Topical agents that inhibit lymphatic transport – a first aid against snakebite

D.F. van Helden,[1] P.A. Thomas,[2] P.J. Dosen,[1] M.S. Imtiaz,[1] D.R. Laver[1] and G.K. Isbister,[3] [1]School of Biomedical Sciences & Pharmacy and Hunter Medical Research Institute, University of Newcastle, NSW 2308, Australia, [2]Department of Nuclear Medicine, Royal Brisbane and Women's Hospital, QLD 4029, Australia and [3]School of Medicine and Public Health, University of Newcastle, NSW 2308, Australia.

This study examined the use of two topical pharmacological agents that inhibit the propulsion of lymph as a snakebite first aid where slowing venom reaching the circulation reduces systemic toxicity. It was based on the fact that toxin molecules in most snake venoms are large molecules and generally first enter and traverse the lymphatic system before accessing the circulation. It followed on from a previous study where it was shown that topical application of a nitric oxide donor slowed lymph flow to a similar extent in humans and rats and increased the time to respiratory arrest for subcutaneous injection of an elapid venom (*Pseudonaja textilis*, Ptx; Eastern brown snake) into the hind feet of anaesthetized rats.

Non-recovery experiments were performed on rats anaesthetised with *i.p.* urethane at 1 to 1.5 g/kg without recovery. All procedures were approved by the University of Newcastle Animal Care and Ethics Committee (Ethics Approval A-2009-153) according to the Australian Code of Practice for the care and use of animals for scientific purposes as laid down by the National Health and Medical Research Council of Australia (2004). The effects of topical application of the L-type Ca^{2+} channel antagonist nifedipine and the local anaesthetic lignocaine in inhibiting lymph flow and protecting against envenomation were examined in an anaesthetized rat model. The agents significantly increased dye-measured hind limb lymph transit times by 500% and 390% compared to controls ($p < 0.0001$ for both) and increased the time to respiratory arrest to foot injection of Ptx venom at 1 mg/kg by 60% ($p < 0.001$) and 40% ($p < 0.05$) respectively. The study also examined the effect of different doses of Ptx venom finding that for doses over the range 0.4 to 1.5 mg/kg there was a negative linear relationship between increase in venom dose and time to respiratory arrest. Animals always survived at doses of less than or equal to 0.2 mg/ml ($n = 8$).

The finding that the different lymphatic inhibitors slowed but did not block venom entry could be due to several factors. First, it is to be noted that lymph drainage occurs through both superficial lymphatics, which lie just under the skin and the deep lymphatic vessels, which drain the limb musculature and other deep tissues. Thus while venom absorption by the lymphatics will generally first occur through the superficial lymphatics following snakebite from short fanged snakes (*i.e.*, most elapids), anastomoses also allow entry of lymph into the deep lymphatics (Caplan, 1980). Therefore, inhibition of lymph flow in the superficial lymphatics will not entirely block active lymph flow, which may still be mediated by the deep lymphatics, these being less influenced by the topical inhibitors. Second, there may also be passive lymph flow as will arise if there is a net positive interstitium-lymphatic pressure gradient causing lymph to flow centrally. Such flow will be aided by factors such as arterial pulsations, fluctuations of central venous pressure and skeletal muscle contractions (Gashev, 2002), though there was no evidence of the latter in these deeply anaesthetized rats. Third, there may be low-level permeability of the vasculature to venom toxins, allowing entry of the toxins directly into the circulation.

The findings suggest that a range of agents that inhibit lymphatic flow could potentially be used as an adjunct treatment to pressure bandaging with immobilization (PBI) in snakebite first aid. This is important given that PBI (a snakebite first aid recommended by the Australian National Health and Medical research Council) is often incorrectly applied (Canale *et al.*, 2009). The fact that there was a relatively sharp cut off in the dose response such that venom doses of 0.2 mg/kg or less were not lethal whereas doses of 0.4 mg/kg or higher were always lethal highlights the importance of first aid procedures that slow and preferably limit venom entry into the circulation. The use of a local anaesthetic would have the added advantage of reducing pain.

Canale E, Isbister GK & Currie BJ. (2009) *Emergency Medicine Australasia* **21**, 184-90.
Caplan I. (1980) *Phlebologie* **33**, 537-45.
Gashev AA. (2002) *Annals of the New York Academy of Sciences* **979**, 178-187; discussion 188-196.

Supported by the NHMRC and Hunter Medical Research Institute