[原著] An Experimental Study of Emergency Care for Habu Bites : Estimation of Amount of Venom Removed by Suction

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An Experimental Study of Emergency Care for Habu Bites

—— Estimation of Amount of Venom Removed by Suction ——

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In Okinawa Islands, more than 200 Habu (*Trimeresurus flavoviridis*) bites are reported annually[1] Habu venom, when injected into an animal parenteraly, causes localized lesions consisting of hemorrhage, necrosis and edema. When a large amount of venom is injected, nausea, vomiting, hypotension and even death are observed[2]. Treatment with Habu antivenom extremely decreases the mortality rate in Habu bites. Nevertheless, functional and histological disturbances of various organs resulting from the venom still remain a serious problem for snakebite patients. Araki *et al*[3] reported that 33 cases of Habu bites experienced malfunction of the hands or legs and, in severe case, the patients were incapable of grasping or walking. The severity of the disfunctions of the organs depends on the site of the bite and time of beginning treatment with antivenom. But the most important factor is the amount of venom injected. The amount of Habu venom is considered to be correlated with the severity of disfuncions caused[3]. Therefore, it is important in the treatment of snakebites to decrease the amount of venom injected. Thus, an emergency care for Habu bites is recommended as follows : cutting the bitten site and applying suction to reduce venom injected, or binding the bitten arm or leg proximal to the heart in order to prevent the spread of the venom through out the whole body[4].

However, experimental estimation of the effect on the removal of snake venom from animals has not yet been established. The present study is therefore designed to determine the amount of venom that can be removed by suction from the venom-injected animal.

Materials and Methods

Materials: Male chickens 3 to 5 days old and male Wistar rats weighing about 500g were used. Habu venom used in this study was prepared from a pool of Habu venom which was collected and lyophilized in the Okinawa Islands in 1967.

Measurement of Habu venom in chicken foot: Habu venom dissolved in 0.9 % NaCl was intracutaneously injected into the sole of the right foot of the chicken at the rate of 0.05 ml per 40 g body weight. The foot was cut off just above the spur 5 hours after injection and the weight of the foot was measured. As the control, the left foot was also removed at the same time. The amount of the venom was presented as the percentage of the increased weight of the venom -injected foot to the weight of the control foot. Thus, the calibration curve was made of the venom amount and of the foot weight.

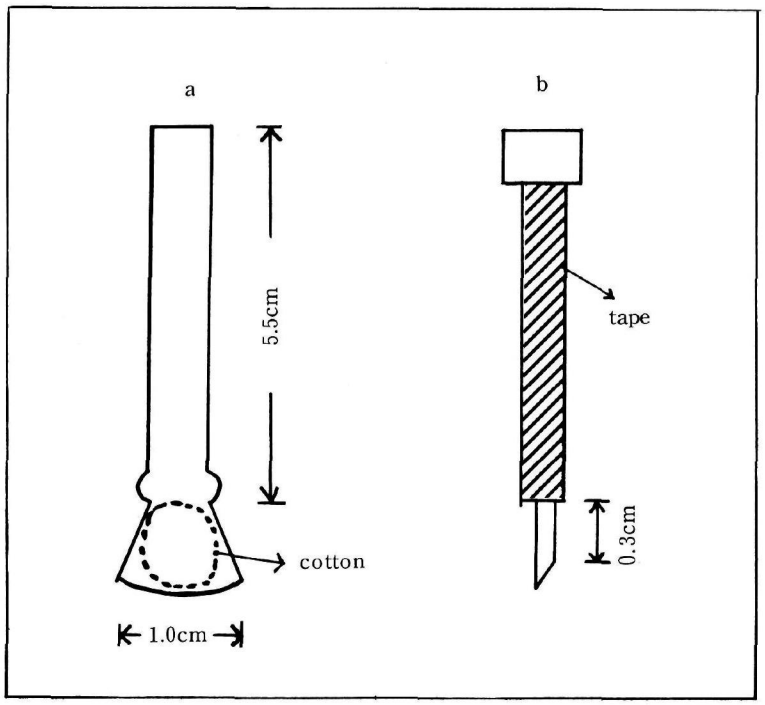


Fig. 1 Illustration of the instrument used for suction.
a: micro glass tube for suction, b: needle for intramuscular injection.

Effect of suction on venom-injected rats: Habu venom (0.1 ml) was intracutaneously injected in two rows (right and left) into the back skin of the rat which had been treated with a depilatory agent 24 hours before venom injection. The rat was anesthetized with a subcutaneous injection of urethane (0.5g per kg body weight). Then, the right side of the venom-injected sites was cut with a razor to 8 mm in length and 1 to 2 mm in depth, and suction was applied for one minute with a micro glass tube connected to the suction pump (Toyo Jet Sucker, Ts-75 A) at 30 cm Hg. In our preliminary study, it was observed that the suction pressure by human mouth reached about 30 cm Hg. The left row was observed as control. The micro glass tube used for the suction is illustrated in Fig. 1-a. The venom removed from the back skin was absorbed in a small pieces of cotton packed at the top of the glass tube. The venom absorbed in the cotton was washed away with 0.9 % NaCl of 0.4 ml. The resulting solution was injected intracutaneously into three chicken feet at a rate of 0.05 ml per 40 g body weight and the weight of the foot was measured 5 hours later. The amount of venom removed by suction was determined by using the calibration curve made on the same day. In the study of the effect of snake venom injected intramuscularly, the same procedures were repeated. After cutting the skin of the buttock of the rat, a Habu venom solution of 0.1 ml was directly injected into the buttock muscle at a depth of 3 mm using a needle illustrated in Fig. 1-b. The muscle was cut with a razor to 4 mm in depth and 3 mm in length, and then suction was applied under the same conditions as in the intracutaneous injection. The amount of venom removed by suction was determined from the calibration curve.

Observation of localized lesions caused by Habu venom: In the intracutaneous injection case, the rat was killed by ether 24 hours after suction and its back skin was removed. The spot of hemorrhage

in both the suction and the control sites of the skin was observed. The rat which received an intramuscular injection of the venom was killed 2 hours after suction, and the lesion of the suction site was compared with that of the control site.

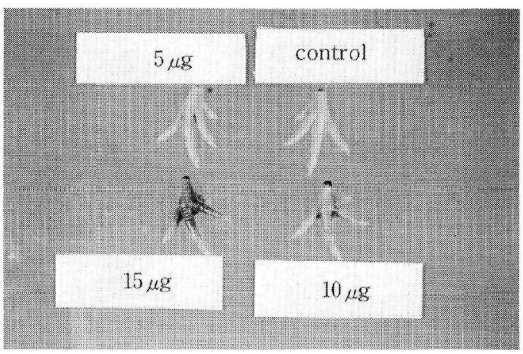
Results

Measurement of Habu venom in chicken foot: As presented in Table 1, the weight of the right foot and the left foot of a nontreated chicken was 0.797 ± 0.07 g and 0.791 ± 0.07 g (n = 20), respectively. The difference in weight of the right foot and the left was of no significance. Thus, we used the right foot for the venom estimation and the left for control. Edema and hemorrhage developed in proportion to the amount of venom (Fig. 2). The relationship between the percentage of increase in the right foot weight and the amount of Habu venom is indicated in Fig. 3. The weight of the right foot was linearly increased with 3 to 15 μg doses of the venom on semilogarithmic graph paper. Thus, this linear line was used as the calibration curve for determination of the amount of venom removed by suction.

Table 1. Weight of untreated chicken foot

No.	left (g)	right (g)	$\left(\frac{\text{right}}{\text{left}}\right) \times 100$
1	0.739	0.729	98.65
2	0.818	0.806	98.53
3	0.749	0.730	97.46
4	0.746	0.746	100
5	0.829	0.817	98.55
6	0.760	0.732	96.32
7	0.675	0.675	100
8	0.858	0.851	99.18
9	0.829	0.842	101.57
10	0.889	0.879	98.88
11	0.849	0.837	98.59
12	0.703	0.712	101.28
13	0.709	0.709	100
14	0.820	0.825	100.61
15	0.810	0.810	100
16	0.855	0.843	98.60
17	0.923	0.896	97.07
18	0.680	0.682	100.29
19	0.835	0.830	99.40
20	0.864	0.862	99.77
$\bar{x}$	0.797	0.791	99.24
s *	0.071	0.069	1.33

* Standard deviation



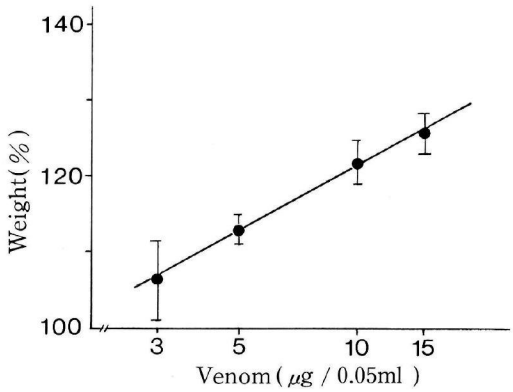


Fig. 2. Venom-injected foot of the chicken (left) . Habu venom was intracutaneously injected into the sole of the chicken foot and the foot was cut off just above the spur 5 hours later.

Fig. 3. Relationship between the increase in foot weight and the amount of Habu venom (right).

Habu venom was injected into the sole of chicken foot and the weight of the foot was determined as described in Materials and Methods. Weight (%) represents the percentage of increased weight of venom-injected foot to the weight of control foot. Each point shows mean ± S.D. from 12 chickens.

Effect of suction: The amount of Habu venom removed by suction is presented in Table 2. When 200, 300, and 500 μg of the venom were intracutaneously injected into rats, about 26, 20, and 21 % of the venom, respectively, was removed by suction. In the intramuscular injection case, 11 to 13 % of the venom was removed by suction. Thus, the amount of venom removed by suction was almost constant regardless of the dose of venom applied. The spot of hemorrhage on the back skin was slightly reduced by suction. In the intramuscular injection case, improvement by suction of local lesion of the venom-injected site was not clear.

Table 2. Effect of suction on venom-injected rats

Injection	Dose (μg/0.1 ml)	Sucked-venom * (μg)	Sucked-venom * (%)
Intracutaneous	200 (n=9)	53.16 ± 15.40	26.58 ± 7.70
	300 (n=6)	61.20 ± 25.98	20.40 ± 8.66
	500 (n=6)	107.45 ± 44.60	21.49 ± 8.92
Intramuscular	500 (n=6)	66.85 ± 16.05	13.37 ± 3.21
	1000 (n=8)	118.70 ± 70.90	11.87 ± 7.09
	1500 (n=6)	203.40 ± 168.90	13.56 ± 11.26

*Mean ±S.D.

Discussion

In Okinawa, suction of a bitten site has been recommended as an emergency treatment for Habu bites. However, it is not clear how much venom is removed by suction and it is also not clear whether or not such treatment is useful for reduction of venom toxicity. Since sucked-venom will contain tissue fluids and is of a very small volume, the chemical method such as measurement of protein content is not useful for determining the amount of venom removed by suction. So we used the bioassay method for determining the amount of venom, and observed the edema forming activity of the venom in the chicken foot. In this method the foot was cut off just above the spur, and the weight of the foot was measured exactly. The weight of the chicken foot was linearly increased with 3 to 15 μg of the venom injected intracutaneously and this range in amount was enough to determine the amount of sucked-venom. As the foot weight was almost the same from 1 to 7 hours after the venom injection (data not shown), the foot was cut off at 5 hours when weight variation caused by a dose of the venom was least. Yamakawa *et al*[5] measured the edema forming activity in mouse paw. Weight difference between the right and the left paws of normal mouse is large compared with that of chicken foot. This variation is considered to come from the cutting site or from the presence of hair of the skin. In this respect, the spur of the chicken foot is a good indicator for cutting off.

About one fifth of the venom injected intracutaneously into rats was removed by suction, but the improvement in localized lesions such as hemorrhage was not so clear when observed with the naked eye. In the rat which received intramuscular injection of the venom, only one tenth of the venom was removed by suction treatment. We observed in the preliminary study that a water solution of brilliant blue, when injected into the muscle under the same conditions as the venom injection, spread into the muscle tissue along the muscle fiber. Since the venom was soluble in water, it may be assumed that Habu venom injected into the muscle is rapidly diffused, consequently the venom remaining at the injection site is small. Thus, it seems reasonable that less Habu venom would be removed by suction in the intramuscular injection. As large doses of venom were given to rats in order to acertain the amount of venom in this study, the remaining amounts after suction were so large that the reduction of the venom toxicity would not clearly be observed.

The Habu snake has two fangs of about 1.5 cm in lenght and the venom is ejected from pores at 0.1 cm from the tip of the fang[6]. About 20 mg of the venom is ejected in one attack, but sometimes 100mg is administered in one attack[7]. When the back of the hand of humans is attacked, more venom will be removed by suction treatment compared with that which can be removed in a calf bite because the calf has abundant muscles. The amount of venom removed by suction may vary with the depth of the cut at the bitten site, and with suction methods used such as an instrument or the mouth, or with place attacked. However, our experimental results suggest the possibility that suction treatment may be helpful in emergency care for snakebites.

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References

1) Araki Y. and Teruya K. : Snakebites of the Okinawa Prefecture in 1981. Reports of ecological and epidemiological studies to diminish Habu bites in Okinawa Prefecture (V) p19-34, Okinawa Prefecture, 1982 (In Japanese)

2) Sawai Y. : Studies on snakebites in the Asian areaes. In Natural Toxins (Ed. Eaker D. and Wadstorm T.) p25-32, Pargamon Press , 1980.

3) Araki Y., Teruya K. and Kayo M. : Sequelae of Habu snakebites in south area of Okinawa Island. Reports of ecological and epidemiological studies to diminish Habu bites in Okinawa Prefecture (V). p35-41, Okinawa Prefecture, 1982 (In Japanese)

4) Yoshida C. : Habu and Human. p132-140, Naha Shuppan, Okinawa , 1979 (In Japanese)

5) Yamakawa M., Nozaki M. and Hokama Z. : Studies on the quantitative method for determination of edema forming activity of Habu *(Trimeresurus flavoviridis)* and Sakishima Habu*(Trimeresurus elegans)* venom. The SNAKE 5, 168-173, 1973 (In Japanese).

6) Yoshida C. : Habu and Human. p17-22, Naha Shuppan, Okinawa , 1979 (In Japanese).

7) Hokama Z. : Study on exqerimental envenomation by the Habu. The SNAKE 10, 107-113, 1978 (In Japanese)