Anti-Inflammation Mechanism of Extract from *Eisenia bicyclis* (Kjellman) Setchell

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Abstract □ An extract from *Eisenia bicyclis*, previously shown to possess anti-inflammatory activity, was found to stabilize lysosomal membranes *in vitro* as determined by measurement of inhibition of the marker enzyme β-glucuronidase. Some anti-inflammatory activity was also due to counterirritancy.

Keyphrases □ *Eisenia bicyclis* extract—mechanism of anti-inflammatory activity, lysosomal stabilization □ Anti-inflammatory activity—*Eisenia bicyclis* extract, lysosomal stabilization

Although a number of important steroidal and nonsteroidal agents are presently in use for the treatment of inflammation, the search continues for new anti-inflammatory drugs. Previous studies from this laboratory (1) indicated that an extract from the alga *Eisenia bicyclis* (Kjellman) Setchell, collected from the shores of Korea, contains a compound possessing anti-inflammatory activity. This material has been reported (2) to be a complex polymer which, on chemical degradation, yields glucose, phloroglucinol, and trimethylamine.

Several methods were used in the original studies (1) to demonstrate anti-inflammatory activity, including the inhibition of the denaturation of bovine serum albumin and the prevention of carrageenan-induced edema in the rat paw. However, the material was ineffective in suppressing rat paw edema due to the administration of complete Freund's adjuvant. Little could be determined from these initial studies regarding the mechanism of the anti-inflammatory action other than that it was not due to a thermogenic effect and that it did not involve the pituitary–adrenal axis.

It was of interest, therefore, to determine if the compound might be active by virtue of its ability to stabilize lysosomal membranes and thereby inhibit the release of lysosomal enzymes from the organelle. The release of these enzymes and subsequent tissue damage have been implicated in several types of inflammation such as chemical burns (3) and arthritis (4). That stabilization of the membrane could be achieved by anti-inflammatory drugs was demonstrated utilizing the release of the lysosomal marker enzymes β-glucuronidase and acid phosphatase (5). Comparison of the ability to stabilize the membranes was made with phenylbutazone[1], a well-known anti-inflammatory agent. In addition, it was important to determine if all or some of the anti-inflammatory effect might be due to counterirritancy.

Table I—Effect of Drugs on Lysosomal Membrane Stability *In Vitro*

Drug	Enzyme Activity[a] ± *SE* (4)[b]	
	Acid Phosphatase	β-Glucuronidase
None	4.46 ± 0.16[c]	0.66 ± 0.02[c]
Phenylbutazone (0.309 mg/ml)	4.13 ± 0.08[c,d]	0.20 ± 0.01[d]
Eisenia extract (3.087 mg/ml)	3.66 ± 0.22[d]	0.24 ± 0.03[d]
Eisenia extract (0.309 mg/ml)	3.64 ± 0.16[d]	0.24 ± 0.01[d]
Eisenia extract (0.031 mg/ml)	3.83 ± 0.22[d]	0.64 ± 0.02[c]

[a] Expressed as μmoles of product/ml of lysosomal fraction/20 min. [b] Number of animals. [c,d] Groups not having same superscript are significantly different, $p < 0.05$.

EXPERIMENTAL

Materials and Methods—Adult male albino rats[2] were housed in air-conditioned rooms and allowed food and water *ad libitum*. The procedures for the preparation of hepatic lysosomal fractions and the determination of the activities of lysosomal β-glucuronidase and acid phosphatase were basically those of Ignarro (5). A 10% liver homogenate was prepared in 0.25 *M* sucrose–0.02 *M* tromethamine acetate buffer, pH 7.4, and centrifuged at 600×*g* for 5 min at 4° in a refrigerated centrifuge[3]. The supernate was decanted, mixed with an equal volume of the buffer, and recentrifuged at 3500×*g* for 15 min.

The resulting pellet was rinsed gently twice with 0.45 *M* sucrose–0.02 *M* tromethamine acetate buffer, pH 7.4, and resuspended in 4.0 ml of the buffer. The suspension was warmed to 25°, and 0.20-ml aliquots were added to 20-ml beakers containing 2.0 ml of 0.18 *M* sucrose–0.04 *M* tromethamine acetate buffer, pH 7.4, with either 0.1 ml of drug solution or water. Incubation was carried out at 37° for 0 or 15 min in a metabolic shaker[4] and terminated by centrifugation at 27,000×*g* for 15 min at 4° in polyethylene tubes.

To determine if the drug had any direct effect on the marker enzymes, the drug was added after the incubation period. To determine the total enzyme activity, 2.0 ml of 0.2% octoxynol[5] in 0.04 tromethamine acetate buffer, pH 7.4, replaced the buffer.

Acid phosphatase was determined by measuring the formation of *p*-nitrophenol from *p*-nitrophenyl phosphate, and β-glucuronidase activity was determined by measuring the formation of phenolphthalein from phenolphthalein glucuronide (5). One milliliter of the high-speed supernate and 1 ml of water were added to 1 ml of 0.3 *M* citrate buffer, pH 4.8. Incubation was initiated by the addition of 0.04 ml of substrate [*p*-nitrophenyl phosphate (79 mg/ml) or phenolphthalein glucuronide (63 mg/ml)]. Acid phosphatase incubations were terminated with 0.2 ml of 4 *N* NaOH, and absorbances were read at 405 nm. β-Glucuronidase assays were stopped with 0.4 ml of 2.2 *M* glycine–sodium hydroxide buffer, pH 12, and read at 540 nm.

Measurement of anti-inflammatory activity was made using a carrageenan-induced rat paw edema test system. The rat hindpaw was injected in the subplantar region with 0.1 ml of 1.0% carrageenan in saline, and the paw volume was determined by a plethysmographic method described by Van Arman *et al.* (6). The volume was measured 3 hr later, and edema formation was calculated as the difference.

Statistical analysis of the data from the lysosomal stabilization data comparing various doses was carried out using Duncan's new

[1] Butazolidin.
[2] Charles River Breeding Laboratories.
[3] Sorvall RC2-B.
[4] Dubnoff.
[5] Triton X-100.

Table II—Effect of Buffering *Eisenia* Extract on Carrageenan-Induced Rat Paw Edema

Treatment[a]	Mean Edema, $mm^3 \pm SE$ (6)[b]
Saline	1174 ± 99
Buffered[c] *Eisenia* extract	678 ± 176[d]

[a] Administered intraperitoneally 1 hr before carrageenan. [b] Number of animals. [c] Buffered at pH 7.4 with 0.2 *M* KH_2PO_4–Na_2PO_4. [d] $p < 0.05$.

multiple-range finding test. Comparison of the anti-inflammatory activity data utilized the Student two-tailed *t* test (7).

RESULTS AND DISCUSSION

The results of testing the activity of the *Eisenia* extract for ability to stabilize lysosomal membranes are presented in Table I. They have been corrected for any direct inhibition of the enzyme. Acid phosphatase was a poor indicator of lysosomal membrane condition, since the powerful stabilizing agent phenylbutazone, which was used as a positive control, inhibited enzyme release by only 7%. However, phenylbutazone (0.309 mg/ml) and the extract at a concentration of either 3.087 or 0.309 mg/ml offered significant inhibition of release of β-glucuronidase. At the lower extract concentration of 0.031 mg/ml, there was no protection.

Since some compounds have been shown to be anti-inflammatory because of their irritant properties (8), it was important to determine if the compound might be acting as an anti-inflammatory agent by virtue of its acidity; it normally was injected at pH 4.5. When the material was buffered at pH 7.4 and anti-inflammatory activity was assessed (Table II) using the carrageenan-induced rat paw edema model, there was only 42% inhibition of inflammation. This value is somewhat lower than the expected 88% (1), indicating that the protection afforded by the *Eisenia* extract is due in part to a nonspecific counterirritancy.

It appears that there are two mechanisms for the anti-inflammatory activity of the complex polymer isolated from *E. bicyclis* (Kjellman) Setchell. One is its ability to stabilize the membranes of lysosomes and inhibit the release of the destructive lysosomal enzymes, and the other is a counterirritancy effect.

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Synthesis and Antimicrobial Activity of *N*-Substituted *N'*-Cyano-*S*-(triorganostannyl)isothioureas

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Abstract □ Six *N*-substituted *N'*-cyano-*S*-(trimethylstannyl)isothioureas were synthesized by the reaction of (trimethylstannyl)cyanamide with various organic isothiocyanates. The IR spectrum of each compound was obtained over the 4000–30-cm^{-1} range, and some bands were assigned. The six new compounds and five previously synthesized *N*-substituted *N'*-cyano-*S*-(triphenylstannyl)isothioureas were tested for and were found to exhibit antifungal activity. *N*-Phenyl-*N'*-cyano-*S*-(triphenylstannyl)isothiourea was also investigated for antibacterial activity and was observed to be especially inhibitory toward Gram-positive species. The antimicrobial activity of two compounds was compared to that of the oxygen analogs of these compounds.

Keyphrases □ *N'*-Cyano-*S*-(triorganostannyl)isothioureas, *N*-substituted—synthesis, IR spectra, antimicrobial activity □ Antifungal activity—synthesis of six and evaluation of 11 *N*-substituted *N'*-cyano-*S*-(triorganostannyl)isothioureas □ Antibacterial activity—evaluation of *N*-phenyl-*N'*-cyano-*S*-(triphenylstannyl)isothiourea

Recently, the synthesis of 13 *N*-substituted *N'*-cyano-*O*-(trimethylstannyl)isoureas (I*a*) and 12 *N*-substituted *N'*-cyano-*O*-(triphenylstannyl)isoureas (I*b*) was described (1). Six of these compounds were tested for and were found to exhibit antifungal activity; one (I*b*, R' = C_6H_5) was also investigated for an-