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Melittin used as a Protective Agent against X-irradiation

THE chief component of bee venom is melittin, a strong basic polypeptide of molecular weight 2850 and known amino-acid sequence[1-3]. It has a variety of pharmacological effects[4], principally a very potent direct haemolytic and a strong cardiotoxic activity[5].

Shipman and Cole[6] recently found that the resistance of mice against X-irradiation increases greatly after subcutaneous injection of bee venom. In a single experiment they found one fraction of bee venom effective. This was identified as melittin. We used highly purified melittin and a larger number of animals to determine whether melittin is the protective agent in whole bee venom.

Bee venom (from Champlain Valley Apiaries, Middlebury, Vermont) was collected by a method utilizing electric shock[7]. Melittin was prepared according to known methods[1,3] and its purity tested by amino-acid analyses[8]. The results showed that the sample we used was as pure as the melittin prepared for other investigations[1,3].

LAF_1 mice (12–16 weeks old) were injected subcutaneously 24 h before irradiation with whole bee venom and melittin in isotonic saline solution. The mice were placed in a stationary circular lucite cage, directly in contact with the end of a 32 cm treatment cone of a 250 kVp, 30 mA deep therapy X-ray unit. The added filtration was 0·25 mm Cu + 1 mm Al with a half value layer of 0·97 mm Cu. All animals received 850 r. In two characteristic experiments with a total of 110 mice the results were as shown in Table 1.

Another 110 mice received 100 mg per cent 'Neomycin' and 840 u/ml. of 'Polymixin B' in their drinking water throughout the experiment, as before[6]. This treatment did not influence the results.

Whereas the effect of whole been venom in the described conditions showed inconclusive evidence for protection, the results with melittin were statistically significant ($P < 0{\cdot}005$)[9].

Earlier experiments with immature Swiss-Webster mice did not produce definite results because the resistance of such young animals against other influences of environment is more variable. For example, it became obvious that not more than five animals should be placed in one cage.

Bee venom and melittin have a high toxicity when injected intravenously or intraperitoneally ($LD_{50} = 4$ mg/kg), as we and others[10] have found. Young and adult Swiss-Webster and LAF_1 mice, however, survived for at least 30 days after subcutaneous injections with doses as large as 60 mg/kg. On the other hand, optimal protection can be achieved with doses of approximately 5 mg/kg[6]. This considerable discrepancy between the maximum tolerated and the effective dose indicated that the protection by melittin is caused by a pharmacological process. This opens up possibilities for further studies in physiological and pharmacological directions.

Table 1

Drug	No. of animals	No. of survivors after 30 days	Percentage survival
	Experiment 1		
Whole bee venom	15	15	100
Control	10	5	50
Melittin	15	15	100
Control	10	4	40
	Experiment 2		
Whole bee venom	15	5	33·3
Control	15	6	40
Melittin	15	15	100
Control	15	7	46·7
Total	110		

There have recently been reports in the literature[11] of the radioprotective action of various compounds. All these compounds differ from melittin in two decisive respects. First, they are relatively simple substances, not of protein structure; and second, they are effective only when injected immediately, that is within 30 min, before irradiation[11].

The particular structure of melittin is comparable with a detergent[3]. This similarity may be responsible for the slow absorption and diffusion of melittin into the organism. This fact may also be responsible for its radioprotective effects.

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