Printed in U.S.A.

EFFECT OF NONSTEROID ANTI-INFLAMMATORY AND ANTIPYRETIC DRUGS ON PROSTAGLANDIN BIOSYNTHESIS BY HUMAN SKIN*

MALCOLM W. GREAVES, M.D., PH.D., M.R.C.P., AND WENDY McDONALD-GIBSON

ABSTRACT

There is increasing evidence that prostaglandins are mediators of inflammation in skin and that prostaglandins are synthesised locally in response to the inflammatory stimulus. The effect of four nonsteroid anti-inflammatory or antipyretic drugs on prostaglandin biosynthesis by human skin has therefore been studied. Aspirin (0.56 mM) and indomethacin (0.28 mM) produced a small but significant inhibition of synthesis of prostaglandin E_2. Indomethacin and chloroquine, but not aspirin, inhibited synthesis of prostaglandin $F_{2\alpha}$. Acetaminophen inhibited synthesis of prostaglandin $F_{2\alpha}$ but did not inhibit prostaglandin E_2 synthesis. None of the drugs studied are therapeutically effective anti-inflammatory agents in human skin and it may be significant that the inhibitory effects of aspirin and indomethacin on prostaglandin synthesis by skin are small compared with the effects of the same drugs on prostaglandin synthesis in other tissues.

Prostaglandins have been identified in several different types of experimental inflammatory skin lesions including ultraviolet-induced inflammation [1, 2], contact dermatitis [3], and scalded skin [4]. Little or no prostaglandin activity is present in normal skin [5] and it is probable that the prostaglandin activity in inflamed skin is the result of local biosynthesis. Prostaglandin synthetase activity has been identified both in rat and human skin [5–8]. In previous work we found that in vitro prostaglandin biosynthesis by homogenates of rat and human skin was inhibited by the potent corticosteroid, fluocinolone acetonide. Vane [9], who used cell-free supernatants from homogenized guinea-pig lung as a source of prostaglandin synthetase, was unable to demonstrate significant inhibition of prostaglandin synthesis by hydrocortisone, although aspirin and indomethacin produced marked inhibition in his experiments. Similar results with nonsteroid anti-inflammatory drugs have been obtained using prostaglandin synthetase from sheep seminal vesicles, human platelets, and dog spleen [10–12]. We have investigated the effect of aspirin and indomethacin on prostaglandin biosynthesis by homogenates of human skin. Aspirin and indomethacin have both anti-inflammatory and analgesic activities. By contrast, chloroquine although a potent anti-inflammatory drug is devoid of analgesic activity. Acetaminophen is a potent antipyretic and analgesic agent without anti-inflammatory activity. It was therefore of great interest to determine the effect of chloroquine and acetaminophen on prostaglandin biosynthesis by skin.

MATERIALS AND METHODS

Healthy human breast skin was used in all experiments within 2 hr of mastectomy operations. The subcutaneous fat was trimmed off, the skin was weighed and divided into small fragments with scissors. Approximately 0.4-gm skin fragments were placed in 1.5 ml phosphate buffer (pH 7.4) containing hydroquinone (0.27 mM) and glutathione (0.325 mM) [13]. The skin was homogenized with a Polytron tissue homogenizer and 0.5 ml bovine serum albumin solution was added to a final concentration of 5 mg/ml. The homogenate was then incubated aerobically in the presence of an excess of the prostaglandin E_2 and $F_{2\alpha}$ precursor arachidonic acid (Sigma Chemical Co., 99% pure grade 1) containing 0.1 μc tritium labelled arachidonic acid (specific activity 9.6 C per mM) (New England Nuclear Inc.) for 40 min at 37°C in a shaking water bath. The final concentration of arachidonic acid was 12.5 μg/ml and the reaction volume was 2 ml. The reaction was terminated by adding 10 ml of ice-cold ethanol and the mixture maintained at 4° C for 4 hr. After centrifugation, the supernatant was retained and the residue washed in ice-cold ethanol. Acidic lipids were extracted from the combined washings and supernatants with petroleum ether and diethyl ether followed by thin-layer chromatography using the method of Greaves and McDonald-Gibson [14].

The prostaglandin E_2 and prostaglandin $F_{2\alpha}$ activity in the extract was estimated by elution of the zones of the chromatoplate corresponding to simultaneously developed standard prostaglandins E_2 and $F_{2\alpha}$ followed by determination of radioactivity of the eluates using a Packard Tricarb Liquid Scintillation counter. In a single experiment prostaglandin activity in the zones of the chromatoplate corresponding to prostaglandins E_2 and $F_{2\alpha}$ was determined concurrently by the radioactive method described above and by bioassay using the isolated rat uterus preparations. There was no significant difference in results obtained by radioactivity determinations and by bioassay of these chromatoplate eluates, confirming that the E_2 and $F_{2\alpha}$ zones of the chromatoplate contained, respectively, prostaglandin E_2 and $F_{2\alpha}$. Recoveries of prostaglandin E_2 and $F_{2\alpha}$ applied to the skin prior to

Manuscript received January 12, 1973; in revised form May 2, 1973; accepted for publication May 8, 1973.
This work was supported by Grant No. G 971/33 of the Medical Research Council.

* From the Department of Dermatology, The University of Newcastle upon Tyne, Newcastle upon Tyne, England.

incubation exceeded 90 percent and there was less than 10 percent variation in the rate of synthesis between different replicates of the same skin sample.

The effect of anti-inflammatory or antipyretic drugs was studied by adding the drug to the reaction mixture immediately before incubation. All experiments included negative control determinations in which arachidonic acid and other reagents were incubated with buffer alone before addition of skin homogenates, extraction, and thin-layer chromatography. Values obtained from the negative control were subtracted from all experimental incubation values before determination of prostaglandin E_2 and $F_{2\alpha}$ synthesis.

RESULTS

Skin samples from 7 donors were studied. Conversion of arachidonic acid to prostaglandin E_2 and prostaglandin $F_{2\alpha}$ took place in all experiments. The mean total prostaglandin synthesis in 40 min was 6.09 μg/gm wet weight of skin (mean percent conversion per 0.4 gm skin sample per 40 min = approx 9). 66.8 percent of the synthesized prostaglandin activity was PGE_2. There was no significant synthesis of prostaglandins by skin homogenates in the absence of added arachidonic acid. The results in the presence of the anti-inflammatory drugs are summarized in Figures 1 and 2.

Aspirin. Aspirin in a concentration of 0.56 mM produced a small inhibition of prostaglandin E_2 biosynthesis of 19 ± 8% (S.E.M.) and this was statistically significant ($p < 0.05$). There was no significant change in prostaglandin $F_{2\alpha}$ synthesis in the presence of aspirin although the effects of aspirin on synthesis of prostaglandin $F_{2\alpha}$ were extremely variable between different experiments. Total prostaglandin synthesis (prostaglandin E_2 + prostaglandin $F_{2\alpha}$) was reduced by 19 percent but this was not statistically significant ($p > 0.05$).

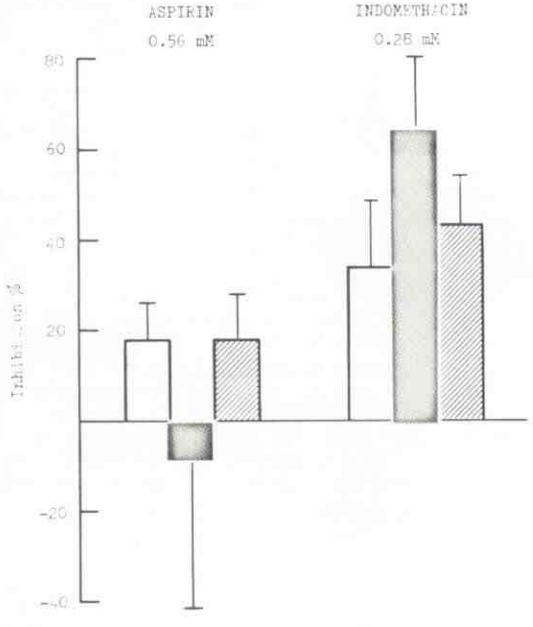

FIG. 1: Effect of aspirin and indomethacin on prostaglandin E_2 and $F_{2\alpha}$ synthesis by homogenates of human skin in the presence of excess arachidonic acid substrate. All results expressed as percent inhibition compared with control synthesis in the absence of the drug. Open rectangles: prostaglandin E_2. Solid rectangles: prostaglandin $F_{2\alpha}$. Shaded rectangles: total prostaglandin synthesis (E_2 and $F_{2\alpha}$). Drugs added immediately before incubation.

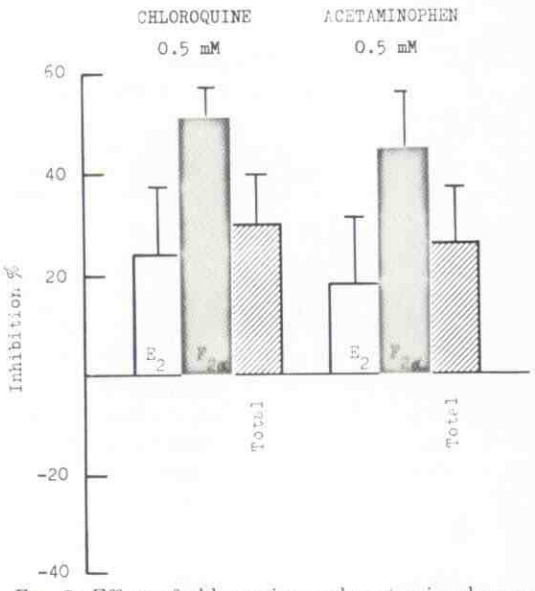

FIG. 2: Effect of chloroquine and acetaminophen on prostaglandin E_2 and $F_{2\alpha}$ synthesis by homogenates of human skin, in the presence of excess arachidonic acid substrate. All results expressed are percent inhibition compared with control synthesis in the absence of the drug. Open rectangles: prostaglandin E_2. Solid rectangles: prostaglandin $F_{2\alpha}$. Shaded rectangles: total prostaglandin synthesis (E_2 and $F_{2\alpha}$). Drugs added immediately before incubation.

Indomethacin. Indomethacin was a slightly more potent inhibitor of prostaglandin synthesis (Fig. 1). In a concentration of 0.28 mM, indomethacin caused a significant inhibition of biosynthesis of prostaglanin E_2. (34 ± 13% [S.E.M.] $p < 0.025$), prostaglandin $F_{2\alpha}$ (64 ± 15% [S.E.M.] $p < 0.005$), and total prostaglandin synthesis (43% ± 10% [S.E.M.] $p < 0.005$).

Chloroquine. Chloroquine 0.5 mM caused marked inhibition of prostaglandin $F_{2\alpha}$ synthesis (51 ± 6% [S.E.M.] $p < 0.0005$) (Fig. 2) but had little inhibitory effect on prostaglandin E_2 synthesis (24 ± 13% [S.E.M.] $p > 0.05$). However, inhibition of total prostaglandin synthesis was significant (30 ± 10% [S.E.M.] $p < 0.025$).

Acetaminophen (Fig. 2). Like chloroquine, acetaminophen in a concentration of 0.5 mM caused significant inhibition of synthesis of prostaglandin $F_{2\alpha}$, and total prostaglandin synthesis (45 ± 11% [S.E.M.] $p < 0.0125$; 26 ± 11% [S.E.M.] $p < 0.05$, respectively) but there was no inhibition of prostaglandin E_2 synthesis.

DISCUSSION

These results show that none of the four nonsteroid anti-inflammatory or antipyretic drugs studied were potent inhibitors of prostaglandin E_2 biosynthesis by human skin homogenates although aspirin and indomethacin caused a small but significant inhibition of synthesis of prostaglandin E_2. By contrast, indomethacin, chloroquine, and acetaminophen produced 45 percent or more inhibition of prostaglandin $F_{2\alpha}$ synthesis. The E prostaglandins are highly vasoactive but the F prostaglandins produce little or no inflammation in skin [15]. If the four drugs owe their anti-inflammatory or antipyretic activity to inhibition of prostaglandin biosynthesis, then these results may explain their ineffectiveness in inhibiting inflammation in skin. In experiments published elsewhere [8] we found that fluocinolone acetonide, a corticosteroid which is an extremely effective anti-inflammatory agent in human skin, produced greater inhibition of prostaglandin E_2 synthesis by human skin than either aspirin or indomethacin did in the present experiments. By contrast, Vane [9], who used enzyme preparations from guinea-pig lung, found that aspirin and indomethacin in similar concentrations were 2–10 times more potent inhibitors of synthesis in his system than ours, whereas he was able to obtain little or no inhibition with corticosteroids. There are at least two possible reasons for this difference. There is evidence [16] that prostaglandin synthetase from different tissues may show differing sensitivities to the inhibitory effects of the same anti-inflammatory drugs. Alternatively, the difference could be related to the use in our experiments of a simple homogenate of human skin whereas Vane used cell-free supernatants from guinea-pig lung homogenates. Our negative results with nonsteroid drugs are unlikely to be due to use of inadequate concentrations since the concentrations used were in excess of blood and tissue concentrations achieved after therapeutic dosage [17–19].

The results with chloroquine and acetaminophen are of special interest. Chloroquine probably owes its anti-inflammatory activity at least in part to stabilization of lysosomal membranes [20]. In the present experiments there was a small inhibition of synthesis of prostaglandin E_2 by chloroquine but synthesis of prostaglandin $F_{2\alpha}$ was reduced by about 50 percent. We have recently reported the effects of chloroquine on prostaglandin synthesis by rat skin [21] and found that there was a small significant inhibition of prostaglandin $F_{2\alpha}$ synthesis. Although acetaminophen is a potent analgesic and antipyretic it is devoid of anti-inflammatory activity. Our results do not, however, support the suggestion of Willis et al [22] that the antipyretic action of acetaminophen may be due to inhibition of prostaglandin E_2 synthesis.

REFERENCES

1. Greaves MW, Sondergaard J: Pharmacologic agents released in ultraviolet inflammation studies by continuous skin perfusion. J Invest Dermatol 54:365, 1970
2. Mathur GP, Gandi MV: Prostaglandin in human and albino rat skin. J Invest Dermatol 58:291, 1972
3. Greaves MW, Sondergaard J, McDonald-Gibson W: Recovery of prostaglandins in human cutaneous inflammation. Br Med J 2:258, 1971
4. Ängaard E, Arturson G, Jonsson EE: Efflux of prostaglandins in lymph from scalded tissues. Acta Physiol Scand 80:46A, 1970
5. Van Dorp D: Recent developments in the biosynthesis and the analyses of prostaglandins. Ann NY Acad Sci 180:181, 1971
6. Ziboh VA, Hsia SL: Prostaglandin E_2: biosynthesis and effects on glucose and lipid metabolism in rat skin. Arch Biochem Biophys 146:200, 1971
7. Jonsson C-E, Ängaard E: Biosynthesis and metabolism of prostaglandin E_2 in human skin. Scand J Clin Lab Invest 29:289, 1972
8. Greaves MW, McDonald-Gibson W: Prostaglandin biosynthesis by human skin and its inhibition by corticosteroids. Br J Pharmacol 46:172, 1972
9. Vane JR: Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nature [New Biol] 231:232, 1971
10. Smith WL, Lands WEM: Stimulation and blockade of prostaglandin biosynthesis. J Biol Chem 246:6700, 1971
11. Smith JB, Willis AL: Aspirin selectively inhibits prostaglandin production by human platelets. Nature [New Biol] 231:235, 1971
12. Ferreira SH, Moncada S, Vane JR: Indomethacin and aspirin abolish prostaglandin release from the spleen. Nature [New Biol] 231:237, 1971
13. Foss P, Takeguchi C, Tai H, Sih C: Mechanism of prostaglandin biosynthesis. Ann NY Acad Sci 180:126, 1971
14. Greaves MW, McDonald-Gibson W: Extraction of prostaglandin-like activity from whole human blood. Life Sci 11:73, 1972
15. Crunkhorn P, Willis AL: Cutaneous reactions to intradermal prostaglandins. Br J Pharmacol 41:49, 1971
16. Sykes JAC, Maddox IS: Prostaglandin production by experimental tumours and effects of anti-inflammatory compounds. Nature [New Biol] 237:59, 1972
17. Greenberg LA, Lester D: The metabolic fate of acetanilid and other aniline derivatives. J Pharmacol Exp Ther 90:150, 1947
18. Brodie BB, Axelrod J: The fate of acetophenetidin (PHenacetin) in man and methods for the estimation of acetophenetidin and its metabolites in biological material. J Pharmacol Exp Ther 97:58, 1949
19. Prouty RW, Kuroda K: Spectrophotometric determination and distribution of chloroquine in human tissues. J Lab Clin Med 52:477, 1958
20. Homewood CA, Warhurst DC, Peters W, Baggeley VC: Lysosomes, pH and the anti-malarial action of chloroquine. Nature [New Biol] 235:50, 1972
21. Greaves MW, McDonald-Gibson W: Anti-inflammatory agents and prostaglandin biosynthesis. Br Med J 3:527, 1972
22. Willis AL, Davison P, Ramwell PW, Brocklehurst WE, Smith B: Release and action of prostaglandins in inflammation and fever: inhibition by anti-inflammatory and anti-pyretic drugs, Prostaglandins in Cellular Biology. Edited by PW Ramwell, BB Pharriss. Alza Conference Series, vol 1, 1972, p 227