EFFECT OF THIOL-COMPOUND ON TOXICITY OF HABU SNAKE (TRIMERESURUS FLAVOVIRIDIS HALLOWELL) VENOM

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In the previous paper on the effects of various chelating agents on the systemic action of Habu snake venom, we reported that the treatment with EDTA enhanced the toxicity of the venom while thiol-compounds such as BAL and d-penicillamine markedly reduced it (1). In the present work three thiol-compounds, that is l-cysteine, thioglycolate-Na and dihydrothioctic acid were examined for their effects on the systemic action of Habu snake venom.

MATERIALS AND METHODS

Male, Wistar strain rats, weighing about 250 g, propagated and bred in our department were used. The Habu snake venom used was obtained from Trimeresurus Flavoviridis Hallowell in 1967 at Naze Health Center in Amami-Oshima and was lyophilized there. The drugs used were l-cysteine (Kanto Chemical Co., Inc.), thioglycolate-Na (Minophagen Pharmaceutical Co. Inc.) and dihydrothioctic acid (Fujisawa Pharmaceutical Co. Inc.). The animals were anesthetized by intraperitoneal injection of 1 g/kg of urethane, and the carotid blood pressure and respiration were surgically recorded on the kymograph. Electrocardiogram was also recorded, and heart rate, respiration rate, and times of cardiac and respiratory arrests were determined within the range of 180 minutes. Immediately after the termination of these experiments, autopsy was performed chiefly to examine for visceral hemorrhage. Preparation of the drug solution and dose of the drug are given in Table 1. Controls were given an aqueous solution containing 10 mg of the venom per ml of distilled water. Doses of the drugs were l-cysteine 30 mg, thioglycolate sodium

| Table 1. Experimental conditions. |
|-----------------------------------|
| Treatment (i.v. injection) | Mixed time (min) | No. of rats | Body weight (g) |
|-------------------------------|-----------------|-------------|-----------------|
| HV alone                      |                 | 5           | 241.0±23.1*     |
| HV + l-Cysteine 30 mg/kg      | 5               | 5           | 240.8±11.5      |
| HV + Thioglycolate-Na 60 mg/kg | 120             | 5           | 243.6±15.7      |
| HV + Dihydrothioctic acid     | 5               | 5           | 258.0±4.7       |
|                               | 120             | 5           | 239.4±21.4      |
|                               | 5               | 5           | 244.6±22.9      |
|                               | 120             | 5           | 254.2±31.3      |

HV : Habu venom 10 mg/kg,  * : Standard deviation
60 mg and dihydrothioctic acid 10 mg. These were each added to 10 mg of the venom, and the mixtures were diluted with distilled water to final volumes of 1 ml. Both the venom solution and venom plus drug solutions (at 5 and 120 minutes after mixture) were centrifuged at 2500 r. p. m. for 3 minutes, and the supernatants were immediately given into the femoral vein. Results were compared between venom group and venom plus drug groups.

RESULTS

1) Effect of Habu venom

Immediately after the administration of 10 mg/kg of Habu venom, blood pressure began to fall sharply, becoming below 60 mm Hg in about 10 minutes. About 20 minutes the lowered blood pressure was once recovered to some extent, but after 60 minutes the blood pressure dropped again, attaining 0 in 120 minutes. Respiration and heart rates likewise lowered in association with blood pressure fall, and the fall of their rate were especially prominent after 60 minutes, then induced respiratory arrest at 110.2±10.7 minutes,

![Fig. 1. Effect of Habu snake venom on heart rate, respiration rate and blood pressure (with standard deviation) in rats.](image)

![Fig. 2. Effect of Habu snake venom on ECG in rat.](image)
and cardiac arrest at 120.8±12.3 minutes. Thus all the cases died in about 120 minutes (Fig. 1). In ECG, depression of ST segment and enlargement of R height were seen in about 10 minutes after the administration. Then, conversely elevation of ST segment and prolongation of QRS interval were observed in 90 minutes after the injection (Fig. 2). Autopsy revealed marked bleeding in the heart, especially remarkable one in the cardiac apex, bleeding and edema in the lung, and petechiae in the pancreas, mesentery and adrenal glands (Table 2).

2) Effect of I-cysteine on the action of Habu snake venom

The venom treated with I-cysteine for 5 minutes produced result not so different from that in the control. Thus, abrupt fall of blood pressure and decrease in respiratory rate were observed soon after the administration of the venom treated with I-cysteine, and

| Treatment (i.v. injection) | Mixed time (min) | Heart | Lung | Pancreas | Mesentery | Adrenal glands |
|---------------------------|------------------|-------|------|----------|-----------|----------------|
| HV alone                  |                  | ++    | +    | ++       | +         | +              |
| HV + 1-Cysteine 30 mg/kg  | 5                | ++    | +    | +        | +         | ±              |
| HV + Thioglycolate-Na     | 5                | ±     | -    | +        | -         | -              |
| HV + Dihydrothioctic acid | 5                | -     | -    | -        | -         | -              |
| HV alone                  | 120              | ++    | +    | ++       | +         | +              |
| HV + 1-Cysteine 60 mg/kg  | 120              | ±     | -    | +        | -         | -              |
| HV + Dihydrothioctic acid | 120              | -     | -    | -        | -         | -              |

HV: Habu venom 10 mg/kg

Fig. 3. Effect of Habu snake venom with I-cysteine (5 minutes incubated) on heart rate, respiration rate and blood pressure (with standard deviation) in rats.
at about 80 minutes, cardiac rate decreased abruptly. Respiratory arrest occurred at 107.4 ±10.0 minutes, and cardiac arrest at 119.0 ±10.5 minutes. All the cases died at about 120 minutes (Fig. 3). When, however, the venom treated with 1-cysteine for 120 minutes was given, the systemic action by administration of the venom was found markedly attenuated. Thus, immediately after the administration of the treated-venom, blood pressure dropped abruptly to the extent of about 70 mm Hg, then the dropped-pressure began to be restored at about 40 minutes after the administration, and thereafter the blood pressure showed fairly high level until 180 minutes; also respiratory and cardiac rates were depressed only slightly, and all the cases survived during the observation period of 180 minutes (Fig. 4). The ECG after the administration of 5 minute-treated venom was not different from the control, showing depression of ST at about 10 minutes, and elevation of ST after 90 minutes. After the administration of 120 minute-treated venom, change in ECG was clearly slighter than in the control, showing only depression of ST with slight increase in R height at about 10 minutes after the injection. Autopsy disclosed visceral hemorrhage milder than in the control, though hemorrhage and exudation were still visible in the lung after the administration of the 5 minute-treated venom (Table 2).

3) Effect of thioglycolate-Na on the action of Habu snake venom

When treated with thioglycolate-Na, toxicity of the venom was markedly reduced. Surely abrupt fall of blood pressure was observed immediately after the administration of the venom treated with the drug for 5 or 120 minutes, but the rate of the fall was obviously slighter, and its recovery was faster than in the control; it began to be restored at 10 minutes after the treated venom administration, returned nearly to the initial level in about 60 minutes after the administration, and remained sufficiently high in all the cases at 180 minutes. Also cardiac and respiratory rates decreased clearly less than in the cont-
rol (Figs. 5 and 6). No marked change was visible in ECG in either case. At autopsy, mild hemorrhage was seen in the heart and pancreas, but not in any other organs. Nor was found any pulmonary edema (Table 2).

4) Effect of dihydrothioctic acid on the action of Habu snake venom

When treated with dihydrothioctic acid for 5 minutes or 120 minutes, the venom showed marked attenuation in toxicity; although blood pressure lowered soon after the treated venom administration, its rate was extremely small, and its recovery was also prompt.
FIG. 7. Effect of Habu snake venom with dihydrothiocic acid (5 minutes incubated) on heart rate, respiration rate and blood pressure (with standard deviation) in rats.

And at 180 minutes after the administration, the blood pressure of all the cases maintained the level of about 100 mm Hg. Respiratory and cardiac rates gave slighter decreases than after treatment with L-cysteine or thioglycolate-Na (Figs. 7 and 8). ECG showed increase in R height and slight inversion of T at 5 minutes after the 5 minute-treated venom, but nearly normal wave 30 minutes after the administration. The 120 minute-treated
FIG. 9. Effect of Habu snake venom with dihydrothioctic acid (5 minutes incubated) on ECG in rat.

venom elicited scarcely any change in ECG (Fig. 9). Autopsy revealed no visceral hemorrhage (Table 2).

DISCUSSION

In the previous work we presumed that EDTA enhanced the toxicity of the snake venom probably because it would have increased pulmonary hemorrhage (1). The present experiment disclosed that the toxicity was considerably reduced by 120 minute treatment with 1-cysteine though not by 5 minute treatment. And this was supported by autopsy at which neither bleeding nor exudation of lung was found in the former, though both were still visible in the latter despite milder visceral hemorrhage than in the control. Thioglycolate-Na and dihydrothioctic acid markedly attenuated toxicity of the venom, perfectly preventing the production of pulmonary hemorrhage and exudation. It is therefore evident that the presence or absence of pulmonary hemorrhage and exudation is closely related with the potency of the systemic action of the snake venom. Also the grade of cardiac hemorrhage had connection with the systemic action, since all the cases died in about 120 minutes after administration of the non-treated venom. After the administration of the venom treated with 1-cysteine for 5 minutes, bleeding of cardiac apex was severe, and ECG indicated myocardial infarction.

BAL, a dithiol-compound, more strongly reduced the toxicity of the venom than penicillamine, a monothiol-compound (1). Thioglycolate-Na, also a monothiol compound, showed detoxifying power as strong as dihydrothioctic acid. As for bleeding, however, dihydrothioctic acid, which is a dithiol compound like BAL, more perfectly prevented its occurrence than thioglycolate-Na. Consequently dithiol-compounds are considered to have stronger antitoxic effect than monothiol-compounds. Many works have been made on detoxicating actions of thiol-compounds on Habu venom, using as criteria hemorrhage and myolysis at the site of the venom injection. Maeno (2) reported that a thiol compound weakened heat-labile proteinase and hemorrhagic component of the venom. Aoki (3), who investigated anti-Habu venom action of α-thiolactoyl-glycine-Na, reported that this compound inhibited hemorrhagic action of Habu venom, but not so markedly its myolytic action. There are, however, few reports in effects of thiol substances on the systemic action of Habu venom.

As for the mechanism of the anti-Habu venom action of the thiol-compound, two possibilities are considered. One is detoxication by direct action on the venom itself.
Habu venom is a high molecular substance rich in S content. Its active radical is considered to be S-S bond. A low molecular SH-compound is likely to react with S-S bond of the venom, to alter its activity by substitution reaction. This seems to be indicated by the fact that Habu venom, which is nearly transparent though with slight turbidity, presents a milky opaque appearance when thioglycolate-Na or dihydrothioctic acid is added to it. Katayama (4) explains the utility of “Hypo” and GSH for bite by Agkistrodon halys blomhoffi (Mamushi), like Habu snake, it belongs to Crotalidae. A second possibility is the inhibition of the venom’s action on the living body. Habu venom inhibits the activity of succinic dehydrogenase (SH enzyme), which plays an important role in the oxidation of carbohydrate in the living body (5, 6). Thiol-compounds are considered to antagonize this inhibitory action. As for dihydrothioctic acid, Sawai et al. (7-9) and Miyagi et al. (10) added it to the same volume of Habu venom, and after 37°C 1 hour incubation gave this mixture repeatedly to horses and rabbits. And they succeeded in this way to elevate the circulating antibody titer without producing any marked side effect, they inoculated the thus prepared toxoid to volunteer in inhabitants of Amami Islands for the prevention of poisoning from Habu bite. The present experiments demonstrated that when treated with thioglycolate-Na or dihydrothioctic acid for 5 minutes, the toxicity of Habu snake venom was considerably attenuated. Therefore, these findings shows the utility of these drugs for the therapy of Habu snake bite.

**SUMMARY**

Ten mg of Habu venom were mixed in vitro with 30 mg of L-cysteine, 60 mg of thioglycolate-Na and 10 mg of dihydrothioctic acid, respectively, and after 5 and 120 minute incubation the supernatants were intravenously given to male rats under urethane anesthesia. And blood pressures, respiratory and cardiac rates, ECG and visceral autopsy findings were compared with those in the control group which were given 10 mg/kg of untreated Habu venom.

1. After the administration of 10 mg/kg of crude Habu venom, abrupt blood pressure fall, respiratory suppression and decrease in cardiac rate occurred, and all the cases died from paralysis of respiration in about 120 minutes. ECG showed depression of ST segment at 10 minutes, and elevation of ST segment and prolongation of QRS interval at 90 minutes, and autopsy disclosed cardiac hemorrhage, especially at the cardiac apex, pulmonary hemorrhage and edema, and petechia in the pancreas, mesentery and adrenal glands.

2. After the administration of the venom treated with L-cysteine for 5 minutes, the results were not so different from those of the control given untreated Habu venom, and all animals died in about 120 minutes. After the administration of 120 minute-treated venom, however, the animals demonstrated results of obvious attenuation of the toxicity, surviving beyond 180 minutes. In ECG after the 5 minute-treated venom, changes were remarkable, showing ST depression in the beginning and ST elevation at about 100 minutes after the injection. Autopsy revealed milder visceral hemorrhage, but pulmonary
hemorrhage and exudation were seen after the administration of the 5 minute-treated venom.

3. Attenuation of the toxicity was more pronounced in the thioglycolate-treated venom, which produced blood pressure fall to lesser degree and milder changes in ECG, all surviving more than 180 minutes. At autopsy, the pancreas and heart showed slight bleeding.

4. The toxicity was most reduced by treatment with dihydrothioctic acid; the venom thus treated produced milder fall of blood pressure, and its recovery was prompt. Also respiration and ECG were affected very slightly by such venom, and no visceral hemorrhage was found at autopsy.

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