Expired CSL antivenoms were donated from hospitals around Australia. For the enzyme immunoassay (EIA), the washing solution was 0.02% Tween 20 in PBS, the blocking solution 0.5% BSA in PBS, and 96-well Greiner High Binding plates were used.

Enzyme immunoassays were used to quantify the amount of monovalent antivenom (quantity of monovalent antibodies to a specific snake venom) in vials of commercially produced antivenom, using a previously described method (O'Leary et al., 2006, 2007). To quantify the five types of CSL monovalent antivenoms the plate wells were coated with venom solutions from the five representative snakes: common brown snake (*Pseudonaja textilis*), common tiger snake (*Notechis scutatus*), coastal taipan (*Oxyuranus scutellatus*), common death adder (*Acanthophis antarcticus*) and mulga snake (*Pseudechis australis*). Solutions of antivenom 10 U/ml in PBS were prepared from the five terrestrial snake monovalent antivenoms (brown, tiger, taipan, death adder and black), monovalent sea snake antivenom, polyvalent snake antivenom and redback spider antivenom.

Plate wells were coated with 100 μl of venom solution (1 μg/ml) in carbonate buffer for 1 h at room temperature then at 4 °C overnight. The plate was then washed, and blocking solution (300 μl/well) was applied for 1 h and the plate was washed again. Antivenom solutions were diluted to 1 in 5000 in PBS and applied to the plate in triplicate (100 μl/well). A standard curve was constructed with antivenom concentrations of 0.15–10 mU/ml. After 1 h the plate was washed again and anti-horse peroxidase conjugate 0.5 μg/ml in blocking solution was applied (100 μl/well). After a further hour the plate was washed again and TMB applied (100 μl/well), followed after 5–10 min by 1 M H_2SO_4 (50 μl/well). Plates were read at 450 nm on a BioTek ELx808.

The standard curve was fitted to a sigmoidal dose–response equation using Prism software. Coefficients of variation between triplicates were less than 10%. The amount of antivenom was calculated based on the most recent batch of antivenom available using the standard curves. For example, batch 10201 of BSAV was assumed to have the stated concentration of 1000 U/vial, and the standard curve was created using this batch. The contents of each monovalent antivenom were also expressed as a number/proportion of vials of each type of monovalent antivenom, using the standard curve for the corresponding monovalent antivenom as a conversion.

All CSL antivenoms tested appeared to be polyvalent and contain varying amounts of all five terrestrial snake monovalent antibodies based on their binding to the five representative venoms. The results are summarised in Table 1 in both units (U) of antivenom and vial equivalents. Redback spider antivenom did not have any measurable binding affinity for any of the five snake venoms, showing that the observed binding is not due to non-specific interactions with equine protein. The antivenoms had expiry dates over a period of 15 years, suggesting that the antivenoms have been mixtures for at least this period of time.

The number of units (U) in each monovalent antivenom vial is determined to be the amount of antivenom that will neutralise the average amount of venom obtained from milking the respective snake. One unit (1 U) of antivenom activity is defined to be the amount required to bind/neutralise 0.01 mg of venom from the snake species against which the antivenom is raised (White, 2001). This means that the antivenom volume (or number of units) is proportional to the average venom yield of the snake and therefore the larger snakes—taipan, mulga and death adder—have larger volume antivenoms. For the same reason, the larger volume monovalent antivenoms will contain much larger amounts of the monovalent antivenoms from the snakes that yield less venom when milked (brown and tiger), and this is shown in Table 1.

This information cannot be used to rationalise hospital stocks of monovalent antivenoms in Australia because there is no guarantee that the antivenoms will remain as mixtures. If the manufacturer changed the way they immunise the horses, this would result in monovalent antivenoms not containing some or all of the other antivenoms. However, it would be possible for the manufacturer to reduce and simplify the number of types of antivenom and provide fewer polyvalent terrestrial snake antivenoms. One such possibility would be to produce only two snake antivenom products—a low volume brown/tiger bivalent antivenom and a larger volume pentavalent antivenom. The low volume bivalent brown/tiger antivenom would be the most important and widely used antivenom for about three-quarters of serious envenomings (Isbister et al., 2008). The high volume pentavalent would be the