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SHORT COMMUNICATIONS

Inhibition of thromboxane B_2 and 6-ketoprostaglandin $F_{1\alpha}$ formation by anti-inflammatory drugs in carrageenin-induced granuloma

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Indomethacin, aspirin and salicylate, in descending order of potency, inhibit the synthesis of prostaglandin E_2 and $F_{2\alpha}$ from arachidonic acid by cell-free preparations of a guinea-pig lung [1]. Vane has proposed that the clinical actions of these drugs could be explained by inhibition of prostaglandin production.

In our previous papers [2–4], we reported that thromboxane B_2 and 6-ketoprostaglandin $F_{1\alpha}$ were mainly formed from arachidonic acid, while prostaglandin E_2 and $F_{2\alpha}$ were only formed in negligible amounts by the homogenate of rat carrageenin granuloma. Both thromboxane B_2 and 6-ketoprostaglandin $F_{1\alpha}$ have recently been proven to be formed from arachidonic acid via prostaglandin endoperoxides, i.e. prostaglandin G_2 and H_2 [5, 6]. The formation of endoperoxides is followed by another transformation via labile thromboxane A_2 in human platelets in the case of thromboxane B_2 [7], and via labile prostacyclin in pig and rabbit aortas, pig mesenteric arteries, bovine coronary arteries and rat stomach fundus in the case of 6-ketoprostaglandin $F_{1\alpha}$ [8–13]. The present experiments were undertaken in order to investigate the change in biosynthetic activity on thromboxane B_2 and 6-ketoprostaglandin $F_{1\alpha}$ with the development of inflammation and the effect of anti-inflammatory drugs on their biosynthesis from arachidonic acid by the homogenate of rat carrageenin granuloma.

[1-^{14}C]Arachidonic acid (58 mCi/m-mole) was purchased from the Radiochemical Centre, Amersham, England. Arachidonic acid, 99 per cent purity, indomethacin and hydrocortisone-21-phosphate were purchased from Sigma Chemical Co., Ltd., St. Louis, U.S.A. Thin-layer chromatography (TLC) plates of Silica gel 60 F_{254}, 0.25 mm in thickness, were purchased from E. Merck, Darmstadt, Germany. Aspirin was supplied by Tsukishima Yakuhin Co., Ltd., Tokyo, Japan.

Granuloma pouch was induced in male Sprague–Dawley strain rats weighing from 130 to 150 g by the method described in a previous paper [14]. Eight-day-old granulomas were used unless otherwise stated. The granulation tissues were homegenized in Ca^{2+}-and Mg^{2+}-free phosphate buffered saline (tissue to buffer ratio, 1:2.5, w/v). The homogenate was then centrifuged at 600 *g* for 5 min, and the resulting supernatant was used as the enzyme source. Protein content was measured by the method of Lowry *et al.* [15] using bovine serum albumin as the standard. Each incubation tube contained 4 ml of the enzyme (14 mg protein/ml), 0.2 μCi of [14C]arachidonic acid, 50 μg of unlabeled arachidonic acid and one of the anti-inflammatory drugs in various concentrations. Incubation was carried out in air at 37° for 30 min with shaking. Reaction was terminated by chilling the mixture followed by quickly adding an appropriate amount of 1 N HCl to bring the pH of the reaction mixture to 3.0. The mixture was then extracted with 8 vol. of ethyl acetate. The resulting organic phase was evaporated to dryness under reduced pressure. Residues were dissolved in a small amount of ethanol and applied to TLC plates. The plates were developed in the AI solvent system (benzene–dioxane–acetic acid 20:20:1, v/v [16], and the radioactive products were detected by a Dünnchicht scanner. Zones corresponding to thromboxane B_2 and 6-ketoprostaglandin $F_{1\alpha}$ were separately scraped off, and the radioactivities were counted by a scintillation counter.

As described in the previous papers [2–4], thromboxane B_2 and 6-ketoprostaglandin $F_{1\alpha}$ are the two main metabolites transformed from arachiodonic acid by the homogenate of carrageenin induced granuloma. Thin-layer chromatographic R_f values of thromboxane B_2 and 6-ketoprostaglandin $F_{1\alpha}$ in AI solvent system were 0.62 and 0.42, respectively. The production rate of these metabolites undergoes different alterations with the development of inflammation, i.e. the production of thromboxane B_2 increases, while 6-ketoprostaglandin $F_{1\alpha}$ decreases as shown in Fig. 1. The significance of these alterations still remains unknown, since only two studies reporting biological activities for 6-ketoprostaglandin $F_{1\alpha}$ have been carried out to date. Dawson *et al.* [17] found 6-ketoprostaglandin $F_{1\alpha}$ has prostaglandin $F_{2\alpha}$ like activity in respiratory smooth muscle, and Pace-Asciak [18] found 6-ketoprostaglandin $F_{1\alpha}$ is somewhat active in inhibiting platelet aggregation induced by arachidonic acid. The intermediate precursors of 6-ketoprostaglandin $F_{1\alpha}$ and thromboxane B_2, however, are reported to be the very biologically active substances; prostacyclin has striking properties of vasodilatation and inhibition of platelet aggregation [8–13], while thromboxane A_2 leads to vasocontraction and platelet aggregation [19, 20, 7]. Therefore, our observation that the production of thromboxane B_2 and 6-ketoprostaglandin $F_{1\alpha}$ from arachidonic acid in the homogenate of carrageenin induced granuloma undergoes different alterations with the development of inflammation as shown in Fig. 1 suggests an interesting possibility, namely that prostacyclin and thromboxane A_2 might be implicated in the regulation of exudation and repair responses in inflammation.

The effect of anti-inflammatory drugs on the formation of these two products was examined. Aspirin and indomethacin were chosen as typical non-steroidal anti-inflammatory drugs, and hydrocortisone was chosen as a typical steroidal anti-inflammatory drug. For each drug, the dosage used covered a wide range, i.e. 2–500 μM for aspirin; 0.2–0.50 μM for indomethacin; and 0.01–1000 μM for hydrocortisone. Inhibition of the generation of the two metabolites by these drugs is expressed as per cent of control in the figures. As shown in Figs 2 and 3, both thromboxane B_2 and 6-ketoprostaglandin $F_{1\alpha}$ are inhibited by both aspirin and indomethacin, and the inhibition rates of both thromboxane B_2 and 6-ketoprostaglandin $F_{1\alpha}$ are decreased to almost the same degree by each non-steroidal anti-inflammatory drugs. The concentration for 50 per cent inhibition for indomethacin is about 3 μM, whereas that for aspirin was about 500 μM. On the other hand, hydrocortisone fails to produce either a significant stimulation or inhibition of the generation of these two products, even at the high concentration of 1000 μM.

Although prostaglandin E is widely suspected to be a chemical mediator of inflammation [21], we have suggested that the contribution of endogenous prostaglandin E as a mediator of the response to vascular permeability in granulomatous inflammation is minor at best. The endogenous prostaglandin E level was not high enough to

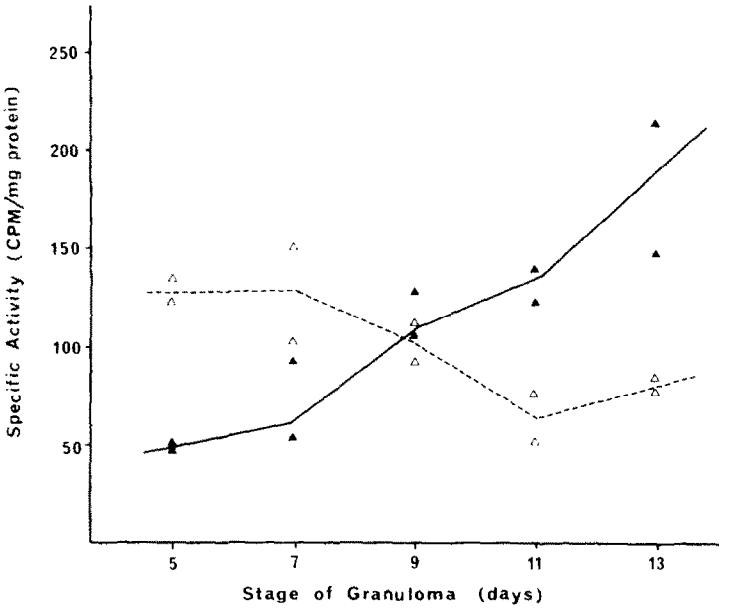

Fig. 1. Alterations of the production of thromboxane B_2 (closed triangles) and 6-ketoprostaglandin $F_{1\alpha}$ (open triangles) from arachidonic acid in the homogenate of carrageenin induced granuloma with the development of inflammation. Each point is the mean of duplicate experiments on one animal. See text for details.

evoke a vascular permeability response in the granulation tissue [2, 22]. Moreover, the granulation tissue was found to be inert to transform 8,11,14-eicosatrienoic acid to prostaglandin E_1, prostaglandin $F_{1\alpha}$ and any other products (unpublished data) and to have negligible biosynthetic and metabolizing activity of prostaglandin E_2 and $F_{2\alpha}$ [2]. As shown in Figs 2 and 3, the generation of thromboxane B_2 and 6-ketoprostaglandin $F_{1\alpha}$ is inhibited in the same degree by indomethacin and aspirin in the homogenate of carrageenin induced granuloma. These findings are in good agreement with the fact that the mode of action of non-steroidal anti-inflammatory drugs is through the inhibition of the activity of prostaglandin synthetase, and consequently through the reduction of prostaglandin generation [1, 23]. On the molar basis, indomethacin is approximately 170-fold more potent than aspirin as an inhibitor of the synthesis of thromboxane B_2 and 6-ketoprostaglandin $F_{1\alpha}$ in the homogenate of carrageenin induced granuloma. The inhibition on a molar basis found here is similar to that on prostaglandin E generation with dog spleen enzyme preparations [24], and that on prostaglandin E_2 and $F_{2\alpha}$ generation with lyophilized bovine seminal vesicle enzyme preparations [25]. Hydrocortisone shows no effect on the generation of thromboxane B_2 and 6-ketoprostaglandin $F_{1\alpha}$ from arachidonic acid in the homogenate of carrageenin induced granuloma as stated above. This result is consistent with the fact that steroidal anti-inflammatory drugs do not inhibit the activity of prostaglandin synthetase [1], but inhibit the release of arachidonic acid from phospholipids in cell membrane [26].

Overall results stated in this communication strongly suggest that the biosynthetic formation of thromboxane

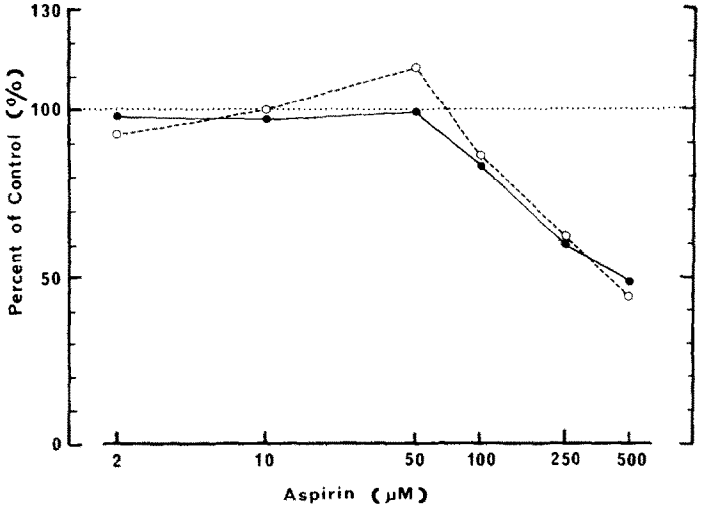

Fig. 2. Inhibition of thromboxane B_2 (closed circles) and 6-ketoprostaglandin $F_{1\alpha}$ (open circles) from arachidonic acid by aspirin in the homogenate of carrageenin induced granuloma. See text for details.

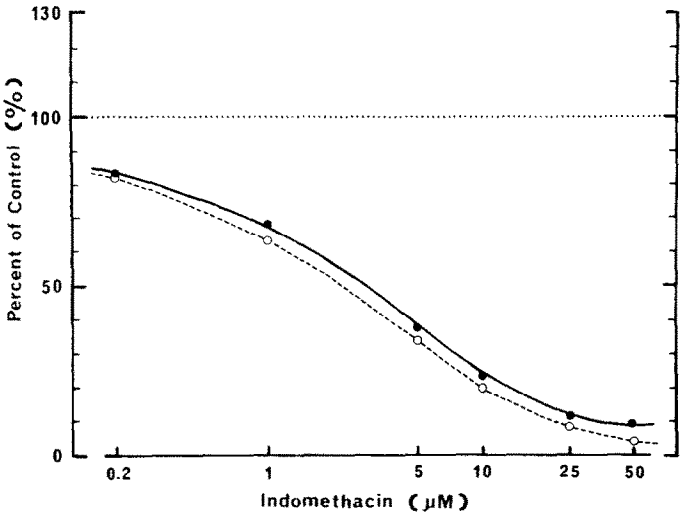

Fig. 3. Inhibition of thromboxane B_2 (closed circles) and 6-ketoprostaglandin $F_{1\alpha}$ (open circles) from arachidonic acid by indomethacin in the homogenate of carrageenin induced granuloma. See text for details.

B_2 and 6-ketoprostaglandin $F_{1\alpha}$ from arachidonic acid in the homogenate of carrageenin induced granuloma is via the pathway of prostaglandin endoperoxides. This is the same as that of thromboxane B_2 in human platelets [5, 7] and that of 6-ketoprostaglandin $F_{1\alpha}$ in pig and rabbit aortas, pig mesenteric arteries, bovine coronary arteries, and rat stomach fundus [6, 8–13]. Moreover, since the formation rate of these two metabolites from arachidonic acid undergoes different alterations with the development of inflammation as shown in Fig. 1, further investigation on the biological role of these two metabolites and their intermediate precursors in inflammation is under way in our laboratory and will be reported elsewhere.

Summary. The effect of anti-inflammatory drugs on the formation of thromboxane B_2 and 6-ketoprostaglandin $F_{1\alpha}$ from arachidonic acid in the homogenate of carrageenin induced granuloma was characterized by a radiometric assay. Indomethacin and aspirin show an inhibitory effect. On a molar basis, indomethacin is about one hundred and seventy times more potent than aspirin. Hydrocortisone-21-phosphate shows no effect. It was also known that the production rate of thromboxane B_2 and 6-ketoprostaglandin $F_{1\alpha}$ undergoes different alterations with the development of inflammation, i.e. the production of thromboxane B_2 increases, while that of 6-ketoprostaglandin $F_{1\alpha}$ decreases.

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