A comparison of the inhibitory activity of compounds on ocular prostaglandin biosynthesis

P. Bhattacherjee and K. E. Eakins

In the present study, we have compared the potency of various compounds as inhibitors of prostaglandin biosynthesis in ocular tissues in vitro under standard conditions of temperature, pH, substrate concentration, and time. The nonacetic anti-inflammatory agent, indoxole, was approximately 100 times as potent as indomethacin on the anterior uvea and 25 times as potent on the conjunctiva. SU 21524 (Pirprofen) was also more potent than indomethacin on the ocular tissues. Other nonsteroidal anti-inflammatory agents such as naproxen, phenylbutazone, and oxynphenbutazone were all essentially equiactive with indomethacin on the anterior uvea, whereas indomethacin was more potent than phenylbutazone or oxynphenbutazone on the conjunctiva. Aspirin, paracetamol, and dexamethasone had little or no activity in these in vitro experiments. The pharmacologic profile of activity of the active compounds was found to differ in the ocular tissues from that in other tissues such as spleen and seminal vesicles. Since other factors such as ease of penetration and local tissue irritation may affect the final selection of compounds for use as topical anti-inflammatory agents, the next step would be to evaluate the effects of compounds found to be active in the present experiments by this route of administration.

The apparent importance of prostaglandins (PG) in ocular inflammation suggests that substances that inhibit the action and/or synthesis of prostaglandins may prove useful as ocular anti-inflammatory agents. Nonsteroidal agents such as aspirin or indomethacin are known to inhibit prostaglandin biosynthesis and this action is thought to be the mechanism by which they exert their therapeutic effects.

Recent evidence indicates that prostaglandin synthetase systems from different tissues show different sensitivities to inhibitory drugs. Flower and Vane showed the antipyretic, analgesic drug, 4-acetamidophenol (paracetamol), to be ten times less effective than aspirin on prostaglandin biosynthesis in cell-free homogenates of dog spleen, whereas it had the same potency as aspirin on preparations made from either dog or rabbit brain. Similar results with aspirin and paracetamol were ob-
tained using mouse or gerbil brains. This differential sensitivity of the prostaglandin synthetase systems was thought to explain the fact that paracetamol has antipyretic but not anti-inflammatory activity. Bhat-tacherjee and Eakins found a thousand-fold variation in the ID50 of indomethacin against the prostaglandin synthetase systems from different tissues of the rabbit. Indomethacin was a far less potent inhibitor of prostaglandin biosynthesis in ocular tissues than it was in spleen or kidney medulla, which may explain why indomethacin is not very potent, when given orally, in the treatment of ocular inflammation. These observations raise the possibility of developing compounds which can selectively inhibit prostaglandin biosynthesis in some tissues with relatively little effect in others. It was concluded that for use as ocular anti-inflammatory agents, it would be necessary to study their effectiveness against the synthetase systems derived from ocular tissues.

In the present study, we have examined the inhibitory activities of a range of compounds on prostaglandin formation by microsomal fractions of ocular tissues, specifically the iris/ciliary body and conjunctiva. By this means we hope to be able to select compounds for potential use as ocular anti-inflammatory agents.

Methods

New Zealand white rabbits of either sex were killed with pentobarbitone sodium. The eyes were rapidly removed, the iris/ciliary body and conjunctiva dissected free, the tissues cut into small pieces with scissors, and washed with ice-cold Kreb’s solution. Pooled samples from 10 eyes were used to prepare each batch of cell-free PG synthetase preparation according to the method of Flower, and co-workers. The tissues were homogenized for two minutes in ice-cold 100 mM phosphate buffer (pH 7.4). After centrifugation at 10,000 g for 10 minutes at 2°C, the precipitate was discarded and the supernatant recentrifuged at 80,000 g for 1 hour at 2°C. The resultant microsomal pellets were then resuspended in phosphate buffer and used as the source of the synthetase. Total protein in this final solution was determined by the method of Lowry and co-workers.

Microsomal fractions containing the prostaglandin synthetase systems were incubated in mixtures of 2 ml. of 50 mM phosphate buffer containing 20 μg of arachidonic acid, 100 μg of reduced glutathione, 10 μg of hydroquinone, and approximately 1 mg. of protein from the pellet suspension. Samples were incubated aerobically with shaking at 37°C for 20 minutes at which time the reactions were stopped by heating the tubes in boiling water for one minute. A zero time control was included in each experiment together with a control 20 minute sample. Drugs under examination were then added to the reaction mixtures in varying concentrations.

Following the incubation, the contents of each tube were then extracted for prostaglandins with ethanol acidified with formic acid, followed by petroleum ether (boiling point: 37.5 to 52.8°C) and chloroform. They were then assayed against PGE2 on rat stomach strips, suspended in 5 ml. of Kreb’s solution at 37°C gassed with 5 per cent CO2 and O2 and containing methysergide, atropine, and mepyramine (all 0.1 μg per milliliter). In some experiments, the identity of the reaction product was tentatively identified as PGE2 by chromatography on paper impregnated with silica gel and silver nitrate.

Drugs used in this study. Indomethacin (Merck, Sharp, and Dohme), phenylbutazone, oxyphenbutazone, SU 21524 (Pirprofen, Ciba-Geigy), naproxen (Syntex), indoxole (Upjohn), aspirin (E. Merck), dexamethasone (Merck, Sharp, and Dohme), and paracetamol (4’-hydroxy-acetanilide, Eastman Kodak).

Results

Tissue homogenates and microsomal pellets were freshly prepared for each experiment. Generation of PG-like activity by the microsomal fractions from the added substrate (arachidonic acid) was determined in each preparation (activity in the 20-minute sample minus activity in the zero time sample). Varying amounts of the substances to be tested as inhibitors were added to the incubation flasks and the percentage inhibition of the control generation of PG-like activity determined. The concentration of these substances was chosen to give a wide range of inhibition and each substance was tested at least four times at each of three to four concentrations. Regression lines were then calculated from the log-dose/response curves and the ID50 values (the concentration in micromoles required to produce a 50 per
cent inhibition of the control biosynthesis) determined from these lines (Table I). The regression lines for all the active compounds appeared to be parallel, and are shown in Fig. 1.

Indomethacin, phenylbutazone, and oxyphenbutazone were approximately equiactive on the anterior uvea, although indomethacin was twice as active as phenylbutazone and four times as active as oxyphenbutazone on the conjunctiva. Naproxen was slightly more active than indomethacin on the anterior uvea. SU 21524 (Pirprofen) was six to seven times as potent as indomethacin on the anterior uvea but only two to three times as active on the conjunctiva. Indoxole was the most potent compound studied, being over 100 times as potent as indomethacin on the anterior uvea and approximately 25 times as potent on the conjunctiva. With aspirin, no inhibition could be detected at the 100 μg per milliliter dose level. In some experiments, even concentrations of 200 to 300 μg per milliliter did not exhibit an inhibitory effect, although when extremely high concentrations (500 to 1,000 μg per milliliter) were used, some inhibition (10 to 15 per cent) was observed. Some possible metabolites of aspirin were also studied but were all found to be without effect (Table III). Neither paracetamol (4-acetamidophenol) nor dexamethasone showed any inhibition at 100 μg per milliliter.

**Table I.** The inhibitory activity of some compounds on PG-formation from added arachidonic acid by microsomal fractions of ocular tissues

<table>
<thead>
<tr>
<th>Compound</th>
<th>Anterior uvea</th>
<th>Conjunctiva</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[μg/mL]</td>
<td>[μM]</td>
</tr>
<tr>
<td>Indoxole</td>
<td>0.16</td>
<td>0.49</td>
</tr>
<tr>
<td>Pirprofen</td>
<td>2.8</td>
<td>11.2</td>
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<td>Naproxen</td>
<td>13.0</td>
<td>59</td>
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<tr>
<td>Phenylbutazone</td>
<td>17.9</td>
<td>58</td>
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<tr>
<td>Indomethacin</td>
<td>18.5</td>
<td>52</td>
</tr>
<tr>
<td>Oxyphenbutazone</td>
<td>21.0</td>
<td>64.8</td>
</tr>
<tr>
<td><strong>No significant inhibition at 100 μg/ml.</strong></td>
<td></td>
<td></td>
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</tbody>
</table>

Aspirin, Paracetamol, Dexamethasone.
Table II. Relative potencies* of some compounds on PG-formation from added arachidonic acid by microsomal fractions on different tissues

<table>
<thead>
<tr>
<th>Compound</th>
<th>Anterior uvea</th>
<th>Conjunctiva</th>
<th>Sheep seminal vesicles</th>
<th>Dog spleen</th>
<th>Bovine seminal vesicles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indomethacin</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<tr>
<td>Pirprofen</td>
<td>6.7</td>
<td>2.7</td>
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<td>—</td>
<td>—</td>
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<tr>
<td>Indoxole</td>
<td>111</td>
<td>25</td>
<td>0.43</td>
<td>—</td>
<td>—</td>
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<tr>
<td>Naproxen</td>
<td>1.4</td>
<td>ND</td>
<td>0.11</td>
<td>0.027</td>
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</tr>
<tr>
<td>Phenylbutazone</td>
<td>1.03</td>
<td>0.45</td>
<td>0.04</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Oxyphenbutazone</td>
<td>0.88</td>
<td>0.26</td>
<td>0.01</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Aspirin</td>
<td>&lt; 0.04</td>
<td>&lt; 0.017</td>
<td>0.011</td>
<td>0.009</td>
<td>0.005</td>
</tr>
</tbody>
</table>

*Relative potency = $\frac{\text{ID}_{50} \text{ Compound}}{\text{ID}_{50} \text{ Indomethacin}}$

Table III. Some possible metabolites of salicylic acid which did not inhibit generation of PG-like activity from arachidonic acid in microsomal preparations of iris/ciliary body

No inhibition by 200 µg per milliliter:
- 3,4-Dihydroxybenzoic acid
- 2,4-Dihydroxybenzoic acid (gentisic acid)
- 3,4,5-Trihydroxybenzoic acid (gallic acid)
- O-Hydroxy hippuric acid
- Sodium salicylate

Discussion

In the present study, we have compared the potency of various compounds as inhibitors of prostaglandin biosynthesis in ocular tissues. It should be borne in mind that the present results were obtained under standard incubation conditions of temperature, time, pH, and substrate concentration. It may well be that the optimal requirements of the synthetase enzyme(s) in ocular tissues may differ from those in other organs. The nonacidic anti-inflammatory agent, indoxole, was the most potent compound studied, being approximately 100 times as potent (on a molar basis) as indomethacin on the anterior uvea and 25 times as potent on the conjunctiva. SU 21524 (2-[3-chloro-4-(3-pyrrolin-1-yl)-phenyl] propionic acid (Pirprofen) was also considerably more potent than indomethacin on the ocular tissues. The arylyacetic acid derivative, naproxen, and the pyrazolone derivatives, phenylbutazone and oxyphenbutazone, were all essentially equiactive with indomethacin on the anterior uvea, whereas indomethacin was somewhat more potent than either of the pyrazolone derivatives on the conjunctiva.

Where possible, we have compared the relative potencies of different anti-inflammatory compounds on the ocular tissues with other tissues such as the dog spleen and sheep and bovine seminal vesicles. As shown in Table II, it is apparent that the pharmacologic profile of activity of the active compounds differs in the ocular tissues. Indoxole is 100 times as potent as indomethacin on the anterior uvea, whereas it is only approximately half as active as indomethacin on the sheep seminal vesicle preparation. In addition, phenylbutazone was approximately 20 to 30 times less active than indomethacin on the preparations of seminal vesicles and spleen, but was equipotent with indomethacin on the anterior uvea. On the other hand, aspirin was considerably less effective than indomethacin in all of the studies, similarly, paracetamol and dexamethasone were essentially without activity in all the tissues.

In the present experiments, aspirin was found to have little or no inhibitory activity on prostaglandin biosynthesis in either of the ocular tissues in vitro. However, variations in the relative potencies of aspirin and indomethacin have been noted previously (see Tables II and III). In dog spleen preparations, aspirin was approximately 100 times less active than indomethacin, and in bovine seminal vesicles...
aspirin was approximately 225 times less active than indomethacin. Therefore, taking into consideration the lower absolute potency of indomethacin on the ocular tissues under the present incubation conditions using a relatively high substrate concentration, the lack of effect of aspirin in the present study is not surprising. It is interesting, however, that in vivo aspirin (600 mg rectally) in the rabbit, which yielded plasma salicylate levels of 30 mg per cent, had a marked effect on prostaglandin biosynthesis in the anterior uvea. One possible explanation of this discrepancy would be that a more active metabolite of aspirin may participate in the overall effect of the drug in vivo. However, none of the possible metabolites of aspirin studied had a significant effect on prostaglandin biosynthesis in vitro. Alternatively, the problem may be related to variations in the control of enzymatic activity possibly linked to differing functions of prostaglandins in different tissues. A similar lack of effect of aspirin in vitro (up to 150 μg per milliliter) has recently been reported using the prostaglandin synthetase derived from microsomal fractions of canine myocardium.

Neither the antipyretic drug paracetamol nor the anti-inflammatory steroid dexamethasone were active in these in vitro experiments. The lack of effect of dexamethasone seen in the present study suggests that the lowered levels of prostaglandin-like activity found in patients with acute anterior uveitis treated with topical dexamethasone were not the result of any direct action of the steroid on prostaglandin biosynthesis. However, we cannot rule out the possibility that the very high local concentrations of dexamethasone which can be achieved by topical administration may have had some action on the prostaglandin system.

The ideal ocular anti-inflammatory agent would be effective following topical application. Therefore, the next step would be to evaluate and compare the actions of the compounds found to be active in the present study by this route of administration, since other factors such as ease of penetration and local tissue irritation are bound to affect the final selection.

We dedicate this paper to the memory of Professor G. K. Smelser of Columbia University. Kenneth E. Eakins acknowledges a special debt to George Smelser for the opportunity he gave me to enter the field of Eye Research. We thank Anna Szechter for her technical assistance and Dr. J. Pike, The Upjohn Company, Kalamazoo, Mich. for the prostaglandins used in this study.

REFERENCES