Composition and stability of Jack Jumper Venom by Ion Trap Mass Spectrometry

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Jack Jumper ants (*Myrmecia pilosula*) are widespread in Southern Australia including Tasmania, where the population prevalence of clinical systemic allergy is 2.7%[1]. Their sting can cause severe allergic reactions and even death[2]. Recently, a randomised controlled trial of venom immunotherapy has been established, requiring a detailed analysis of the venom and its stability in solution.

Two major protein allergens had already been sequenced[3,4] via their cDNA and found to contain 112 and 75 amino acid residues, and named Myr pI and Myr pII respectively. Up to ten subsequences of these were proposed as the constituents of the final venom from SDS-PAGE and conventional N-terminal sequencing; of these pilosulin 1 (Myr pI 57→112, 6052 daltons) and pilosulin 2 (Myr pII 49→75, 3212 daltons) are key members. These are extremely basic peptides, with 20% and 33% of their residues respectively being lysines or arginines.

HPLC-MS analysis using an ion trap was therefore undertaken with a view to confirm these previous reports of composition, to directly observe any disulfide-bridged pairs, and to monitor the venom's stability in solutions used for human injection.

The main peptide in both fresh and stored samples was found to have a molecular weight of 5608 daltons. Direct MS/MS data from this, although complex, showed evidence of at least the 10-residue sequence from the N-terminus of pilosulin 2 being present. This suggested that this peptide might contain pilosulin 2 with disulfide link(s) to a smaller peptide; subsequent reduction, alkylation and extensive MS/MS sequence data, however, showed that it was in fact consistent with Myr pII 49→74 (3155 daltons) linked through two disulfide bonds to a peptide of 2457 daltons. This data indicated a possible anomaly in the published structure of pilosulin 2. The observation of this major heterodimer involving a Myr pII subsequence and a previously unreported peptide may explain why some sera, including that from a man dying following a sting, do not strongly recognise synthetic Myr pI and Myr pII produced from cDNA sequencing data, yet react strongly to whole venom[2].

The second most abundant peptide in all samples analysed had a molecular weight of 6067 daltons and was found by MS/MS to be an isoform of pilosulin 1. Pilosulin 1 was confirmed as a relatively minor component by both molecular weight and partial direct sequencing by MS/MS. The only other previously proposed peptide directly confirmed by molecular weight and MS/MS data was Myr pI 68→112 (4938 daltons).

Preliminary stability studies in a polysorbate/mannitol/saline solution at 4°C using LC-MS detection indicated that the 5608 dalton dipeptide degraded by about 50% after 5 weeks, while pilosulin 1 and its isoform degraded by 25%.

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