ANTI-INFLAMMATORY, ANALGESIC AND ANTIPYRETIC ACTIVITIES OF 6-CHLORO-5-CYCLOHEXYLINDAN-1-CARBOXYLIC ACID (TAI-284)

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Since the discovery of a dramatically potent antiphlogistic or antirheumatic effect of cortisone, a number of glucocorticoid derivatives were synthesized for pharmacological evaluation and some of them have been introduced to clinical use in rheumatic diseases and other various inflammatory disorders. The large doses or prolonged use of glucocorticoids, however, produced a variety of clinical side effects such as gastro-intestinal lesion and impaired protection against infection mainly due to adaptive adrenal atrophy and immunosuppression. Enthusiastic efforts to dissociate the therapeutic effects from adverse ones in chemical structure of glucocorticoids have all resulted in failure.

Consequently, chemical and pharmacological investigations to search for new non-steroidal antiphlogistics different in mode of action from glucocorticoids and less in toxicity have been revived with successful introduction of some agents on the market. They consist of a variety of chemical compounds including weak organic acids such as phenylbutazone, ibufenac, and indomethacin, and moderately strong base such as benzydamine.

Research largely concentrated upon various weak organic acids effective against inflammatory processes in experimental animals in this laboratory has resulted in presentation of 6-chloro-5-cyclohexylindan-1-carboxylic acid (TAI-284), which has strong antiinflammatory, analgesic, and antipyretic activities. The structure is as follows:

Since no single assessment can represent the general aspects of the drug and provides a satisfactory evaluation for anti-inflammatory agents in the screening process (1), a variety of phlogistic materials, experimental animals, or sites of inflammation are required. In addition, according to Winter (2) certain less specific means of producing inflammation in the screening methods may give an efficient evaluation of anti-inflammatory agents clinically used.

Therefore, to assess the anti-inflammatory activities of TAI-284, a variety of substances such as carrageenin, kaolin, acetic acid, croton oil, sodium urate, and Freund’s complete adjuvant were used as phlogistic agents, and ultraviolet irradiation and cotton pellets were also applied.
METHODS

In addition to TAI-284, indomethacin, phenylbutazone, mefenamic acid, ibufenac, aspirin and aminopyrine were used. For oral administration all the test agents except for aminopyrine dissolved in water, were suspended with 4% gum acacia in water. For intravenous injection of the former two agents, they were dissolved by adding an equimolar amount of NaOH, or their sodium salts were used.

Animals used were SD-JCL rats, ICR-JCL and CF\textsubscript{1}-JCL mice, Hartley albino guinea-pigs, albino rabbits, and mongrel dogs. Rats and mice were specific-pathogen free.

All the anti-inflammatory, analgesic, and antipyretic assays except for the granuloma pouch and cotton pellet methods were performed under the blind condition. Thus, the administration of test agents and the assay were performed by separate operators who were not informed of the agents and doses.

By statistical treatment of the assayed values obtained, the mean value and standard error were routinely presented. The relative potency of TAI-284 to each referential agent was obtained by the 4-point or 6-point assay.

1. **Anti-inflammatory assays**

1. **Carrageenin edema**

Following the method of Winter et al. (3), the measurement of the volume of unilateral hind paw of male rats weighing 190 ± 10 g was followed by the oral administration of test agents in various doses. One hour after the administration of drugs, 0.05 ml of 1% carrageenin suspension in physiological saline was injected subcutaneously into the plantar side of the hind paw, and 3 hours later the edema thus developed was estimated by measuring the volume of the paw. The edema in the treated rats was compared with that in the control animals.

The maximal edema caused by carrageenin was attained from 3 to 6 hours after the injection and the considerable swelling of the injected paw was still present at 24 hours, though it declined gradually.

In addition, male rats weighing 160 ± 10 g were bilaterally adrenalectomized, then maintained on 1% saline as a drinking water and used for the carrageenin-edema test 4 days after the operation.

2. **Kaolin edema**

Since the subcutaneous injection of kaolin into a hind paw was reported to produce a long-lasting local edema (4), 0.05 ml of 10% kaolin suspended with 4% gum acacia in physiological saline was used following the procedure described above. Due to the presentation of the maximal kaolin edema 5 hours after the injection, the anti-inflammatory effects of test agents orally administered were evaluated at 5 hours.

3. **Edema induced by miscellaneous irritants**

In a similar procedure to the carrageenin edema test, 0.05 ml of 0.02% serotonin, 1% formalin, 2.5% brewer's yeast, 2.5% mustard or 1% dextran as well as 0.1 ml of 10% egg white were injected one hour after administration of test agents.
In addition, the edema induced by 0.1 ml of 0.1% trypsin or 0.02% bradykinin was estimated 2 hours after the injection.

4. Edema in mice

To produce the hind paw edema, ICR-JCL male mice weighing 23±2 g were injected with 0.025 ml of 1% carrageenin suspended in physiological saline subcutaneously at the right foot and with the same volume of the vehicle at the left one. Four hours thereafter those animals were sacrificed by cervical vertebral dislocation and each hind foot was cut at the tars-o-tibial joint. Edema was estimated by the differential weight of both paws. Test agents were orally administered 30 minutes previous to the carrageenin injection.

5. Ultraviolet erythema

Following the procedure of Winder et al. (5), the irradiation of ultraviolet rays delivered from a Hanovia Analytical Model Quartz Lamp for one minute on the depilated skin of a flank in guinea-pigs weighing about 350 g produced the early and secondary phases of vascular dilatation at the site of irradiation. The early response was ascribed by Logan and Wilhelm (1963) to a local release of histamine. The secondary delayed response appeared at about 2 hours lasting for more than 24 hours.

Test agents were administered orally twice, one hour before and immediately after the irradiation. Two hours after the irradiation the degree of the erythema was scored in each animal. The anti-inflammatory activity was expressed as ED50.

6. Intraperitoneal dye leakage

Following the method of Whittle (6), 0.2 ml/animal of 0.25% Evans blue dissolved in physiological saline was injected intravenously in ICR-JCL mice weighing about 20 g at 25 minutes after an oral administration of either test agent. Five minutes thereafter, 0.1 ml/10 g body weight of 0.6% acetic acid was injected intraperitoneally. The dye in the peritoneal exudation, sampled 30 minutes after the injection of acetic acid, was subjected to the spectrophotometric estimation.

In the same procedure in rats, the volume of 0.5% dye solution was 0.5 ml/animal and that of 3% acetic acid was 0.2 ml/100 g body weight.

7. Granuloma pouch

To evaluate an inhibitory effect on inflammatory exudation, following the method of Robert and Nezamis (7), an air pouch of 25 ml was formed subcutaneously at the back of female rats, and 0.5 ml of 1% croton oil dissolved in arachis oil was injected into the pouch. Two days later whole air in the pouch was withdrawn to promote exudation. Whole procedure hitherto was performed aseptically. Test agents were orally administered daily for 5 days from the day of the pouch formation. On the next day of final administration the volume of exudate in the pouch was measured.

8. Cotton pellet

Inhibition against the tissue proliferation resulting from chronic exposure to cotton pellets or irritative substances proved to be one of reliable approaches to the discovery of indomethacin.

Following the method of Winter et al. (8), in each male rat two sterilized cotton pellets
weighing 30±1 mg respectively were embeded subcutaneously at the lateral abdomen aseptically. The test agents were orally administered daily for 7 days. On the next day of final administration, the pellets were removed, dried, and weighed to determine the weight of the granuloma formed around the pellets.

9. Adjuvant arthritis

An arthritic syndrome was reported to develop systemically in rats after an injection of Freund's complete adjuvant (9).

The intradermal injection of 0.05 ml of 0.5% Freund’s complete adjuvant suspended in liquid paraffin at the plantar surface of unilateral hind paw in rats produced the primary inflammation, which developed progressively at the injected foot within 24 hours, and also the secondary lesions (redness and swelling), which appeared progressively at non-injected limbs, tail, and ears from about 10 days.

In addition to the estimation of the paw edema volume, the degree of secondary lesions described above was scored in 0 (no abnormality), 1 (slight), and 2 to 5 according to the inflammatory progression. Test agents were orally administered from the day before the adjuvant injection daily for 14 days.

10. Urate arthritis

The intra-articular injection of sodium urate at the dog knee joint was reported to produce an accumulation of intracapsular exudate (10). The oral administration of test agents to the pentobarbital-anaesthetized dogs was followed, one hour later, by the intra-articular injection of 15 mg of sodium urate suspended in 1.5 ml of physiological saline. The latter procedure resulted in the accumulation of intracapsular exudate 4 hours later. By comparing the exudate thus induced between in the treated animals and in non-treated ones, the anti-inflammatory activity was evaluated.

11. Effect of combination with dexamethasone

To evaluate the combination effect, dexamethasone suspended with 4% gum acacia in water was orally administered alone or in combination with TAI-284 to rats in the carrageenin edema and granuloma pouch tests.

12. Bronchospasm

Following the method of Collier et al. (29), guinea-pigs weighing 400±50 g were anaesthetized with 1.25 g/kg of urethane intraperitoneally injected and were subjected to the artificial respiration through a tracheal canula. Five ml of air was forced to inspire at a frequency of 72/minute and expired air was led to flow out. The increase in resistant pressure to inspired air induced by bronchoconstriction was recorded on a polygraph via transducer. Test agents were given through a polyethylene tube inserted into jugular vein.

1) Bradykinin

After recording a few control responses by 25 μg/animal of bradykinin, test agents were administered in a volume of 0.2 ml/100 g body weight. Five or 30 minutes thereafter the response to bradykinin was recorded again. The inhibitory effects of test agents were evaluated by the differential responses.

2) Histamine
Animals were treated in the same procedure as described above, and histamine in the dose of 2 µg/animal was used as a broncho-constrictor agent at least 10 minutes after the bradykinin response.

3) Serotonin

The broncho-constriction responses were induced by 4 µg/animal of serotonin. The administration of test agents was followed, 5 or 15 minutes later, by the similar response.

4) Acetylcholine

Acetylcholine in 8 µg/animal was used in the same procedure as that of serotonin.

5) ATP

The dose of 6 mg/animal was used.

6) Antigen-antibody reaction

Animals were treated with subcutaneous and intraperitoneal injections of 1 ml of 10% egg albumin (Difco), respectively. Two weeks later, 1 ml of 10% Zn-egg albumin was injected subcutaneously, and one week thereafter the animals thus treated were subjected to the following bronchospasm test. The intravenous administration of test agents was followed 5 minutes later by the bronchospasm induced by 10 mg/kg of egg albumin. Control animals were treated with physiological saline before the challenge. The inhibitory effect of test agents was obtained by comparison between the responses in control animals and those in treated ones.

13. Liver glycogen deposition

It is known that glucocorticoid activity runs parallel with the anti-inflammatory activity of pregnane derivatives (11). As the first indication of the potent anti-inflammatory activity of a novel steroid, it has been a fairly common practice to assess its liver glycogen-depositing activity in mice or rats.

In order to determine such a property of TAI-284, male rats weighing 190 ± 10 g, fasted previously overnight, were administered orally with test agents and 400 mg/kg of glucose. Seven hours thereafter the animals were sacrificed to remove the liver and the hepatic glycogen level was estimated according to the method of Gyermek and Fekete (12).

II. Analgesic assays

1. Randall-Selitto’s method

The analgesic property of the anti-inflammatory agents is counted for their possible interference with formation or action of pain-producing substances liberated in the inflammatory focus.

A hind paw of rats was injected subcutaneously at the plantar side with 0.1 ml of 10% brewer’s yeast suspension in physiological saline (13). Two hours later, an application of pressure stimulus at a constant speed of 10 mmHg/second onto the surface of the inflamed paw showed a decreased threshold to elicit a struggle or withdrawal response. The painful stimuli were applied every one hour for 4 hours after the oral administration of test agents. Thus applied pressure was recorded on a polygraph via transducer in comparison with that of control animals treated with yeast alone.

2. Phenylquinone writhing
The writhing syndrome of ICR-JCL mice caused by an intraperitoneal injection of a variety of irritative substances was reported to be inhibited by analgesic agents. Some selectivity of analgetics towards those irritants was also demonstrated in this test (14, 15).

The writhing and stretching responses were caused by the intraperitoneal injection of 0.1 ml/10 g body weight of 0.02% phenylquinone in mice (16). Test agents were administered orally 30 minutes before the phenylquinone injection, after which the frequency of responses was counted in each animal for 20 minutes.

3. Urate arthritis

According to Rosenthale et al. (17), an intra-articular injection of sodium urate in dogs induced pain responses in gait.

Dogs administered orally with test agents were injected one hour later with 10 mg of fine sodium urate crystals suspended in 0.5 ml of physiological saline intra-articularly to a knee joint of unilateral hind limb. Three and 4 hours thereafter the impaired gait of the animals was observed to count pain degree as following:

- 0: no abnormality
- 1: hypersensitive gait
- 2: dragging gait
- 3: occasional 3-legged gait
- 4: complete 3-legged gait

4. Haffner's method

CF₃-JCL mice weighing 19±2 g were administered orally with test agents, and 30 and 60 minutes later a tail root of the animals was pinched by forceps, when the pseudo-avoidance reflexes such as biting, squeaking, and looking backward were observed (18).

5. D'Amour-Smith's method

CF₃-JCL mice weighing 19±2 g were subjected to radiant heat stimuli. An application of radiant heat from an ultra-red ray lamp at 95 V and 250 W to a tail tip painted with carbon produced the withdrawal response of the tail (19). Before an oral administration of test agents, animals showing the responding time for more than 5 seconds were discarded. The responding time was reestimated 30 and 60 minutes after the administration to evaluate the analgesic activity of test agents by its prolongation. Absence of the response for more than 15 seconds was regarded as the complete analgesia.

6. Hot plate method

CF₃-JCL mice weighing 20±2 g, which were placed on the hot plate at the temperature of 55±0.5°C, showed lickings of a hind foot or jumping behaviors (20). Animals showing such responses within 20 seconds were administered orally with test agents. The responding time was repeatedly determined 30 and 60 minutes later. The prolongation of that time indicated the analgesic activity of test agents.

III. Antipyretic assays

1. Febrile rat

Following the method of Winder et al. (21), male rats weighing 210±10 g were injected subcutaneously with 1 ml/100 g body weight of 15% baker's yeast suspended in
physiological saline. Thus treated animals were fasted, while a drinking water was given ad libitum. At 16 hours after the yeast injection the rectal temperature showed a steady elevation, when a drinking water was withdrawn and test agents were administered orally. Thereafter body temperature was measured every one hour for 5 hours.

2. Febrile rabbit

Male rabbits were made febrile by an intravenous injection of a commercial bacterial polysaccharide (T.T.G). Thereafter rectal temperature was determined every one hour for 5 hours. Test agents were administered orally 90 minutes after T.T.G injection.

RESULTS

1. Anti-inflammatory activities

1. Carrageenin edema

As shown in Fig. 1, the previous treatment of rats with TAI-284 in the doses above 0.75 mg/kg inhibited the edema. The dose-response curves showed that TAI-284 was slightly more effective than indomethacin and markedly more effective than phenylbutazone or mefenamic acid. In another experiment, the time course of the anti-edematous effect caused by single doses of 3 and 6 mg/kg of TAI-284 and indomethacin showed a duration of about 6 hours.

In addition, the inhibitory activity of TAI-284 was still present in the bilaterally adrenalectomized rats.

![Fig. 1. Anti-edematous activities of TAI-284, indomethacin, phenylbutazone and mefenamic acid.](source)
2. **Kaolin edema**

Kaolin edema showed a larger volume and less variance than that caused by carrageenin. The minimal effective dose of TAI-284 was 0.75 mg/kg, while those of indomethacin and phenylbutazone were 1.5 and 25 mg/kg respectively. The dose-response curves revealed that TAI-284 was about 1.5 times more potent than indomethacin (Fig. 1).

3. **Edema caused by miscellaneous irritants**

   On the egg-white edema TAI-284 showed little effect in the doses of 3 and 6 mg/kg, while 6 mg/kg of indomethacin revealed a slight inhibitory activity.

   No effect on the serotonin edema was obtained by TAI-284 (3 and 6 mg/kg), indomethacin (3 and 6 mg/kg) or phenylbutazone (100 mg/kg).

   Neither TAI-284 nor indomethacin showed an effect in the doses of 3 and 6 mg/kg on the formalin edema, while aspirin at 200 mg/kg slightly inhibited it.

   Similarly to the serotonin edema, brewer's yeast edema was not inhibited by either TAI-284, indomethacin or phenylbutazone.

   Although indomethacin in the doses of 3 and 6 mg/kg showed no effect on the mustard edema, TAI-284 (6 mg/kg) and phenylbutazone (100 mg/kg) revealed inhibitory effects.

   TAI-284 (3 and 6 mg/kg), indomethacin (3 and 6 mg/kg), and aspirin (200 mg/kg) inhibited the dextran edema, although the effect of TAI-284 was not statistically significant.

   TAI-284 (3 and 6 mg/kg) and aspirin (200 mg/kg) showed significant effects on the trypsin edema, while indomethacin (3 and 6 mg/kg) had little activity.

   On the bradykinin edema no inhibitory effect was obtained by TAI-284 (3 and 6 mg/kg), indomethacin (3 and 6 mg/kg) or phenylbutazone (100 mg/kg).

4. **Edema in mice**

   The inhibitory effects of TAI-284 at 50 and 100 mg/kg on the mouse paw edema induced by carrageenin were comparable to those of indomethacin in the doses of 5 and 10 mg/kg, respectively. Both agents showed a linear dose-response relationship. Aspirin at 200 mg/kg showed a slight inhibition.

5. **Ultraviolet erythema**

   In the doses between 0.5 and 4 mg/kg TAI-284 and indomethacin similarly inhibited the ultraviolet erythema in dose related way in guinea-pigs. These activities were much higher than those of mefenamic acid and ibufenac (Fig. 2).

   A marked difference in the anti-erythemic activity between TAI-284 and indomethacin was demonstrated when these agents were administered 24 hours before the irradiation. Thus, the erythema was dose-dependently depressed by TAI-284, which abolished the erythema completely in the dose of 5 mg/kg. On the other hand, indomethacin was markedly less effective in this respect (Fig. 2).

6. **Intraperitoneal dye leakage**

   In the dye leakage inhibition in mice, the minimal effective dose of TAI-284 was 6.25 mg/kg, while that of indomethacin was 0.312 mg/kg. Dose response curves revealed that the potency of TAI-284 was 1/10 times that of indomethacin (Fig. 3). However the index (LD50/ED50) of TAI-284 was 35 (880 mg/kg/25 mg/kg), which was much larger
FIG. 2. Anti-UV-erythema activities of TAI-284, indomethacin, mafenamic acid and ibufenac.

FIG. 3. Inhibitory effect of TAI-284, indomethacin and phenylbutazone on increased capillary permeability induced by acetic acid in mice.
than that of indomethacin. The latter was 10 (25 mg/kg/2.5 mg/kg) in the index value.

In rats, as shown in Table 1, both agents showed a similar inhibition against the dye leakage.

7. Granuloma pouch

The minimal effective doses of TAI-284, indomethacin, and phenylbutazone to inhibit the exudation in granuloma pouch were 1.0 mg/kg, 0.5 mg/kg, and 12.5 mg/kg, respectively. By comparison of dose-response curves TAI-284 proved to be one half, 12 times, and 25 times as potent as indomethacin, phenylbutazone, and mefenamic acid, respectively (Fig. 4).

**Table 1. Effect of TAI-284, indomethacin and phenylbutazone on increased capillary permeability induced by acetic acid in rats.**

| Compound       | Oral dose mg/kg | No. of rats | Evans blue concentration µg/ml ± s.e. | % Inhibition |
|----------------|-----------------|-------------|---------------------------------------|--------------|
| Control        | -               | 6           | 20.1 ± 2.60                           | -            |
| TAI-284        | 1.25            | 6           | 14.3 ± 2.76                           | 28.8         |
|                | 2.5             | 6           | 12.2 ± 1.06                           | 39.1*        |
| Indomethacin   | 1.25            | 6           | 14.6 ± 3.00                           | 27.4         |
|                | 2.5             | 6           | 13.4 ± 1.42                           | 33.3*        |
| Phenylbutazone | 25              | 6           | 12.1 ± 2.17                           | 39.5*        |
|                | 50              | 6           | 10.8 ± 1.81                           | 46.0*        |

*: P<0.05

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**Fig. 4. Anti-inflammatory activities of TAI-284, indomethacin, phenylbutazone and mefenamic acid.**
The ratio of LD50 to a minimal effective dose of TAI-284 was 80, while those of indomethacin and phenylbutazone were 40 and 60, respectively.

8. Cotton pellet

The daily oral administration of 0.5 to 3.0 mg/kg of TAI-284 for 7 days in rats inhibited the weight of granuloma induced by the implantation of cotton pellets in dose related way. TAI-284 was slightly more effective than indomethacin and showed much higher activity than those of mefenamic acid and phenylbutazone (Fig. 4).

Although the daily treatment of TAI-284 in the dose of 3 mg/kg increased the weight of spleen, the other treatment with less doses did not affect the weights of thymus, adrenals, and spleen as well as body weight gain.

9. Adjuvant arthritis

The intradermal injection of Freund's complete adjuvant to rats induced an edema in the injected foot on the second day (primary lesion). From about 10 days after the injection, a hind foot not injected, forelimbs, ears, and a tail exhibited erythema and swelling, and body weight decreased gradually (secondary lesions).

![Fig. 5. Effects of TAI-284 and indomethacin on the development of adjuvant arthritis in rats.](image)

The daily administration of TAI-284 in the doses of 1 and 2 mg/kg inhibited both the primary and secondary lesions. The activity of TAI-284 was slightly higher than that of indomethacin (Fig. 5).

10. Urate arthritis

As shown in Table 2, TAI-284 in the doses of 1.25 to 5.0 mg/kg depressed dose-dependedently the exudate accumulation in dog knee joints injected with sodium urate. The potency of inhibitory effect of TAI-284 was almost equal to that of indomethacin, although their dose-response curves were not parallel.
TABLE 2. Effect of TAI-284 and indomethacin on synovial exudate induced by injection of sodium urate crystals into knee joint of dogs.

| Compound       | Exp. No. | Oral dose (mg/kg) | No. of dogs | Mean exudate volume (ml±s.e.) | % Inhibition |
|----------------|----------|-------------------|-------------|-------------------------------|--------------|
| Control        | 1        | -                 | 4           | 4.10±0.41                     | -            |
|                | 2        | -                 | 6           | 4.37±0.34                     | -            |
| TAI-284        | 2        | 1.25              | 6           | 3.33±0.53                     | 23.8         |
|                | 1        | 5.0               | 4           | 1.94±0.33                     | 52.4**       |
| Indomethacin   | 2        | 1.25              | 6           | 2.75±0.43                     | 37.1*        |
|                | 1        | 5.0               | 4           | 1.98±0.24                     | 51.7**       |

*: P<0.05, **: P<0.01

TABLE 3. Inhibition of croton oil-induced exudate in granuloma pouch by TAI-284 and dexamethasone, alone and in combination.

| Oral dose (mg/kg) | Dexamethasone | TAI-284 |
|-------------------|---------------|---------|
|                   | Exp. No.      | No. of rats | Mean exudate volume (ml±s.e.) | % Inhibit. | Adrenal mg | Thymus mg | Spleen mg |
|                   |               |            |                             |           |            |           |           |
| -                 | -             | 1          | 9                           | 9.11±0.48 | -          | 27.4      | 494       | 672       |
| -                 | -             | 2          | 9                           | 9.22±0.49 | -          | 24.4      | 475       | 620       |
| 0.005             | -             | 1          | 9                           | 7.98±0.71 | 12.4       | 25.3      | 423       | 632       |
| 0.010             | -             | 1          | 9                           | 7.63±0.53 | 16.2       | 25.6      | 418       | 602       |
| 0.010             | -             | 2          | 9                           | 6.82±0.68 | 26.0*      | 25.0      | 388*      | 561       |
| 0.020             | -             | 2          | 9                           | 4.97±0.37 | 46.1**     | 24.2      | 302**     | 499**     |
| -                 | 0.5           | 1          | 9                           | 7.83±0.61 | 14.1       | 27.7      | 480       | 668       |
| -                 | 1.0           | 2          | 9                           | 6.66±0.85 | 27.8*      | 29.2      | 503       | 606       |
| 0.0025            | 0.25          | 1          | 9                           | 6.68±0.62 | 26.7**     | 26.8      | 408       | 634       |
| 0.005             | 0.5           | 1          | 9                           | 6.44±0.59 | 29.3*      | 26.5      | 536       | 624       |
| 0.005             | 0.5           | 2          | 9                           | 6.24±0.69 | 32.3**     | 24.8      | 448       | 604       |

*: P<0.05 **: P<0.01

TABLE 4. Inhibitory effects of TAI-284, indomethacin and phenylbutazone on bronchospasms induced by bradykinin or histamine in guinea-pigs.

| Compound       | Exp. No. | Dose (mg/kg) | Bradykinin response | % Inhibition | Histamine response |
|----------------|----------|--------------|---------------------|--------------|-------------------|
| TAI-284        | 1        | 1*           | 40.0                | -10.1        |
|                | 2        | 1**          | 73.3                | 4.7          |
|                | 3        | 1**          | 56.5                | 18.3         |
| Indomethacin   | 4        | 1**          | 40.8                | -11.1        |
|                | 5        | 1*           | 57.7                | -5.0         |
|                | 6        | 1*           | 63.1                | -12.8        |
| Phenylbutazone | 7        | 4**          | 52.4                | 7.1          |
|                | 8        | 4*           | 33.3                | -3.2         |
|                | 9        | 4*           | 70.0                | 5.7          |

*: administered 5 minutes before bronchospasm
**: administered 30 minutes before bronchospasm
11. Combination with dexamethasone

In the inhibitory effect on the carrageenin edema, the combination of TAI-284 with dexamethasone showed a significant synergism. Also in the granuloma pouch test, more marked synergistic effect was obtained (Table 3). Such a combination did not decrease the weights of thymus, adrenals, and spleen, while the administration of dexamethasone alone did so.

12. Bronchospasm

TAI-284 and indomethacin in the dose of 1 mg/kg respectively as well as 4 mg/kg of phenylbutazone inhibited the bronchospasm caused by bradykinin (Table 4).

Neither TAI-284, indomethacin, nor phenylbutazone showed inhibitory effects on the histamine-induced bronchospasm.

TAI-284 and indomethacin in the dose of 5 mg/kg were ineffective to inhibit the bronchospasm caused by serotonin.

The acetylcholine-induced bronchospasm was not inhibited by TAI-284 and indomethacin at 5 mg/kg.

TAI-284 and indomethacin at 5 mg/kg showed inhibitory effects on the ATP-induced bronchospasm.

In the dose of 5 mg/kg, TAI-284 and indomethacin inhibited by 50% the bronchospasm caused by the antigen-antibody reaction which was induced by a challenge injection of egg albumin.

13. Liver glycogen deposition

Administrations of 3 and 6 mg/kg of TAI-284 or indomethacin did not increase the liver glycogen level in rats loaded with glucose. On the other hand, prednisolone in the dose of 2.5 mg/kg elevated the level.

II. Analgesic activities

1. Randall-Selitto's method

TAI-284 in the doses of 1.25 to 5.0 mg/kg produced a dose-dependent elevation of the threshold pressure applied onto an inflamed rat paw. The activity of 2.5 mg/kg of TAI-284 was comparable to that of phenylbutazone in the dose of 50 mg/kg. The potency of indomethacin was almost equal to that of TAI-284 (Fig. 6).

2. Phenylquinone writhing

As shown in Fig. 6, TAI-284 in the dose range from 6.25 to 25 mg/kg depressed dose-dependently the frequency of the phenylquinone writhing syndrome. Comparison of dose-response curves revealed that the potency of TAI-284 was much higher than that of phenylbutazone, and was 1/6 times that of indomethacin. However, the index (LD50/ED50) of TAI-284 was 70, while that of indomethacin was 16, which was much lower than that of the former.

3. Urate arthritis

An administration of TAI-284 at 2.5 mg/kg markedly improved the impaired gait of dogs caused by an intra-articular injection of sodium urate crystals. In this respect, indomethacin was slightly less effective than TAI-284 (Table 5).
TABLE 5. Effect of TAI-284 and indomethacin on functional impairment and exudation induced by injection of sodium urate crystals into knee joint of dogs.

| Compound       | Oral dose mg/kg | Sex | B.W. kg | Score 2 hr | Score 3 hr | Exudate ml |
|----------------|-----------------|-----|---------|------------|------------|------------|
| Control        | ---             | M   | 10.1    | 4          | 4          | 5.1        |
|                |                 | F   | 9.2     | 3          | 3          | 1.5        |
| TAI-284        | 2.5             | M   | 9.5     | 0          | 0          | 2.0        |
|                | 2.5             | F   | 12.6    | 0          | 0          | 0.5        |
| Indomethacin   | 2.5             | M   | 10.8    | 0          | 0          | 1.1        |
|                | 2.5             | F   | 9.4     | 2          | 1          | 4.5        |

* : 1—tenderness
  2—limping
  3—occasional 3-leg gait
  4—complete 3-leg gait

4. Haffner's, D'Amour-Smith's, and hot plate methods

Neither TAI-284 (100 mg/kg) nor indomethacin (10 mg/kg) showed an analgesic activity in the Haffner's method.
TAI-284 (100 mg/kg) was ineffective, however, indomethacin (10 mg/kg) and aminopyrine (200 mg/kg) produced a significant retardation of the tail flick reaction caused by radiant heat.

In the hot plate method, TAI-284 (100 mg/kg) and indomethacin (5 mg/kg) showed no activity.

III. Antipyretic activities

1. Febrile rat

In the febrile rats injected with baker’s yeast, TAI-284 showed a marked antipyretic
effect. The activity of 0.098 mg/kg of this agent was comparable to those caused by 0.78 mg/kg of indomethacin and 12.5 mg/kg of aminopyrine (Fig. 7). In contrast to aminopyrine, TAI-284 and indomethacin showed a long-lasting activity.

In spite of the strong effect on febrile body temperature, TAI-284 even at 6.25 mg/kg did not affect the normal temperature, while aminopyrine at 50 mg/kg lowered it.

2. Febrile rabbit

As shown in Fig. 8, TAI-284 in the doses of 5 and 10 mg/kg showed a marked decrease in febrile body temperature in rabbits injected with T.T.G. The potency of this agent was about 2.5 times that of indomethacin.

DISCUSSION

TAI-284 proved, in the present experiments, to have potent anti-inflammatory, analgesic, and antipyretic activities in experimental animals.

Since Winter (1964) suggested that the use of less specific agents as a phlogistic means gave more reliable evaluation of the anti-inflammatory activity than that of specific agents such as serotonin, histamine, egg white, and dextran, the anti-inflammatory activities of TAI-284 were evaluated mainly in the edema of a rat hind paw caused by a subcutaneous injection of carrageenin or kaolin, the granuloma pouch, cotton pellet granuloma, and adjuvant arthritis in rats as well as in the ultraviolet erythema in guinea-pigs.

Another advantage of the use of carrageenin and kaolin is the considerably long continuance of the edema (Garattini 1964). TAI-284 exhibited a strong and relatively long-lasting depression of the respective inflammations, not inferior to that caused by indomethacin.

Reasonable correlation between anti-erythemic and anti-rheumatic activity of pyrazoles, salicylates, cinchophen, and arylacetic acid was demonstrated by Adams et al. (22, 23), Winder et al. (5), and Winter (2). TAI-284 showed a marked inhibition of the erythema induced by ultraviolet irradiation in guinea-pigs. In addition, this anti-erythemic activity was apparent even when TAI-284 was administered 24 hours before irradiation. Such a long-lasting activity seems to correlate with the retarded metabolism of the compound in this species as demonstrated by Tanayama (24). Indomethacin, on the other hand, failed to show such a continued anti-erythemic activity.

Northover (25) observed that various anti-inflammatory agents inhibited intraperitoneal dye leakage induced by physiological saline in mice. In the present experiments, TAI-284 in the oral doses above 1.5 mg/kg inhibited dose-dependently the intraperitoneal dye leakage caused by acetic acid. The inhibitory activity of TAI-284 was considerably less than that of indomethacin in mice, but of the same degree in rats. A similar inferiority of TAI-284 to indomethacin was also demonstrated in depression of the phenylquinone-induced writhing in mice. In this respect, it is worth to notice that the mouse was markedly resistant to the systemic toxicity of TAI-284 as already observed by Kawai et al. (26). These facts seems to relate with the ready metabolic change of this compound in mice (Tanayama, 27).
TAI-284 in the oral doses above 0.5 mg/kg decreased considerably the exudate accumulation in the granuloma pouch induced by croton oil in rats. Such a depression of exudation has been presented by many of non-steroidal anti-inflammatory agents (28). However, TAI-284 as well as indomethacin showed a relatively mild activity to inhibit the granuloma formation around cotton pellets. Compared with synthetic glucocorticoids, these compounds seemed to be less effective in depressing the proliferation of connective tissue manifested lately at the inflammatory site.

Newbould (9) showed that phenylbutazone and aspirin significantly suppressed the primary lesion caused by an intradermal injection of Freund’s adjuvant in rats, but had only a moderate effect on the secondary lesion, which seemed to be immunological in origin. In the present experiments of adjuvant arthritis, TAI-284 showed a marked inhibition of both the primary and secondary lesions, furthermore the compound prevented the body weight decrease associated with the secondary lesions.

The absence of influence by daily administered TAI-284 or indomethacin on the weights of adrenals, thymus, and spleen indicated that the both compounds produced the anti-inflammatory effects without mediation of adrenal glands. This suggestion was also supported by manifestation of the anti-edematous action in the adrenalectomized rats. However, the inhibitory effect of TAI-284 on the carrageenin edema and granuloma pouch was significantly potentiated by the combined administration with dexamethasone in a subthreshold dose. In addition, such a synergistic effect by combination did not associate with decrease in weights of adrenals, thymus, and spleen.

TAI-284 in the oral doses above 1.25 mg/kg inhibited the intracapsular accumulation of exudate induced by a sodium urate injection and improved markedly the limping walk in dogs. Thus, TAI-284 proved to have definite anti-inflammatory and analgesic activities also in non-rodent species.

TAI-284 failed to show the activity of hepatic glycogen deposition which is a common property of glucocorticoids. This fact again supported that the pharmacological action of TAI-284 seemed to be independent of adrenocortical function.

Many of non-steroidal anti-inflammatory agents have been found to antagonize the broncho-constriction caused by bradykinin in guinea-pigs (29, 30). TAI-284 as well as indomethacin blocked the bradykinin-induced bronchospasm, but they were ineffective on the response caused by histamine or serotonin. In the present experiment, in addition, TAI-284 was effective to inhibit the anaphylactic bronchospasm caused by a challenge injection of egg albumin. In that response the slow reacting substance is said to be a dominating substance.

TAI-284 failed to show analgesic activities in the commonly used methods including mechanical and thermal stimuli, while it proved to be highly effective against the pain response of an inflamed foot of rats, and it depressed significantly the writhing syndrome of mice caused by an intraperitoneal injection of an irritative substance. Therefore the analgesic property of TAI-284 seemed to be exerted peripherally. In this respect indomethacin behaved similarly.
Although TAI-284 and indomethacin even in large doses did not lower the physiological body temperature of rats and rabbits, they restored the elevated body temperature caused by an injection of Baker's yeast in rats or a bacterial polysaccharide in rabbits. This antipyretic activity of TAI-284 was extremely high, since it was exhibited even in such a low dose as 0.1 mg/kg in febrile rats. Though the mechanism of pyretogenesis and its antagonism remain to be clarified, the antipyretic effect of TAI-284 and indomethacin exerted only in the febrile animals with a considerable duration presented a marked difference from the property of a typical antipyretic drug, aminopyrine.

When the ratios of LD50 to ED50 or to a minimal effective dose in anti-inflammatory, analgesic and antipyretic assays were compared between TAI-284 and indomethacin, the former agent showed considerably higher values than those of the latter.

**SUMMARY**

The anti-inflammatory, analgesic, and antipyretic activities of TAI-284 were tested in reference to several antiphlogistics. TAI-284 proved to have strong inhibitory effects on various types of inflammation including carrageenin edema, exudation in granuloma pouch, cotton pellet granuloma, polyarthritis induced by Freund's adjuvant in rats, and urate arthritis in dogs.

The anti-inflammatory action of TAI-284 revealed to be independent of adrenal mediation. On the other hand, the combination effect of TAI-284 with dexamethasone was synergistic in anti-inflammatory action. However, TAI-284 alone did not show glucocorticoidal properties such as hepatic glycogen deposition and depression of thymus spleen and adrenals weights.

Marked analgesic activities of TAI-284 were shown in phenylquinone writhing in mice, an inflamed rat paw (Randall-Selitto's method), and in the urate-induced limping in dogs. Analgesic property of this compound seemed to be peripheral in origin.

The potencies of TAI-284 in anti-inflammatory and analgesic assays were almost the same as those of indomethacin or somewhat higher than the latter.

The antipyretic activities of TAI-284 were much higher than indomethacin. TAI-284 was effective even in the dose of 0.1 mg/kg in febrile rats. However, neither agent in higher doses affected the normal body temperature.

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ANTIPHLOGISTIC ACTION OF TAI-284 639

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