Original Article

Differential effects of ibuprofen, indomethacin, and meclofenamate on prostaglandin endoperoxide H₂ metabolism

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Received 27 June 1988; accepted 28 November 1988

Key words: arachidonic acid, prostaglandin endoperoxide H₂, ibuprofen, indomethacin, meclofenamate, feline lung

Summary

Previous studies have suggested the possibility that the non-steroidal antiflammatory drug (NSAID), ibuprofen, may inhibit thromboxane (TX) A₂ synthase activity in addition to inhibiting cyclooxygenase activity. Microsomal fractions isolated from the cat lung contain cyclooxygenase as well as prostacyclin (PGI₂) synthase, TX synthase, and a GSH-dependent prostaglandin (PG) E₂ isomerase activities. When [1-¹⁴C] PG endoperoxide H₂ (PGH₂) was used as substrate, ibuprofen, indomethacin, and meclofenamate exhibited differential effects on terminal enzyme activities. Ibuprofen, at concentrations up to 1 mM, had no effect on the activities of PGI₂ synthase, TXA₂ synthase of GSH-dependent PGE₂ isomerase, whereas indomethacin selectively inhibited PGI₂ synthase activity at 5 × 10⁻⁴ M and 10⁻³ M. Meclofenamate selectively inhibited TXA₂ synthase activity at 5 × 10⁻⁴ M and 10⁻³ M. At concentrations of 5 × 10⁻³ M, this selectivity was not observed, and indomethacin and meclofenamate decreased the formation of both 6-keto-PGF₁α and TXB₂. These data indicate that the choice of NSAID and the concentration employed may specifically alter PGH₂ metabolism. This action may affect the physiologic consequences of the exchange of PGH₂ between cells. The data further indicate that indomethacin has the potential for use as a tool to specifically attenuate PGI₂ synthase activity in vitro.

Introduction

Metabolites of AA play an important role in lung physiology [1, 2]. NSAIDs inhibit cyclooxygenase activity and are widely used for studying the role of cyclooxygenase products in pathophysiologic processes, such as endotoxin shock [3–6]. In all such studies using cyclooxygenase inhibitors as probes, it is assumed that cyclooxygenase inhibition has occurred in lung tissue and that the formation of all terminal enzyme products will be inhibited to an equal extent. However, it has been demonstrated that indomethacin inhibits phospholipase A₂ activity at high concentrations in renal medullary slices [7]. It has also been suggested that ibuprofen may have an additional effect or even its major effect on thromboxane synthase rather than on cyclooxygenase [8, 9]. This study addressed the hypothesis that ibuprofen alters the metabolism of PGH₂. We, therefore, studied the effect of three structurally dissimilar inhibitors of cyclooxygenase activity, ibuprofen (a derivative of proprionic acid),
meclofenamate (a derivative of N-phenylanthranolic acid), and indomethacin (a methylated indole derivative) on arachidonic acid and PGH₂ metabolism in microsomal fractions prepared from the feline lung. Using an in vitro tissue preparation allowed monitoring of the effects of ibuprofen, meclofenamate, and indomethacin on the cyclooxygenase and terminal enzymes; PGI₂ synthase, TXA₂ synthase, and GSH-dependent PGE₂ isomerase activities. The present studies indicate that ibuprofen has no inhibitory activity on any of the terminal enzymes studied. Meclofenamate, however, exhibited selective inhibitory action on TXA₂ synthase activity, and indomethacin exhibited selective inhibitory action on PGI₂ synthase activity. The data indicate also that indomethacin might serve as an investigational tool in the study of PGH₂ metabolism by virtue of its selective activity on PGI₂ synthase.

**Experimental procedures**

Arachidonic acid and indomethacin were purchased from Sigma, St. Louis, MO. Meclofenamate was obtained from Parke-Davis, Warner Lambert, Ann Arbor, MI. Ibuprofen and prostaglandin standards were obtained from The Upjohn Co., Kalamazoo, MI. [¹-¹⁴C]-AA (59.6 mCi/mnmole) was purchased from Amersham, Arlington Heights, IL. Prostaglandin H synthase was purchased from Oxford Biomedical Research, Inc., Oxford, MI.

**Preparation of prostaglandin endoperoxide H₂**

(L-¹⁴C) PGH₂ was generated from 250 nmol (L-¹⁴C) AA by incubation in 0.1 M potassium phosphate buffer (pH 8.0) for 30 sec at 37 °C with 150 units of prostaglandin H synthase. The reaction was terminated by acidification with HCl to pH 2-3, and the reaction mixture was filtered under vacuum onto an ice-cold SPE Octadesylilane (Cl8) 3 ml column (J.T. Baker, Phillipsburg, NJ). Stepwise elution with hexane:ether mixtures in the ratio 8:2, 6:4, 5:5, and 4:6 at 4 °C yielded pure PGH₂ (>90%) in the 4:6 fractions. This methodology is a modification of the previously published method [10]. Low specific activity (L-¹⁴C) PGH₂ was prepared from seminal vesicle microsomes using 3.3 μmol AA plus 10 nmol (L-¹⁴C) AA [10] and purified in the same fashion. All PGH₂ was stored as a hexane/ether solution at −45 °C.

The incubation mixture for AA metabolism contained 200 μg lung microsomal protein in a total volume of 100 μg of 0.1 M potassium phosphate buffer (pH 8.0), 5 mM tryptophan, 20 μM (L-¹⁴C) AA, 0.12 μCi, and other additions as indicated in the text. The incubation mixture for PGH₂ metabolism contained 200 μg of lung microsomal protein in a total volume of 100 μl of 0.1 M potassium phosphate buffer (pH 7.4), 10 μM (L-¹⁴C) PGH₂ (15000 cpm), and other additions as indicated in the text and tables.

The reaction was initiated by the addition of the microsomal fraction, which had been preincubated in one of the above described mixtures for 3 min at 37 °C, to a cold 1.5 ml Brinkman centrifuge tube containing either the AA or the PGH₂ (which had been previously evaporated to dryness under a stream of N₂). The tube was immediately vortexed and incubated at 37 °C for 60 min (AA) or 2 min (PGH₂). Product formation was maximal after incubation with arachidonic acid or PGH₂ for 1 hr or 2 min, respectively, and, as such, represents the profile of products formed during endpoint incubations. The reaction was stopped and the products extracted by adding 400 μl of an ice-cold solution of ethyl acetate:methanol:0.2 M citric acid (15:2:1). The tubes were vortexed and centrifuged at 12800 × g for 30 sec and quickly frozen on dry ice. The upper