PHARMACOLOGICAL STUDIES ON A PLANT LECTIN ALOCTIN A
II. INHIBITORY EFFECT OF ALOCTIN A ON EXPERIMENTAL MODELS OF INFLAMMATION IN RATS

Hiroko SAITO, Toshiharu ISHIGURO*, Ken’ichi IMANISHI
and Ikuo SUZUKI**
Laboratory of Ultrastructure Research, Aichi Cancer Center Research Institute,
Nagoya 464, Japan
Accepted October 24, 1981

Abstract—A glycoprotein, Aloectin A, which was isolated from Aloe arborescens Mill, markedly inhibits adjuvant arthritis in rats and carrageenin-induced edema in rats.

In the previous paper, we demonstrated that Aloectin A (Alo A) could inhibit the growth of methylcholanthrene-induced fibrosarcoma (Meth A) in vivo but not in vitro (1). The mechanism of this growth inhibition seemed to be host-mediated. Alo A which is isolated from leaves of Aloe arborescens Mill has many biological activities including mitogenic activity for lymphocytes and cytoagglutination (2, 3). Since Aloe arborescens Mill has been known to be used as an anti-inflammatory agent in folklore medicine, we examine in the present paper whether Alo A affects adjuvant arthritis in rats by using the method of Newbould (4) and determine whether Alo A affects carrageenin-induced edema in rats by using the method of Winter et al. (5).

MATERIALS AND METHODS

1. Agents used
Indomethacin (Nippon Merck-Banyu Co., Ltd.), prednisolone (Shionogi & Co., Ltd.), carrageenin (Marine Colloid Co., Ltd., USA), liquid paraffin (Merck, Germany) and heat-killed Mycobacterium butyricum (Difco) were used. Preparation of Alo A was according to a method previously described (2).

2. Animals
Sprague-Dawley rats were obtained from Charles River Japan Inc. Kanagawa, Japan. All rats were female and had weights of about 200 g.

3. Assays for anti-inflammatory effects
a. Adjuvant arthritis formation and compound treatment: The arthritic syndrome was induced by an intradermal injection of 0.1 ml of liquid paraffin containing 0.6 mg of heat-killed Mycobacterium butyricum through a No. 20 needle into the plantar surface of the right hind foot.

Compounds to be tested were administered orally or intraperitoneally, once daily for 15 days, starting from 1 day before injection of the phlogistic agent into the foot. In a preliminary investigation, oral administration of Alo A did not affect adjuvant arthritis at the doses tested in this experiment; consequently, Alo A suspended in 0.9% NaCl solution at proper concentrations was administered intraperitoneally throughout this experiment. Indomethacin or prednisolone in aqueous suspension was administered by
gastric gavage. Controls received adjuvant, but no drug. Body weight and foot volume were recorded nearly every day.

b. Edema formation and compound treatment: Hind paw edema was induced by a s.c. injection of 0.05 ml of 1% carrageenin solution in 0.9% NaCl into the right hind foot pads of rats. Alo A suspended in 0.9% NaCl solution was intraperitoneally administered 30 min prior to the injection of the phlogistic agent. Indomethacin in aqueous suspension was administered by gastric gavage. The volume measurements were made before and at 1, 3, 4 and 5 hr after the injection of the phlogistic agent.

c. Volume measurement and calculation of % inhibition: Hind paw volume was measured by the water displacement method (6).

The effects of compounds were expressed in terms of the percentage inhibition of swelling volume in the treated group as compared with the control. The percentage inhibition was calculated as follows:

\[
\text{% inhibition} = \left(1 - \frac{a-x}{b-y}\right) \times 100
\]

where a is the mean hind paw volume of compound treated rats at the fixed times after inciter injection, b is the mean hind paw volume of control rats at the fixed times after inciter injection, x is the mean hind paw volume of compound treated rats before inciter injection, and y is the mean hind paw volume of control rats before inciter injection.

RESULTS

Effect of Alo A on adjuvant arthritis was studied in comparison with the effect of potent anti-inflammatory drugs, that is, indomethacin and prednisolone. Each compound was given daily for 15 days, the first dose being given 1 day before inciter injection. The development of the arthritic syndrome was observed by injection of adjuvant. About 10 days after inciter injection, inflamed lesions (secondary lesions) were detected on the left hind paw and in other lesions. The volume of the injected hind paw and that of the left hind paw (as a secondary lesion) were measured for calculation of the percentage inhibition of the increase in volume of the injected hind paw and that of left hind paw, respectively. Table 1 shows data obtained on the 14th day after adjuvant injection, immediately after the end of treatment, and on the 21st day, 8 days after withdrawal of the compound. Treatment with Alo A at all doses tested effectively suppressed the swelling in the injected foot and in the secondary lesion, and the optimal dose was 5 mg/kg/day. Anti-arthritic activity of Alo A given i.p. seems to

<table>
<thead>
<tr>
<th>Compound</th>
<th>Daily dose (mg/kg)</th>
<th>Therapy (days)</th>
<th>Route</th>
<th>B.W. gain Day 0−21 (g±S.E.)</th>
<th>Inhibition ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Injected hind paw Day 14</td>
<td>Secondary lesion Day 14</td>
</tr>
<tr>
<td>Aloctin A</td>
<td>0.5</td>
<td>1−13</td>
<td>i.p.</td>
<td>1.5±12</td>
<td>9.3</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>1−13</td>
<td>i.p.</td>
<td>3.9±3</td>
<td>27.0</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>1−13</td>
<td>i.p.</td>
<td>4.5±8</td>
<td>56.6</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>1−13</td>
<td>i.p.</td>
<td>5.1±1</td>
<td>29.5</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>1−13</td>
<td>i.p.</td>
<td>5.1±1</td>
<td>4.6</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>2.0</td>
<td>1−13</td>
<td>p.o.</td>
<td>-10±7</td>
<td>23.9</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>2.0</td>
<td>1−13</td>
<td>p.o.</td>
<td>0±1</td>
<td>43.2</td>
</tr>
<tr>
<td>Control</td>
<td>10±9</td>
<td></td>
<td></td>
<td>0±1</td>
<td>0</td>
</tr>
</tbody>
</table>

*Six rats per group*
be higher than that of indomethacin given p.o., and its activity was almost equal to that of prednisolone given p.o. Body weight loss of rats treated with Alo A was not observed and at the optimal dose for anti-arthritis (5 mg/kg/day), Alo A-treated rats obviously gained body weight. Throughout this experiment, no recognizable side reactions of Alo A were observed.

Since an anti-arthritic agent, N-(2-carboxyphenyl)-4-chloroanthranilic acid disodium salt, which has immunoenhancing activity, as opposed to immunosuppressive activity, has no anti-inflammatory activity (7), it is of interest to know whether Alo A possesses an anti-inflammatory activity or not. Table 2 shows the results of an experiment designed to examine this point. When Alo A was given intraperitoneally 30 min before injection of carrageenin, a marked inhibition of edema was observed 3 hr after injection of carrageenin. The effect of Alo A was dose-dependent up to 10 mg/kg, the highest dose tested.

**DISCUSSION**

The present experiments showed that Alo A inhibits adjuvant arthritis in rats and also carrageenin-induced edema. As previously reported, Alo A is a glycoprotein and exhibits various biological activities such as mitogenic activity for lymphocytes, binding reactivity for serum proteins, complement third component activation via the alternative pathway, cytoagglutinating activity, and anti-tumor activity (1–3). Alo A is a new type of anti-inflammatory agent since anti-inflammatory drugs hitherto reported are classified as steroids, non-steroids, immunosuppressive drugs, and antiphlogistic enzymes. In order to construct a reasonable hypothesis for the mode of action of Alo A, further studies are required. Although it has been reported that subplantar administration of lectins such as phytohaemagglutinin-P (PHA) and concanavalin A (Con A) into the rat hind paw produced a dose related edema (8), anti-inflammatory effects for any lectins has not been reported. Further studies are needed to determine if any other lectins affect adjuvant arthritis and carrageenin-induced edema.

**REFERENCES**


3) Suzuki, I., Saito, H. and Inoue, S.: A study of cell agglutination and cap formation on various
cells with Aloctin A. Cell Struct. Funct. 3, 379 (1979)


