THE EFFICACY OF COMPRESSION IMMOBILIZATION TECHNIQUE IN RETARDING SPREAD OF RADIO-LABELED RUSSELL'S VIPER VENOM IN RHESUS MONKEYS AND 'MOCK VENOM' NaI^{131} IN HUMAN VOLUNTEERS

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Abstract. The efficacy of the modified compression immobilization technique in retarding spread of radio-labeled Russell's viper venom in 3 rhesus monkeys *(Macaca mulata)* and "mock venom" NaI^{131} in 14 human volunteers was studied. 0.1 μg of Russell's viper venom having 10 μCi radioactivity in 0.2 ml normal saline containing 0.5% bovine serum albumin was injected subcutaneously at the lateral aspect of the right hind limb of a rhesus monkey. A hand-tight bandaging of a rubber pad measuring 55 × 28 × 16 mm over the injection site and splinting effectively retard spread of radio-labeled venom for the entire length of time applied, although complete immobilization was not achieved. In human volunteers, application of a pad measuring 60 × 50 × 17 mm over the subcutaneous injection site of 20 μCi or 12 μCi/0.2 ml NaI^{131} with a hand-tight bandaging (60 ± 10 mmHg) and immobilization of limb was found to be effective in retarding the movement of radioactive NaI^{131}. These results suggested that the compression pads tried in this study effectively retard the spread of radio-labeled Russell's viper venom (MW ranging from 20,000-90,000) and radioactive NaI^{131} (MW 150) from the site of injection. Thus, it is highly likely that the present compression pad will be useful as a first-aid measure in Russell's viper bite victims.

INTRODUCTION

Use of various first-aid measures in human snakebite cases has been reported in the literature and some have been locally practised for many years. Tourniquets used in Russell's viper bite victims were ineffective in reducing systemic envenoming and retarding spread of venom from the site of bite (Bhat, 1974; Tun-Pe *et al,* 1987). Anker *et al* (1982) reported that splinting with crepe CSL bandaging of the (Sutherland *et al,* 1979) did not prevent spread of "mock venom" NaI^{131} in human volunteers whereas application of compression immobilization technique by Monash method (Anker *et al* 1982) did, We attempted to study the efficacy of the latter technique in retarding spread of radio-labeled Russell's viper *(Daboia russelli siamensis)* venom (I^{125} RVV) and "mock venom" NaI^{131} in rhesus monkeys and human volunteers, respectively, with a view to promting the application of this first-aid technique in Russell's viper bite victims.

MATERIALS AND METHODS

First-aid pad trial in monkeys injected with I^{125} RVV

Russell's viper venom was iodinated with NaI^{125} by the chloramine-T method (Greenwood *et al,* 1963) to a specific activity of 95 μCi/μg. Three rhesus monkeys *(Macaca mulata)* weighing 4 to 5.9 kg with four limbs tied to a restrainer were injected subcutaneously (5mm deep) with I^{125} RVV, 10 μCi containing 0.1 μg venom and 0.5% bovine serum albumin (BSA) in 0.2 ml saline, 4 cm proximal to the lateral malleolus of the right hind limb. A rubber pad measuring 55 × 28 × 16 mm with 25 mm × 1 m long cotton bandage (Fig 1) was applied over the injected site with a hand-tight bandaging and the limb was immobilized with a bamboo splint. In spite of all efforts, a complete immobilization was not achieved in conscious monkeys. One ml heparinized blood was taken from cubital vein at 15 minute intervals for 45 minutes while pad was on and for 30 minutes after its release. Radioactivity was counted in an Autogamma spectrometer (Packard, Model 5230). Ninety-eight percent of the total radioactivity in the blood was found to be tri-chloroacetic acid precipitable.

First-aid pad trial in human volunteers injected with "mock venom" NaI^{131}

Twenty-two healthy male volunteers (14 pad-treated and 8 untreated controls) with a mean age

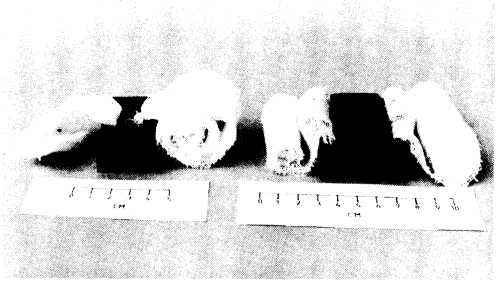

Fig 1—Picture of first-aid rubber pads.

of 35 years (range 22-58 years) and a mean body weight of 50 kg (45-65 kg) were studied. Thyroid uptake of NaI^{131} was blocked by administration of 150 mg of potassium iodide (lugol's iodine) in two divided doses, 24 hours before the experiment. A "mock venom" used was that of radioactive iodine NaI^{131} (Amersham UK code no: 1 BS-2B) with a short nuclear half-life of 8 days (Anker *et al*, 1982). A total dose of NaI^{131} (12 μCi/0.2 ml/subject) was used in 6 subjects (3 tests and 3 controls) (Table 2, 3); due to low radioactive counts in the blood, the dose was increased to 20 μCi//0.2 ml/subject for the remaining subjects. The "mock venom" was injected subcutaneously 5 mm deep except at a site 10 cm distal to head of fibular (15 mm deep) (Table 2, 3) using a 30 mm × 0.5 mm (25G) needle. A firm rubber pad measuring 60 × 50 × 17 mm with a rubber strip of 60 × 28 × 5 mm glued to one side of the pad (Fig 1) and a cotton bandage of 25 mm × 2.5 M long was applied immediately over the site of injection. The limb was immobilized with bamboo splints. It took about 2 minutes to complete the whole procedure. To get uniformity in treatment, only one person (TP) was assigned to carry out injection, pad application and bandaging. Two ml blood was collected through a three way canula into tubes containing 100 μl each of 1,000 units heparin/ml at 0 time and at every 15 minute intervals for 1 hour while the pad was on and 30 minutes after its release.

Ten male volunteers were asked to apply a rubber pad and bandaging of the leg while a rubber baloon of adult sphygmomanometer was interposed between pad and skin of the leg. Pressure exerted by each turn of hand-tight bandaging was found to be on an average of 60 ± 10 mmHg.

Table 1

Results of pad-treated monkeys injected with I^{125} RVV.

Sr	Duration of pad application (min)	Time to 80% max : blood count (min)
M1	52	70 (52+18)
M2	51	53.6 (51+2.6)
M3	44	65.6 (44+21.6)

RESULTS

Table 1 shows the results of pad-treated monkeys injected subcutaneously with I^{125} RVV. Time to reach 80% of the maximum blood radioactivity (used by Anker *et al*, 1982) was taken as a marker for effectiveness of the applied compression pad in preventing spread of "mock venom" from the injected site into the circulation. Inspite of failure

Table 2
Results of untreated human controls injected with NaI^{131}.

Sr	NaI^{131} dose	Site of injection	Duration of treatment (min)	Time to 80% max: blood count (min)
C1	12 μCi/subject	10 cm proximal to lat malleolus	-	40
2	,,	,,	-	40
3	,,	,,	-	40
4	20 μCi/subject	10 cm distal to head of fibula	-	45
5	,,	,,	-	40
6	,,	,,	-	40
7	,,	,,	-	40
8	,,	,,	-	54

to achieve complete immobilization, the compression immobilization technique contained I^{125} RVV while pad was on in 3 monkeys.

The results of untreated and pad-treated human volunteers injected subcutaneously with a dose of "mock venom" NaI^{131} are shown in Tables 2 and 3, respectively. Time to achieve 80% of the maximum blood radioactivity in all 14 pad-treated cases was reached at or after discontinuation of pad treatment.

The trend of appearance of radioactivity in the blood in pad-treated monkeys injected subcutaneously with a dose of I^{125} RVV is shown in (Fig 2). In human volunteers, pad treatment was effective in all subjects except in two (T6 and T13) in containing "mock venom" NaI^{131} for the entire length of time it was applied (Fig 3).

DISCUSSION

Different first-aid techniques advocated for use in snakebite has been studied for their efficacies in retarding the spread of "mock venom" from the site of injection in human volunteers (Anker *et al,* 1982). Of these, the Monash method (Anker *et al,* 1982) using a firm cloth pad and broad cotton bandaging was found to be effective in retarding the uptake of "mock venom" for the entire time applied. We obtained a similar result by employing the compression immobilization principle of the Monash method, using a firm rubber pad with cotton bandaging and splinting. Since the cotton bandage used (Fig 1) cannot withstand stretching, a long length of bandage is needed to cover the whole pad so that a uniform pressure can be applied. In our new version, a piece of cloth with a shorter length was used instead. When time to reach 80% of the maximum blood radioactivity was used as a marker to measure the rate of appearance of radioactivity in the systemic circulation from the injected sites, this technique is effective in preventing spread of both high molecular weight venom components (I^{125} RVV) and the lower molecular weight NaI^{131} "mock venom" in monkeys and human volunteers, respectively. A slow leak of I^{125} RVV in 2 monkeys probably resulted from movement of the injected leg since muscular contraction is found to promote spread of HMW venom components of RVV (Barnes and Trueta, 1914). Also, leakage of radioactivity in 2 out of 14 human volunteers injected with "mock venom" is probably a result of insufficient pressure being ap-

Table 3

Results of pad-treated human volunteers injected with NaI^{131}

Sr	NaI^{131} dose	Site of injection	Duration of treatment (min)	Time to 80% max: blood count (min)
C1	12 μCi/subject	10 cm proximal to lat malleolus	45	52 (45+7)
2	,,	,,	60	72 (60+12)
3	,,	,,	60	72 (60+12)
4	20 μCi/subject	,,	60	72 (60+12)
5	,,	,,	60	60
6	,,	,,	60	60
7	,,	,,	60	60
8	,,	,,	79	87 (79+8)
9	,,	Just below lateral malleolus	60	68 (60+8)
10	,,	,,	60	72 (60+12)
11	,,	10 cm distal to head of fibula	55	68 (55+13)
12	,,	,,	60	72 (60+12)
13	,,	,,	60	60
14	,,	,,	45	50 (45+5)

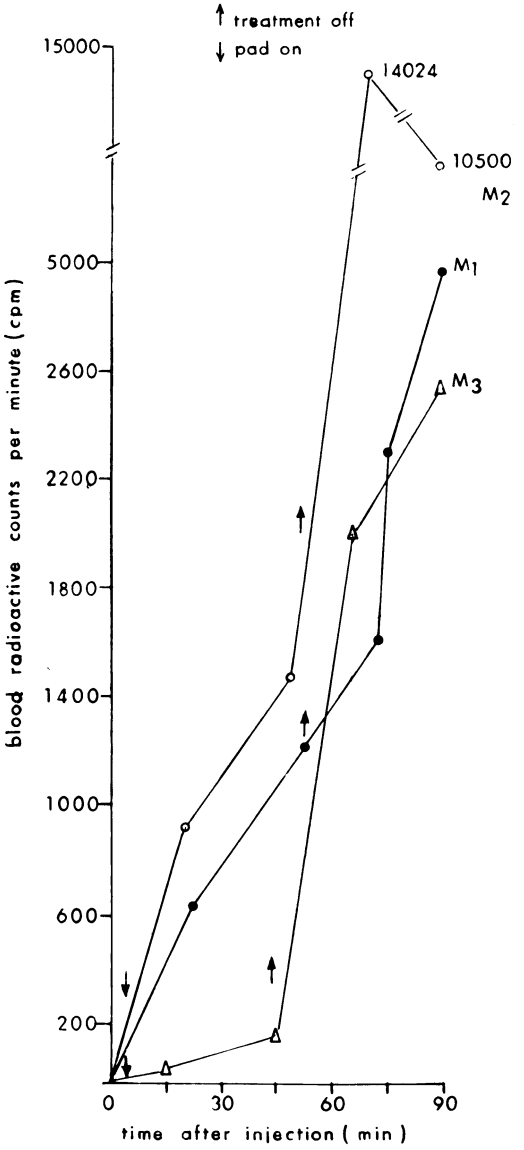

Fig 2—Trend of appearance of radioactivity in the blood of monkeys injected with a dose of I^{125} RVV, 10 μCi containing 0.1 μg venom and 0.5% BSA, subcutaneously.

plied on the pad (a pressure of at least 70 mmHg is required to prevent spread of "mock venom" (Anker *et al,* 1982) or movement of the injected limb rather than the smaller size of rubber pad used. On the other hand, the probability of leakage will be small if a HMW compound like RVV is used instead. Although there will be some local diffusion of venom into the neighboring subcutaneous tissue around pad, further spread of it is prevented by impeding lymphatic flow following immobilization of the limb. In field practice, most snakebite victims apply tight tourniquets (Tun-Pe *et al,* 1987) and one would have thought that pressure applied over the pad would far exceed than that used in our volunteers.

In the present study, a small pad primarily intended to be used in places like bites on fingers or toes of human subjects was used in the monkey experiment. The pressure under the applied pad and local effects following its use in monkeys were not studied. The ill effects following the use of the rubber pad and cotton bandaging in human volunteers were paraesthesia of the legs and slight engorgement of the superficial veins distal to the pad which can be alleviated by keeping the leg in slightly elevated position. Cyanosis of toes and loss of pulsation of dorsalis pedis were not encountered. Most subjects tolerated paraesthesia of the leg which appeared at 30 minutes after application of the pad and lasted to end of the study (1 hour). Since most snakebite victims rarely complain of paraesthesia of the leg in spite of its presence following use of tight tourniquets for hours, the symptoms encountered with the use of compression pad and cotton bandaging should be equally tolerated. As this first-aid technique was found to be effective in delaying spread of both low and high molecular weight compounds, it is expected to retard the spread of HMW components of RVV in human snakebite victims. Hence, a clinical trial of compression pad in Russell's viper bite victims is currently under study.

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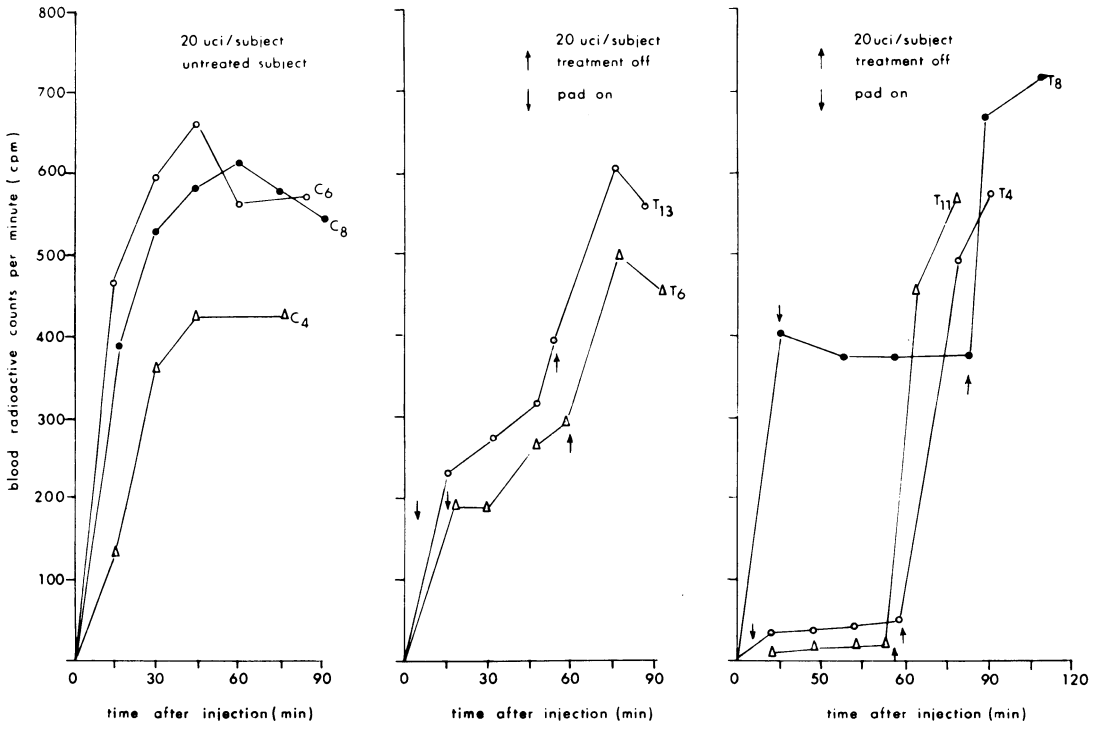

Fig 3—Trend of appearance of radioactivity in the blood of untreated controls and pad-treated human volunteers injected with a dose of "mock venom" NaI^{131}, subcutaneously.

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