Biological Activity of Crude Venom From the Crown-of-Thorns Starfish Acanthaster planci

Kazuo SHIOMI,* Kiyoshi ITOH,* Hideaki YAMANAKA,* and Takeaki KIKUCHI*
(Accepted December 7, 1984)

The crude venom (protein concentration 9640 μg/ml) was extracted with buffered saline from the spines of the crown-of-thorns starfish Acanthaster planci and assayed for some biological activities. The venom was lethal to mice when administered intraperitoneally and the LD₅₀ was determined to be 2.7 mg/kg. In addition to the hemolytic activity against animal erythrocytes in the absence of lecithin, much higher activity was observed in the presence of lecithin, suggesting that the venom contains both direct hemolytic factor(s) and indirect hemolytic factor(s) (phospholipase A). The edema-forming, capillary permeability-increasing and hemorrhagic activities were all positive; the venom dose causing a 130% of the edema ratio in mouse foot, this causing a 10-mm blue spot in rat skin and this again causing a 10-mm hemorrhagic spot, were 9.0, 5.3 and 120 μg, respectively. Moreover, histological observations showed qualitatively that the venom induced severe necrosis in mouse skeletal muscle.

It is well known that the crown-of-thorns starfish Acanthaster planci damages coral reefs by ingestion of corals. Furthermore, this animal is the only known venomous asteroid and contact with the pungent spines covering the outer surface of the entire body causes ill symptoms in human such as extremely painful wound, redness and swelling. The venom is believed to be produced by the acidophilic cells in the epidermis of spines but little is known about its properties. To our knowledge the only available data is that by TAIRA et al. who found that the proteinous venom exhibits lethal and hemolytic activities. However, the above symptoms in human induced by the venom cannot be fully explained by these two activities.

In order to shed more light on the biological activity of the A. planci venom, we first re-examined the lethal and hemolytic activities and then made a survey of the following activities which seemed to be responsible for the symptoms caused by the venom: edema-forming activity, capillary permeability-increasing activity, hemorrhagic activity and myonecrotic activity. The results are reported below.

Materials and Methods

Starfish
A specimen of A. planci was caught at Chichijima in the Ogasawara Islands in September 1983, transported to our laboratory in frozen state and stored at -20°C until use.

Extraction
Spines collected from the specimen were homogenized with an equal volume of buffered saline (0.01 M phosphate buffer containing 0.15 M NaCl, pH 7.0) and centrifuged at 15,000 rpm for 20 min. The supernatant (crude venom) was subjected to assay experiments for some biological activities. The protein concentration of the crude venom was determined to be 9640 μg/ml by the method of LOWRY et al. with bovine serum albumin as a standard.

Estimation of Lethal Activity

Male mice (ddY strain) weighing about 20 g were injected intraperitoneally with 1 ml of serial two-fold dilutions of the crude venom. Two mice were used at each dilution. The LD₅₀ value was estimated by the method of LITCHFIELD and WILCOXON.

Estimation of Hemolytic Activity

Hemolytic activity against cow, horse, sheep, rabbit and guinea pig erythrocytes was determined in the absence of lecithin (l-α-phosphatidylcholine prepared from egg yolk, Sigma) as reported previously. The percentage of hemolysis was...
measured from the absorbance at 542 nm of the hemoglobin released. One hemolytic unit (HU) was defined as the amount of protein required to cause 50% hemolysis. Hemolytic activity against sheep and rabbit erythrocytes in the presence of lecithin was also examined. In this case, to the assay system was added 200 µg of lecithin dissolved in 0.1 ml of ethanol. The addition of the lecithin solution had no ill effect on the estimation of hemolytic activity.

Estimation of Edema-forming Activity

Edema-forming activity was assayed according to the method of Yamakawa et al. Groups of five mice were injected with 25 µl of venom solution (containing various amounts of venom in buffered saline) in the right foot pad and with 25 µl of buffered saline in the left foot pad. After 2 h, the mice were sacrificed by chloroform inhalation and both feet were cut and weighed. Edema ratio was expressed as the percentage in weight of the envenomated foot relative to the weight of saline injected foot. Minimum edema dose (MED) was defined as the least quantity of venom inducing 130% of the edema ratio.

Estimation of Capillary Permeability-increasing and Hemorrhagic Activities

For the estimation of capillary permeability-increasing activity the venom solution (0.1 ml) was administered intradermally in the depilated skin of Wistar male rats weighing 100-150 g and then 1% Evans Blue solution (0.1 ml) was injected intravenously to trace permeability changes. The rats were killed 2 h later and the cross diameters of each blue spot were measured from the visceral side. To determine the hemorrhagic activity the injection of Evans Blue solution was omitted and the cross diameters of each hemorrhagic spot were measured. Minimum permeability-increasing dose (MPID) and minimum hemorrhagic dose (MHD) were defined as the least quantity causing a 10-mm blue spot and a 10-mm hemorrhagic spot, respectively.

Qualitative Assay of Myonecrotic Activity

The venom (0.1 ml) was injected intramuscularly into the thigh of mice. After 3 h, the mice were sacrificed and tissue samples from skeletal muscle of the thigh were obtained and immediately fixed with formaldehyde solution. The tissues were embedded in paraffin, sectioned, stained with hematoxylin-eosin and observed microscopically.

Results

Lethal Activity

The venom was lethal to mice when administered intraperitoneally and the LD₅₀ was 2.7 mg/kg. Although only two mice were used at each dose, a fairly good correlation between the venom dose and the death time of mice was observed (Fig. 1). The venom induced flaccidity, numbness and occasional convulsion in mice and eventually killed them. The speed of the ill symptoms seemed to be dose-dependent. At the doses of more than 1000 µg/mouse the symptoms were observed immediately after injection of the venom and mice killed within 10 h while at nearly the LD₅₀ spontaneous movements were observed during a period of about 24 h after injection and then the symptoms, followed by death in 48-96 h, appeared.

Hemolytic Activity

Although the activity was rather weak (65–211

![Fig. 1. Dose-death time curve for crude venom from A. planci.](image)

Table 1. Hemolytic activity of crude venom from A. planci

<table>
<thead>
<tr>
<th>Erythrocytes from:</th>
<th>Hemolytic activity (HU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-Lecithin*¹</td>
</tr>
<tr>
<td>Cow</td>
<td>65</td>
</tr>
<tr>
<td>Horse</td>
<td>67</td>
</tr>
<tr>
<td>Sheep</td>
<td>85</td>
</tr>
<tr>
<td>Rabbit</td>
<td>182</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>211</td>
</tr>
</tbody>
</table>

*¹ Assay system: venom in buffered saline (3 ml)+2% erythrocyte suspension (1 ml).

*² Assay system: venom in buffered saline (2.9 ml)+200 µg of lecithin in ethanol (0.1 ml)+2% erythrocyte suspension (1 ml).
Biological Activity of Acanthaster planci Venom

Fig. 2. Relationship between venom dose and edema ratio. Each point represents mean ± S.D. of 5 mice.

Edema-forming Activity
The edema-forming activity was positive and the maximum edema ratio was obtained 2 h after injection. As illustrated in Fig. 2, the edema ratio correlated linearly with the venom dose. From the dose-edema ratio relationship, the MED was calculated to be 9.0 μg.

Capillary Permeability-increasing and Hemorrhagic Activities
As shown in Fig. 3, the venom exhibited both capillary permeability-increasing and hemorrhagic activities. The capillary permeability-increasing activity was much higher than the hemorrhagic activity; the MPID was 5.3 μg and the MHD was approximately 120 μg. The blue spot induced by 964 μg of the venom was extremely dark in color.

HU/ml), the venom apparently caused lysis of erythrocytes from different animals in the absence of lecithin in the assay system (Table 1). Significantly, the hemolytic activity against sheep erythrocytes was 20 fold higher in the presence of lecithin than in its absence and that against rabbit erythrocytes 15 fold higher.

Edema-forming Activity
The edema-forming activity was positive and the maximum edema ratio was obtained 2 h after injection. As illustrated in Fig. 2, the edema ratio correlated linearly with the venom dose. From the dose-edema ratio relationship, the MED was calculated to be 9.0 μg.

Capillary Permeability-increasing and Hemorrhagic Activities
As shown in Fig. 3, the venom exhibited both capillary permeability-increasing and hemorrhagic activities. The capillary permeability-increasing activity was much higher than the hemorrhagic activity; the MPID was 5.3 μg and the MHD was approximately 120 μg. The blue spot induced by 964 μg of the venom was extremely dark in color.

HU/ml), the venom apparently caused lysis of erythrocytes from different animals in the absence of lecithin in the assay system (Table 1). Significantly, the hemolytic activity against sheep erythrocytes was 20 fold higher in the presence of lecithin than in its absence and that against rabbit erythrocytes 15 fold higher.

Edema-forming Activity
The edema-forming activity was positive and the maximum edema ratio was obtained 2 h after injection. As illustrated in Fig. 2, the edema ratio correlated linearly with the venom dose. From the dose-edema ratio relationship, the MED was calculated to be 9.0 μg.

Capillary Permeability-increasing and Hemorrhagic Activities
As shown in Fig. 3, the venom exhibited both capillary permeability-increasing and hemorrhagic activities. The capillary permeability-increasing activity was much higher than the hemorrhagic activity; the MPID was 5.3 μg and the MHD was approximately 120 μg. The blue spot induced by 964 μg of the venom was extremely dark in color.
(Fig. 3A-1), suggesting the synergistic effect of the hemorrhagic activity.

Myonecrotic Activity

The myonecrotic activity was assayed qualitatively and found to be positive. The results of the microscopic examination of the muscle tissue at the site of injection are shown in Fig. 4. At a dose of 96 μg the venom caused vacuoles in some muscle fibers and necrosis in some. Normal muscle fibers were also present together with vacuolated and necrotic fibers. At a dose of 964 μg much severer necrosis with complete destruction of cell structure was observed. The necrosis was accompanied with infiltration of leucocytes.

Discussion

The presence of lethal and hemolytic activities, which had been reported by TAIRA et al.,2) was re-confirmed in the A. planci venom. The lethal activity was observed only qualitatively by TAIRA et al.2) while it was examined semi-quantitatively in the present study; the dose-death time curve (Fig. 1) was roughly drawn and the LD₅₀ value determined. TAIRA et al.2) previously reported that the venom caused hemolysis of washed erythrocytes in the absence of lecithin. Similarly, the hemolytic activity against washed animal erythrocytes was found in the absence of lecithin. It is, however, worth mentioning that the venom can lyse sheep and rabbit erythrocytes much more strongly in the presence of lecithin than in its absence (Table 1). These results indicate that the A. planci venom contains both direct hemolytic factor(s) and indirect hemolytic factor(s), namely phospholipase(s) A which can convert lecithin into lysolecithin having hemolytic activity.

In addition to the lethal and hemolytic activities, edema-forming, capillary permeability-increasing, hemorrhagic and myonecrotic activities were newly detected in the A. planci venom. These activities seem to account for the symptoms induced by contact with the spines of A. planci much better than the lethal and hemolytic activities. In this connection it should be pointed out that mediators such as histamine and kinins such as bradykinin have edema-forming activity, capillary permeability-increasing activity, and so on.7) However, mediators and kinins are absent or, if present, trace in the A. planci venom since our preliminary experiments showed that the edema-forming and capillary permeability-increasing factors behaved as large molecular weight substances on Sephadex G-75. It is, therefore, most likely that the venom displays a variety of activities by release of mediators from various organs and tissues and/or by formation of kinins from the activation of kininogens in plasma. In fact, the venom of the sea urchin Toxopneustes pileolus, one of echinoderms in which A. planci is classified, can release histamine from guinea pig smooth muscle and rat mast cells.8,9) To establish a medical treatment for the A. planci sting, it is necessary to disclose whether the A. planci venom can release mediators and/or kinins.

Acknowledgements

The authors thank Mrs. A. ISAYAMA, Ogasawara Marine Center, for collecting the A. planci specimen and Mr. N. OKAMOTO, Tokyo University of Fisheries, for his kind advice in preparing tissue sections.

References

8) A. KIMURA and H. NAKAGAWA: Toxicon, 18, 689-693 (1980).