BIOLOGICAL ACTIVITIES OF METABOLITES OF 6-CHLORO-5-CYCLOHEXYLINDAN-1-CARBOXYLIC ACID (TAI-284: ANTI-INFLAMMATORY AGENT)

Seiji KUZUNA, Naohiko MATSUMOTO and Kiyohisa KAWAI

Biological Research Laboratories, Central Research Division, Takeda Chemical Industries, Ltd., Yodogawa-ku, Osaka, Japan

Accepted May 23, 1974

Abstract—The pharmacological activities of five metabolites of an anti-inflammatory compound, 6-chloro-5-cyclohexylindan-1-carboxylic acid (TAI-284) were investigated and compared with TAI-284 and phenylbutazone. The metabolite IIb (cis-3'-hydroxy-cyclohexyl, β-form) was almost equivalent to TAI-284 regarding anti-inflammatory activity as estimated by the carrageenin edema test, and was more active than TAI-284 in the analgesic activity estimated by the phenylquinone writhing test and determination of the ulcerogenic activity. The metabolites IIa (cis-4'-hydroxy-cyclohexyl) and IV (cis-3'-hydroxy-cyclohexyl, α-form) had about half the anti-inflammatory activity and one third to one quarter the analgesic activity of TAI-284, however the degree of ulcerogenicity was considerably lower than that of TAI-284. I (4'-oxo-cyclohexyl) was less active, and III (trans-4'-hydroxy-cyclohexyl) showed the weakest activity comparable to that of phenylbutazone. Antipyretic activities of all five metabolites were much lower than those of TAI-284. As the pharmacological activities of metabolites varied greatly according to the position of the hydroxyl group on the cyclohexane ring of TAI-284, it is feasible that the distribution pattern and affinity of the metabolites for receptors could be modified.

Anti-inflammatory drugs administered at a high dosage or for a long period of time often induce gastro-intestinal disorders. TAI-284 with potent anti-inflammatory, analgesic and antipyretic activities (1) produced at high doses penetrating ulcers in rat jejunum and ileum. The toxicological properties (2) and detailed features of the ulcer development following administration of the compound have been previously described (3, 4).

It was observed that a phenobarbital pretreatment of animals reduced the toxicity of TAI-284 without altering the anti-inflammatory activity. As phenobarbital is known to be an enzyme inducer in drug metabolism (5), the metabolic mechanisms presumably play a role in the action of this drug. Recently, Aspinall reported that spironolactone pretreatment exerted no effect on the anti-inflammatory activity of indomethacin but markedly suppressed ulcerogenic activity in adjuvant arthritis of rats (6). He suggested that such a differential effect of spironolactone may be explained by the difference between the minimum effective doses of indomethacin exerting the two actions, the same of which is indeed worthy of consideration.

The present report deals with the anti-inflammatory, analgesic, antipyretic and ulcerogenic activities of five metabolites of TAI-284 in comparison with the parent compound and phenylbutazone, and characteristic properties of the metabolites are discussed.
Metabolites of TAI-284, shown in Table 1, were isolated from the perfusate of isolated rat livers by Kanai et al. in our Biological Research Laboratories (7), and also chemically synthesized by Kishimoto et al. in our Chemical Research Laboratories.

Male SD-JCL rats and ICR-JCL mice were used. Test agents were suspended with 4% gum acacia in water and administered p.o. in 1 ml per 100 g body weight to rats, and in 0.2 ml per 10 g body weight to mice. All experiments were carried out following a double-blind schedule.

1. Anti-inflammatory test

Following the method of Winter et al. (8), 0.05 ml of 1% carrageenin suspension in

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chemical structure</th>
<th>Chemical name</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAI-284</td>
<td><img src="TAI-284.png" alt="Chemical structure" /></td>
<td>6-chloro-5-cyclohexylindan-1-carboxylic acid</td>
</tr>
<tr>
<td>I</td>
<td><img src="I.png" alt="Chemical structure" /></td>
<td>6-chloro-5-(4'-oxycyclohexyl) indan-1-carboxylic acid</td>
</tr>
<tr>
<td>IIa</td>
<td><img src="IIa.png" alt="Chemical structure" /></td>
<td>6-chloro-5-(cis-4'-hydroxycyclohexyl) indan-1-carboxylic acid</td>
</tr>
<tr>
<td>IIb*</td>
<td><img src="IIb.png" alt="Chemical structure" /></td>
<td>6-chloro-5-(cis-3'-hydroxycyclohexyl) indan-1-carboxylic acid (β-form)</td>
</tr>
<tr>
<td>III</td>
<td><img src="III.png" alt="Chemical structure" /></td>
<td>6-chloro-5-(trans-4'-hydroxycyclohexyl) indan-1-carboxylic acid</td>
</tr>
<tr>
<td>IV*</td>
<td><img src="IV.png" alt="Chemical structure" /></td>
<td>6-chloro-5-(cis-3'-hydroxycyclohexyl) indan-1-carboxylic acid (α-form)</td>
</tr>
</tbody>
</table>

*: IIb and IV are diastereoisomeric with each other.
(Cited from Kanai et al.)
physiological saline was injected s.c. into the plantar side of the hind paw of male rats weighing 190 ± 10 g, and 3 hr later the edema developed was estimated. Test agents were administered p.o. one hr before the injection of carrageenin.

2. Analgesic test

Mice weighing 20 ± 2 g were used for the phenylquinone writhing syndrome test. Following the procedure of Siegmund et al. (9), 0.02% phenylquinone dissolved in water by adding 5% ethanol was injected i.p. in 0.1 ml per 10 g body weight to induce writhing and stretching responses. The frequency of those responses was counted for 20 min after the injection. Test agents were administered 30 min before the phenylquinone injection.

3. Antipyretic test

Following the method of Winder et al. (10), rats weighing 210 ± 10 g were made febrile by a s.c. injection of 15% baker's yeast suspended in physiological saline in 1 ml per 100 g body weight. The animals were fasted but allowed free access to drinking water until 16 hr later, after which rectal temperature was estimated three times at one-hr intervals. Oral administration of test agents thereafter was followed by hourly estimations of rectal temperature for 5 hr.

4. Ulcerogenicity

Rats weighing 140 ± 10 g were sacrificed 6 hr after the oral administration of test agents. Gastric mucosal ulcers as well as those in the small intestine and other areas of digestive tract were counted and the ulcer area measured.

RESULTS

1. Anti-inflammatory activity

The activity of metabolites of TAI-284 against carrageenin edema in rats is shown in Fig. 1. The activity of metabolite II b was equivalent to that of TAI-284. Metabolites I, Ila and IV were significantly effective at 3 to 12 mg/kg, indicating the relative activities of one half to one fifth of those of TAI-284. Metabolite III displayed much less activity.

![Fig. 1. Comparative anti-inflammatory activity of TAI-284 and its metabolites in carrageenin edema test in rats.](image-url)
2. Analgesic activity

The analgesic activity in the phenylquinone writhing test is presented as the ED50 in Table 2. Metabolite IIb, the most potent anti-inflammatory one, was about 4 times

<table>
<thead>
<tr>
<th>Compound</th>
<th>ED50 mg/kg (95% C.L.) p.o.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAI-284</td>
<td>10.4 (7.2–14.8)</td>
</tr>
<tr>
<td>I</td>
<td>32.8 (13.8–59.2)</td>
</tr>
<tr>
<td>IIa</td>
<td>28.9 (16.3–50.2)</td>
</tr>
<tr>
<td>IIb</td>
<td>2.8 (1.1–4.3)</td>
</tr>
<tr>
<td>III</td>
<td>122.6 (81.1–184.2)</td>
</tr>
<tr>
<td>IV</td>
<td>43.0 (25.9–64.0)</td>
</tr>
<tr>
<td>Phenylbutazone</td>
<td>49.5 (29.8–69.2)</td>
</tr>
</tbody>
</table>

C.L. : Confidence limits

![Fig. 2. Antipyretic activity of TAI-284 and its metabolites in febrile rats injected with Baker's yeast.](image)

○ : \( P < 0.05 \)

* : \( P < 0.01 \)
as analgesic as TAI-284. Metabolites I, IIa and IV had considerably less analgesic activity than did TAI-284 but were nevertheless more active than phenylbutazone. Metabolite III, the least active of all, was observed to have only one twelfth the activity of TAI-284.

3. Antipyretic activity

In contrast to the anti-inflammatory and analgesic actions, antipyretic activities of the five metabolites were much lower than those of TAI-284 as shown in Fig. 2. Metabolite IIb which had the strongest antipyretic activity proved to be only one third as potent as TAI-284. Metabolite I at 12.5 mg/kg, IV at 12.5 mg/kg, III at 25 mg/kg and IIa at 25 mg/kg were equivalent to TAI-284 at 0.3 mg/kg or to phenylbutazone at 25 mg/kg.

4. Ulcerogenicity

TAI-284 at 10 mg/kg p.o. produced quite a number of small round ulcers, mostly about 1 mm in diameter, in the jejunum and ileum, however, none were found in the stomach, duodenum and large intestine (Table 3). Ulcers induced by metabolites I, IIa, III and IV showed a similar distribution pattern, but were significantly less in number than when TAI-284 had been administered. Metabolite IIb was more potent than TAI-284 regarding the ulcerogenicity factor.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Oral dose mg/kg</th>
<th>No. of rats</th>
<th>No. of gastric ulcers</th>
<th>Small-intestinal ulcers Mean±S.E.</th>
<th>Area (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAI-284</td>
<td>10</td>
<td>6</td>
<td>0</td>
<td>60.3±10.7</td>
<td>72.5±12.6</td>
</tr>
<tr>
<td>I</td>
<td>10</td>
<td>6</td>
<td>0</td>
<td>0.3±0.3**</td>
<td>0.3±0.3**</td>
</tr>
<tr>
<td>IIa</td>
<td>10</td>
<td>6</td>
<td>0</td>
<td>0.2±0.2**</td>
<td>0.2±0.2**</td>
</tr>
<tr>
<td>IIb</td>
<td>10</td>
<td>6</td>
<td>0</td>
<td>83.0±9.6</td>
<td>101.0±12.8</td>
</tr>
<tr>
<td>III</td>
<td>10</td>
<td>6</td>
<td>0</td>
<td>0.3±0.3**</td>
<td>0.3±0.3**</td>
</tr>
<tr>
<td>IV</td>
<td>10</td>
<td>6</td>
<td>0</td>
<td>1.3±0.6**</td>
<td>1.3±0.6**</td>
</tr>
</tbody>
</table>

** : P<0.01 as compared with TAI-284.

DISCUSSION

From a stereochemical study of indomethacin by the X-ray analysis, Shen proposed the existence of a specific receptor for the anti-inflammatory action (11). Kamiya et al. in our Chemical Research Laboratories also studied the stereochemical structure of TAI-284 using X-ray analysis (12). As the stereochemical structures of the two compounds are quite similar, there is some possibility that TAI-284 also may have biological activities through a specific receptor (Fig. 3).

TAI-284 was biotransformed in rats to several metabolites the chemical structures of which are quite similar, yet the therapeutic and ulcerogenic activities varied. Generally, a great difference in pharmacological activity between two stereoisomers suggests a different affinity to common receptors (13, 14). Regarding anti-inflammatory activity, metabolite IIb was the most potent, followed by metabolites IIa, IV, I and III respective-
ly. Metabolite IIb had greater analgesic activity than did TAI-284, but the other metabolites IIa, I, IV and III in the respective decreasing order were less potent than TAI-284. Metabolites IIb and IV are diastereoisomers the activities of which were considerably different despite a great similarity in chemical structures. The same feature was observed regarding metabolites IIa and III. It is postulated that the differences in activity can be attributed to the stereochemical structure which influences receptor adaptability.

In contrast to anti-inflammatory and analgesic activities, all the metabolites displayed a much lower antipyretic activity than did TAI-284. This is attributed to the introduction of a hydroxy group to the cyclohexane ring of TAI-284 which would lead to reduction of lipid solubility and thus a decreased distribution to the central nervous system, as many non-steroidal anti-inflammatory agents (15, 16, 17, 18) display antipyretic action via the central nervous system.

Since so many anti-inflammatory agents also produce ulcers in the gastro-intestinal tract, a method for elimination of this side effect is the subject of intensive research. In our previous study, phentolamine, an adrenergic α-blocker, prevented ulceration induced by TAI-284 (3) without influencing the anti-inflammatory action. This differential effect of phentolamine suggests that the mechanism of ulceration is clearly distinguished from the anti-inflammatory one. In the same way metabolites IIa and IV have highly anti-inflammatory properties but are less ulcerogenic.

According to Tanayama et al. (19), the major metabolites found in rat plasma were I and III, but metabolites IIa, IIb and IV were also detectable. The pharmacological activities of TAI-284 in rats seem to be derived, at least partly, from these active metabolites.
Acknowledgements: Thanks are due to Dr. S. Noguchi, Mr. S. Kishimoto and Mr. Y. Kanai for providing samples of metabolites of TAI-284, and Mr. S. Morimoto for expert technical assistance. Gratitude is also due to Dr. M. Nishikawa for advice regarding stereochemistry of TAI-284 and indomethacin.

REFERENCES