# FURTHER STUDIES ON ANTI-INFLAMMATORY ACTIVITY OF TWO POTENT INDAN-1-ACETIC ACIDS

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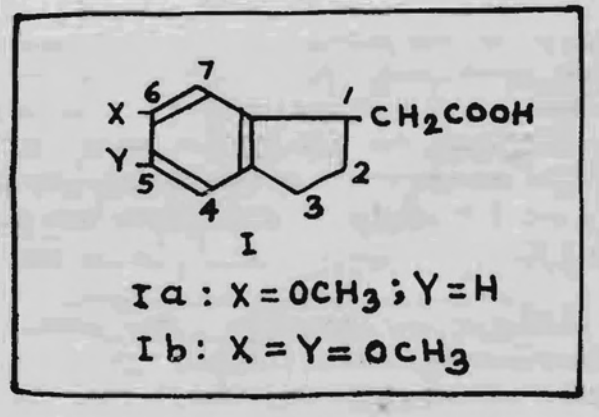
**Summary :** The anti-inflammatory activity of 6-methoxyindan-1-acetic acid (1a) and 5,6-dimethoxyindan-1-acetic acid (1b) was evaluated in various acute, subacute and chronic models of inflammation. The results of these studies suggest that they have equal or slightly more anti-inflammatory activity than phenylbutazone, a standard anti-inflammatory drug. Of the two compounds, 5,6-dimethoxyindan-1-acetic acid appeared to be slightly more active than 6-methoxyindan-1-acetic acid.

**Key words :** anti-inflammatory activity  Indan-1-acetic acids  phenylbutazone

## INTRODUCTION

In a previous publication (4) we reported significant anti-inflammatory activity among a series of indan-1-acids in acute carrageenin-induced oedema method. Two of those compounds namely, 6-methoxyindan-1-acetic acid (1a) and 5,6-dimethoxyindan-1-acetic acid (1b) possessed anti-inflammatory potency close to that of phenyl-butazone, a standard anti-inflammatory drug. Therefore, these two compounds were further evaluated in various acute, subacute and chronic inflammation models. The results of the tests conformed well with our earlier observation (4) and constitute the subject matter of the present paper.

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Ia : X = OCH3 ; Y = H

Ib : X = Y = OCH3

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*d) 5-HT-induced oedema* (2) : 0.05 *ml* of 0.02% 5-HT was injected into the right hind paw of each rat 1 hr after oral administration of test agent, phenylbutazone, or saline and the paw volumes were measured at 2 and 3 hr after the administration of the phlogistic agent. The average increase in paw volume was compared with that of the control group (saline).

*e) Cotton-pellet granuloma :* The method used was essentially that of Winter and Porter (7) with slight modification. Dry sterilized cotton pellets weighing 10±0.5 *mg* were inserted subcutaneously at both the groin regions of each rat. From the next day, the test agent, phenylbutazone, or saline was administered orally daily for 7 days to groups of 6 animals each. Twentyfour hr following the last administration, the animals were sacrificed, the pellets surrounded by granulation tissue were removed, cleaned and then dried at 60°C for 24 hr. The dry weights of these pellets minus the weight of cotton inserted gave the dry granuloma weights. The average values of each treated group were compared with that of the control group (saline).

*f) Adjuvant-induced arthritis method :* The method was essentially that of Newbould (3). Rats were divided at random into groups of 6 animals each. Starting from the day before the administration of 0.05 *ml* of Freund's complete adjuvant (Difco) into the plantar surface of right hind paw of each rat, each group received orally either the test agent, phenylbutazone, or saline daily for 14 days Both the hind paw volumes upto a fixed mark at the level of lateral malleous were measured before and daily after adjuvant administration till 13th day Results were expressed as the percent increase in paw volumes above the initial volume and the average value of each drug-treated group was compared with that of the control group (saline). The formation of nodules and appearance of erythema in tail, nose and ears were observed and graded arbitrarily as mild, moderate and severe for comparison. Change in body weight of the animals during the test period was also recorded for comparison.

## RESULTS AND DISCUSSION

The results with carrageenin-oedema and kaolin oedema tests indicated that the peak activities of indan-l-acetic acids and of phenylbutazone were reached at 3 hr. In all the cases the activity declined very slowly; significant activity was observed even at 24 hr after the drug administration. The residual activity of both the indan-1-acetic acids at 24 hr was significantly higher than that of phenylbutazone (Tables I and II). The

longer duration of action of the indan-1-acetic acids than phenylbutazone may be due to their rapid binding to protein and subsequent slow release when the unbound acids having high pKa values (6.66 to 6.74) (1) are slowly dissociated to their anions which are expected to be the pharmacophores.

Formalin-induced oedema was only weakly inhibited by both the test agents (Table III) whereas none of them produced any effect on 5-HT-induced oedema at 50-100 *mg/kg* dose levels. This observation suggests that the anti-inflammatory activity of these compounds is not dependent on 5-HT antagonism and the mechanism of formalin-induced inflammation is somewhat different from that produced by carrageenin or kaolin and is only partly affected by these compounds.

TABLE I : Anti-inflammatory activity of indan-1-acetic acids compared to that of phenylbutazone in carrageenin-induced oedema in rats.

(Values are average 6 animals per dose)

| *Compound No.* | *Dose mg/kg (oral)* | *Percent increase in paw volumes (mean±SE)* | | | |
|---|---|---|---|---|---|
| | | *2 hr*• | *3 hr* | *4 hr* | *23 hr* |
| Ia | 100 | 56.8±3.35[b] (34.64)† | 64.5±3.72[a] (40.50) | 75.6±2.40[a] (37.83) | 48.2±1.86[b] (29.12) |
| | 50 | 62.7±3.12[b] (27.85) | 72.8±2.87[a] (32.84) | 84.3±3.41[a] (30.67) | 53.4±2.24[c] (21.47) |
| Ib | 100 | 54.2±2.96[a] (37.63) | 60.4±2.48[a] (44.28) | 73.8±2.93[a] (39.31) | 48.6±1.68[b] (28.53) |
| | 50 | 62.0±2.85[b] (28.65) | 71.2±3.54[b] (34.32) | 83.5±2.76[a] (31.33) | 52.9±2.81[d] (22.21) |
| Phenylbutazone | 100 | 48.5±2.75[a] (44.19) | 61.7±3.31[a] (43.08) | 75.3±2.58[a] (38.07) | 54.1±2.32[d] (20.44) |
| | 50 | 58.7±3.28[b] (32.45) | 73.2±2.38[a] (32.47) | 84.6±3.30[a] (30.43) | 57.4±2.98[e] (15.59) |
| Saline (control) | — | 86.9±3.48 | 108.4±4.13 | 121.6±3.82 | 68.0±2.81 |

a-e Probability values (calculated as compared to control using Student's t-test) : a<.001, b<.005, c<.01, d<.025, e<.05.

† Figures in parentheses represent the percent inhibition of oedema.

• Time after carrageenin administration.

TABLE II : Anti-inflammatory activity of indan-1-acetic acids compared to that of phenylbutazone in kaolin-induced oedema in rats.

(Values are average of 6 animals per dose)

| *Compound No.* | *Dose mg/kg (oral)* | *Percent increase in paw volumes (mean±SE)* | | |
|---|---|---|---|---|
| | | *3 hr* | *4 hr* | *5hr* |
| Ia | 100 | 33.0±2.42[a] (48.28)[d] | 40.2±3.14[a] (44.63) | 47.4±2.61[a] (42.54) |
| | 50 | 39.8±3.06[b] (37.62) | 46.6±3.78[b] (35.81) | 54.2±2.94[a] (34.30) |
| Ib | 100 | 30.5±3.18[a] (52.19) | 36.8±2.44[a] (49.31) | 44.5±2.75[a] (46.06) |
| | 50 | 36.2±2.7[b] (43.26) | 42.8±3.11[b] (41.05) | 51.4±3.42[b] (37.70) |
| Phenylbutazone | 50 | 38.7±2.81[b] (39.34) | 44.5±3.04[b] (38.70) | 52.6±3.32[b] (36.24) |
| | 25 | 42.9±2.98[b] (32.76) | 49.8±3.88[c] (31.40) | 57.6±2.66[b] (30.18) |
| Saline (control) | — | 63.8±3.12 | 72.6±3.46 | 82.5±2.89 |

[a-c] Probability values (calculated as compared to control using Student's t-test) : a<.001, b<.005, c<.01.

[d] Figures in parentheses represent the percent inhibition of oedema.

Both the indan-1-acetic acids (Compound Ia and Ib) and phenylbutazone almost equally inhibited the granuloma formation around cotton pellets (Fig. 1). Animals treated with compound Ia, Ib and phenylbutazone gained weight almost equally whereas those treated with saline (control group) lost weight. Actually, the drug-treated animals regained their initial weight loss within 5-6 days after the insertion of cotton pellets.

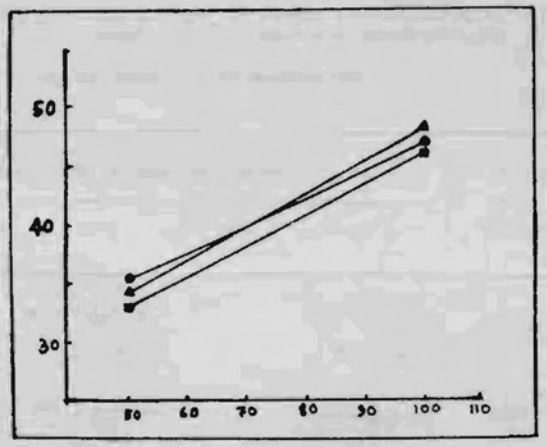


Fig. 1 : Inhibition of granuloma formation by indan-1-acetic acids compared to phenylbutazone in cotton-pellet granuloma method in rats

Ordinate : Percent inhibition of granuloma formation.

Abscissa : Oral dose (*mg/kg*).

Compound Ia = ●, Compound 1b = ▲, Phenylbutazone = ■

Adjuvant-induced arthritis test in rats produced a biphasic response. The initial response started immediately after adjuvant administration and reached maximum on

TABLE III : Inhibitory effects of indan-1-acetic acids compared to that of phenylbutazone on formalin-induced oedema in rats.

(Values are average of 6 animals per dose)

| *Compound No.* | *Dose (mg/kg, p.o.)* | *Percentage inhibition of oedema at 3 hr** |
|---|---|---|
| Ia | 100 | 18.6 |
| | 50 | 15.3 |
| Ib | 100 | 22.7 |
| | 50 | 18.1 |
| Phenylbutazone | 100 | 28.4 |
| | 50 | 20.5 |

*Time after formalin administration.

3rd day and then it started subsiding. The 2nd phase response started from the 9th day and continued till the end of the experiment (13th day). Secondary lesion (appearance of nodules and erythema in tail, nose and ears) started developing from the 10th day. Results of this test as summarized in Table IV show that indan-1-acetic acids (compound no. Ia and Ib) and phenylbutazone are almost equally active in inhibiting the initial increase in paw volumes (3rd day) but the former are slightly more active than the latter in the subsequent phase. This may be due to the higher residual activity of indan-1-acetic acids (compouod no. Ia and Ib) than phenylbutazone at 24 hr as observed in carrageenin-oedema test (Table I). It is seen from Table IV that both the indan-1-acetic acids (compound no. Ia and Ib) almost equally inhibit the development of secondary lesions and are better than phenylbutazone in this respect. Table IV also shows that the animals treated with the test agents or phenylbutazone gained more weight than those treated with saline during the course of the experiment.

TABLE IV. Inhibitory effects of indan-1-acetic acids compared to that of phenylbutazone on adjuvant-induced arthritis in rats

(Values are average of 6 animals per dose)

| *Compound No.* | *Daily oral dose mg/kg* | *Percentage increase in hind paw volumes (mean±SE)* | | | | *Degree of sec. lesion* | *Mean body weight change g/100 g* |
|---|---|---|---|---|---|---|---|
| | | *injected* | | | *Uninjected* | | |
| | | *3rd day* | *8th day* | *13th day* | *13th day* | | |
| Ia | 100 | 78.3±3.11[b] (25.71)[f] | 47.6±3.66[a] (42.37) | 48.6±2.42[a] (50.05) | 24.2±2.84[c] (44.37) | Mild | +11.2 |
| | 50 | 86.7±3.90[d] (17.74) | 54.0±3.18[b] (34.62) | 57.2±3.45[a] (41.21) | 29.0±3.34[e] (33.33) | Moderate | + 8.1 |
| Ib | 100 | 79.2±3.40[b] (24.85) | 47.4±2.81[a] (42.61) | 46.8±3.86[a] (51.90) | 22.3±1.68[b] (48.73) | Mild | +11.6 |
| | 50 | 85.4±3.26[d] (18.97) | 55.2±2.94[b] (33.17) | 55.2±3.38[a] (43.27) | 27.6±3.72[d] (36.55) | Moderate | +7.5 |
| Phenyl-butazone | 100 | 76.6±4.18[b] (27.32) | 49.2±3.43[a] (40.44) | 50.0±3.60[a] (48.61) | 23.5±2.77[b] (45.98) | Moderate | +9.4 |
| | 50 | 83.8±3.45[e] (20.49) | 55.5±2.86[b] (32.81) | 59.7±3.28[a] (38.64) | 29.8±3.30[e] (31.49) | Moderate | +6.8 |
| Saline (control) | | 105.4±3.82 | 82.6±3.35 | 97.3±3.62 | 43.5±3.05 | Severe | +3.5 |

[a–e] Probability values (calculated as compared to control using Student's t-test) : a<.001, b<.005, c<.01, d<.025, e<.05.

[f] Figures in parentheses represent percent inhibition of foot oedema.

It appears from these studies that the test compounds (indan-1-acetic acids) are equipotent or slightly more potent anti-inflammatory agents than phenylbutazone having longer duration of action than the latter and their responses followed a dose-related pattern. Preliminary studies on toxicity of these potent indan-l-acetic acids also indicate that they are much less toxic (acute oral $LD_{50}$>1000 *mg/kg*) and ulcerogenic (minimum effective dose ~ 300 *mg* per *kg*) than phenylbutazone. Such reduced ulcerogenicity of these indan-l-acetic acids is likely due to their high pKa values (6.66 - 6.74) which remain very close to the normal pH of blood (7 4) (1).

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