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# INHIBITION OF CARRAGEENAN EDEMA FORMATION BY ORGANOTIN COMPOUNDS

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Summary: The effects of a single p.o. dose of 0.3-10 mg/kg of organotin compounds such as dibutyltin dichloride ($Bu_2SnCl_2$) and triphenyltin chloride ($Ph_3SnCl$) on the development of edema after subplantar injection of 0.5 mg of carrageenan in 0.05 ml of pyrogen-free saline were examined as compared with those of hydrocortisone. $Bu_2SnCl_2$, $Ph_3SnCl$ and hydrocortisone did not significantly inhibit the development of the first phase of edema at any dose level, but produced more than 90, 70 and 90% inhibition of the second phase, respectively, at a dose of 10 mg/kg 1 hr before the irritant. Moreover, these inhibitions of the second phase were dose-dependent.


Generally, the steroids such as glucocorticoids exert their anti-inflammatory action by preventing the release of arachidonic acid from phospholipids, chemotaxis and release of lysosomal enzymes (1-5). On the other hand, organotin compounds such as $Bu_2SnCl_2$ and $Ph_3SnCl$ have also the similar inhibitory effects on these biological inflammatory processes (6-8). These findings have focused our interest on the anti-inflammatory action of these organotin compounds.

Many assay procedures have been developed to predict the anti-inflammatory potency of compounds. Of these methods, the carrageenan edema assay is the most useful in terms of simplicity and accuracy (9). Carrageenan-induced foot edema can be severely inhibited by pretreatment with anti-inflammatory drugs. Moreover, there are two phases of edema formation over the first few hours: the first phase begins within a few minutes and is complete by one hour, while the second phase begins at about one hour and continues

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through at least three hours (10). The first phase is maintained by the release of histamine and serotonin and the second phase by prostaglandins. The kinins phase (1.5-2.5 hr) is minor and overlaps the two other phases.

This report concerns the effect of organotin compounds such as $Bu_2SnCl_2$ and $Ph_3SnCl$ on rat carrageenan foot-edema.

## MATERIALS AND METHODS

Animals: Male Sprague-Dawley rats of the SPF strain, obtained from the Charles River Breeding Laboratories, were used. Their weights were restricted to 200 ± 10 g. Food was withheld the night before an experiment.

Chemicals: Carrageenan was purchased from Nakarai Chemical Co. A 1% suspension of this irritant was made up in pyrogen-free saline and constantly agitated by ultrasonic waves. Dibutyltin dichloride ($Bu_2SnCl_2$) and triphenyltin chloride ($Ph_3SnCl$) were obtained from Tokyo Kasei Chem. Co. (Japan). Hydrocortisone was purchased from Sigma Chem. Co.. The drugs were homogenously suspended in a 1% aqueous solution of a medium viscosity methylcellulose (MC) obtained from Nakarai Chem. Co..

Assay: A 1% suspension of carrageenan in saline (0.05 ml) was injected into the plantar surface of the right hind paw, as suggested by Winter et al. (9). The control group received saline only. Right- and left-foot were cut off for weighing before or 1, 2, 3, 4 and 5 hr after the carrageenan injection. Each edema weight was represented by calculating the difference in weights of right-foot and left-foot, since the left-foot weights were usually constant regardless of treatment of drugs. Each drug (0.5 ml) was given p.o. at a dose of 0, 0.3, 1, 3 or 10 mg/kg 1 hr before the irritant.

## RESULTS

The effects of a single p.o. dose of 10 mg/kg of organotin compounds such as $Bu_2SnCl_2$ and $Ph_3SnCl$ on the course of development of carrageenan edema were examined as compared with the same dose of hydrocortisone (Fig. 1). Any of $Bu_2SnCl_2$, $Ph_3SnCl$ and hydrocortisone did not significantly inhibit the development of the first phase of edema. However, $Bu_2SnCl_2$ produced more than 90% inhibition of the second phase; this inhibitory effect was almost the same extent as that of hydrocortisone. $Ph_3SnCl$ produced more than 70% inhibition of the second phase.

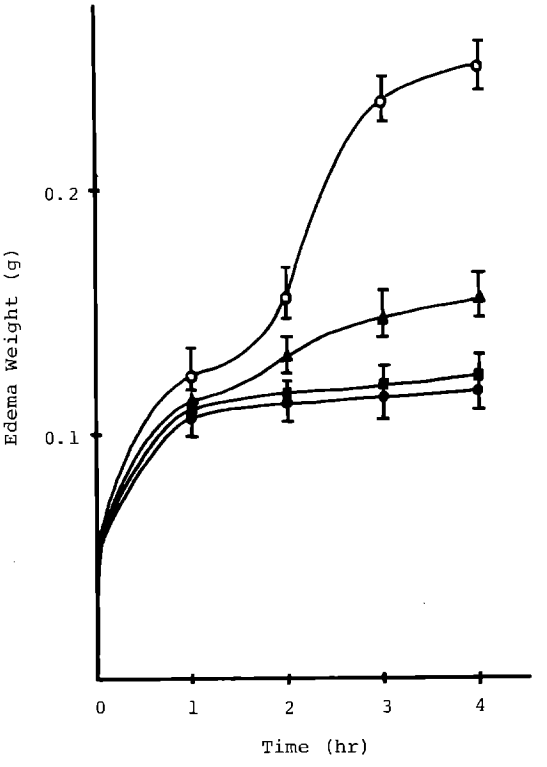


Fig. 1. Effect of organotin compounds on the development of edema after subplantar injection of 0.5 mg of carrageenan in 0.05 ml of pyrogen-free saline. The drugs were given p.o. at a dose of 10 mg /kg 1 hr before the irritant. Each point is corrected for the reading taken at each time after the saline injection as blank control. Vertical bars denote S.E. of the mean for eight determinations. (○) Positive control, (●) $Bu_2SnCl_2$, (▲) $Ph_3SnCl$, (■) Hydrocortisone.

The inhibitory effect of $Bu_2SnCl_2$ against each inflammatory phase as a function of dose is presented in Table I. $Bu_2SnCl_2$ produced strong dose-dependent inhibition of the second phase without affecting the development of the first phase. Moreover, the slope of this dose-response curve was parallel to that of the response obtained with hydrocortisone, although the range of effective dose was different each other.

## DISCUSSION

Carrageenan in a rat's foot causes not only edema and increased permeability of blood vessels, but also immigration of leuk-

TABLE I
EFFECT OF $Bu_2SnCl_2$ ON BIPHASIC CARRAGEENAN EDEMA

| Compound[a] | Dose | Edema Weight[c] | |
|---|---|---|---|
| | | First phase | Second phase |
| | mg/kg, p.o. | g ± S.E. | |
| Control, 1% MC[b] | 0 | 0.125 ± 0.011 | 0.113 ± 0.017 |
| $Bu_2SnCl_2$ | 0.3 | 0.117 ± 0.018 | 0.116 ± 0.012 |
| $Bu_2SnCl_2$ | 1.0 | 0.113 ± 0.016 | 0.074 ± 0.010 |
| $Bu_2SnCl_2$ | 3.0 | 0.111 ± 0.013 | 0.043 ± 0.006 |
| $Bu_2SnCl_2$ | 10.0 | 0.110 ± 0.015 | 0.007 ± 0.001 |

a. Drug was given 1 hr before the carrageenan.
b. Suspending agent was a medium viscosity methylcellulose.
c. Foot weight measurements were made before, 1 and 3 hr after the carrageenan injection. The first-phase weight is an increase in weight between the 1-hr reading and the reading taken before the carrageenan injection minus the reading taken 1 hr after the saline injection as blank control. The second-phase weight is the 3-hr reading minus the 1-hr reading. The values are the means ± S.E. of ten determinations.

ocytes and increase of paw temperature. Both of these latter effects are less reliable as assay methods. One direct method of measuring edema -- which was used in this study -- is to remove the rat's injected hind paw and weigh it. The weight is compared with that of the other hind paw. This method is simple and precise, although it is relatively slow and only a single datum is obtainable from each rat.

The drugs were given p.o., since i.v. and i.p. administrations are generally liable to induce inflammation-like phenomena by the drug irritation. P.O. administration did not induce such an inflammatory effect at all.

Carrageenan edema is more characteristic of acute inflammation than of chronic. Although it is not yet clear what the mediators may be that are important for acute inflammations, most likely several mediators such as prostaglandins (PGs) are brought by white cells to the site of certain inflammations and are released there

and play certain roles at various times. Arachidonic acid is a precursor of prostaglandin metabolites and the release of it seems to be the main reason for the development of inflammation.

From these point of view, it is noteworthy that organotin compounds such as $Bu_2SnCl_2$ and $Ph_3SnCl$ exert the same inhibitory effects as glucocorticoids on not only the release of arachidonic acid (6,7) and lysosomal enzymes (unpublished observation) but also chemotactic response of neutrophils (8), and that the inhibitory effects of these compounds on the release of arachidonic acid depend upon the inhibition of phosphorylation of lipomodulin (7,11). Probably, the inhibitory effects of these organotin compounds on carrageenan-induced inflammation must be also exerted by preventing the phosphorylation of lipomodulin and the subsequent release of arachidonic acid which plays an important role in the release of inflammatory mediators from leukocytes and mast cells.

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