RELATIONSHIP BETWEEN INHIBITION OF PROSTAGLANDIN SYNTHESIS AND DRUG EFFICACY: SUPPORT FOR THE CURRENT THEORY ON MODE OF ACTION OF ASPIRIN-LIKE DRUGS

R. V. Tomlinson, H. J. Ringold,
M. C. Qureshi and E. Forchielli
Institute of Hormone Biology
Syntex Research Center
Palo Alto, California

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SUMMARY

The synthesis of Prostaglandin E_2 (PGE_2) from 5,8,11,14-eicosatetraenoic acid (arachidonic acid) by bovine seminal vesicle microsomes can be inhibited by the non-steroidal anti-inflammatory agents indomethacin, naproxen, its enantiomer and aspirin. The relative inhibitory potencies are 2140 to 150 to 2 to 1. These values correlate in general with anti-inflammatory, anti-pyretic and analgesic activities of these compounds, as evaluated in a number of animal models. Since the prostaglandins have been implicated in inflammation, pyresis and pain, this positive correlation adds support to the hypothesis that a mode of action of aspirin-like drugs is to inhibit the biosynthesis of prostaglandins.

In 1969 Collier proposed that aspirin acted by blocking the actions of humoral inflammatory mediators that are formed as a consequence of the body's defense reactions[1]. Recently, two groups of workers, Vane[2], Ferreira, Moncada and Vane[3], and Smith and Willis[4] have shown that the non-steroidal anti-inflammatory agents aspirin, sodium salicylate and indomethacin inhibit prostaglandin synthesis in guinea pig lung homogenates, perfused canine spleen, and human blood platlets. The observations that injected prostaglandins evoke inflammatory[5] or pyretic[6] responses *in vivo* while their synthesis *in vitro* can be inhibited by aspirin or indomethacin have formed the basis for a proposal that Collier's humoral mediators are the prostaglandins.

In the aforementioned examples, the characterization of prostaglandins and blockade of synthesis was established and quantitated only via biological assay because of the limited biosynthetic capability of

the systems. Therefore, it was deemed of importance to study a well-defined prostaglandin synthetase with sufficient capacity and yield to permit physical quantitation. For this purpose we selected the bull seminal vesicle microsome system which converts the requisite unsaturated fatty acid precursors into PGE_1 or PGE_2 in high yield and we report in this communication a firm correlation between *in vivo* anti-inflammatory and anti-pyretic activity in animals of a number of non-steroidal anti-inflammatory agents and their ability to inhibit the biosynthesis of prostaglandins. Further great specificity is demonstrated by comparison of a d- and l-isomer pair.

MATERIALS AND METHODS

5,8,11,14-eicosatetraenoic acid (arachidonic acid) was purchased from the Hormel Institute; 5,6,8,9,11,12,14,15-[^{3}H]-eicosatetraenoic acid from New England Nuclear and 1-[^{14}C]-eicosatetraenoic acid from Applied Science Labs., Inc. [^{14}C]-PGE was prepared enzymatically by incubating 1-[^{14}C]-arachidonic acid with the microsomal enzyme preparation from bull seminal vesicles as described below. Reduced glutathione and l-epinephrine were purchased from Sigma; indomethacin was the generous gift of the Merck-Sharpe and Dohme Research Laboratories, West Point, Pa., naproxen and its enantiomer† were generously supplied by Dr. Ian Harrison of our Institute of Organic Chemistry and the aspirin was obtained from commercial sources. The bull seminal vesicle microsome prostaglandin synthesizing enzyme system (BSVM) was prepared as described by Sih *et al*[7].

The incubation mixtures, consisting of 1 μCi [^{3}H]-ammonium arachidonate (0.33 mM), reduced glutathione (1.7 mM), l-epinephrine (0.5 mM),

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Indomethacin = 1-(p-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid
Naproxen = d-2-(6'-methoxy-2'-naphthyl)-propionic acid and its enantiomer: l-2-(6'-methoxy-2'-naphthyl)-propionic acid

EDTA (0.07 mM), 10 mg lyophilized BSVM (equivalent to 1 gm wet weight tissue) and inhibitor as indicated, in 1.5 ml 0.2 M tris-HCl buffer, pH 8.5 were incubated in 12 x 100 mm test tubes at 37° for 1 hr with gentle shaking in a Dubnoff Metabolic Shaking Incubator. At the end of the incubation they were treated in one of the two following ways:

1. The mixtures were diluted with 1.5 ml distilled water, acidified with 0.5 ml of 1.0 N HCl and immediately extracted with 2 x 5 ml ethyl ether. The extracts were back washed with one ml water, dried with Na_2SO_4, filtered through phase-separating paper and concentrated under a stream of nitrogen gas. The concentrates were chromatogramed on thin-layer silica plates in ethyl acetate-acetic acid-isooctane-water (110:20:50:100). The developed plates were scanned in a Packard Radiochromatogram Scanner to locate the radioactive zones. Under the experimental conditions described the BSVM system yielded only [^{3}H]-PGE_2 from the [^{3}H]-arachidonic acid, even in the presence of the inhibitors. The degree of inhibition of synthesis was established by comparing the area under the [^{3}H]-PGE_2 peak in the test with that found for the control. Average conversion of arachidonic acid to PGE_2 under normal conditions was about 60 percent.
2. The incubation mixtures were diluted with 2.0 ml distilled water, then acidified by addition of 0.5 ml of 1.0 N HCl. One ml aliquots of the acidified extracts were removed, further diluted with 2.0 ml water and a known quantity of [^{14}C]-PGE_2 was added. The solutions of [^{3}H,^{14}C]-PGE_2 were allowed to stand for 10 min at 5° then they were extracted and the extracts chromatogramed as described above. The doubly labelled PGE_2 was eluted from the silica plate with ethyl acetate and the $^3H/^{14}C$ ratio determined by scintillation counting in Bray's solution using a Packard Scintillation Counter. The decrease in the $^3H/^{14}C$ ratio in the test compared

to that ratio found in the control was taken as the measure of inhibition of prostaglandin synthesis.

RESULTS AND DISCUSSION

Figure 1 shows the results obtained when indomethacin, naproxen, its enantiomer and aspirin were evaluated as inhibitors of PGE_2 synthesis in the BSVM system††. All four compounds were inhibitory with indomethacin being the most potent, followed in decreasing order by naproxen, its enantiomer and aspirin. Summarized in Table I are the $I.D._{50}$ values ($I.D._{50}$ =concentration of inhibitor resulting in 50% inhibition of PGE_2 synthesis). The relationship between indomethacin and aspirin is qualitatively in agreement with that reported by others[2-4]. However, under the conditions in this study with BSVM the absolute difference in potencies is 25 to 100 times those previously

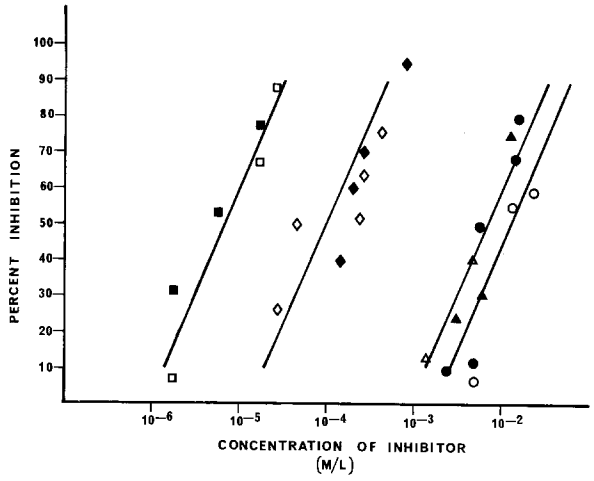

FIGURE 1 - Inhibition of PGE_2 Synthesis from Arachidonic Acid by the BSVM System by Various Anti-inflammatory Agents

Incubation and assay conditions are described under Materials and Methods. Open symbols represent results obtained by direct assay of ^3H (procedure 1). Closed symbols represent results determined by assay of $^3H/^{14}C$ ratios (procedure 2). Indomethacin, □ ■ ; naproxen, ◇ ◆ ; enantiomer, △ ▲ and aspirin, ○ ●.

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††In a separate experiment, synthesis of PGE_1 from eicosatrienoic acid by the same enzyme system was inhibited by indomethacin, meclofenamate, naproxen, aspirin and sodium salicylate (unpublished results).

TABLE I

COMPARISON OF THE INHIBITORY POTENCIES OF VARIOUS ANTI-INFLAMMATORY AGENTS ON THE *IN VITRO* SYNTHESIS OF PGE_2 FROM ARACHIDONIC ACID BY A BOVINE SEMINAL VESICLE MICROSOMAL ENZYME PREPARATION

Compound	ID_{50}*
Indomethacin	7×10^{-6}M
Naproxen	1×10^{-4}M
Naproxen enantiomer	7×10^{-3}M
Aspirin	1.5×10^{-2}M

*ID_{50} = Concentration of inhibitor resulting in 50% inhibition of PGE_2 synthesis.

reported, 2140 in the BSVM against 47 in the guinea pig lung homogenate[2] system. This most likely reflects either a more sensitive or a purer and more concentrated enzyme system with a relative lack of interfering materials and enzymes which may metabolize the PGE_2 formed. Table I also shows the very interesting difference which exists between the potencies of naproxen and its enantiomer as inhibitors. Even though these compounds exhibit similar binding affinities with certain plasma proteins[8] and have identical solubilities, naproxen is 70 times more potent an inhibitor of PGE_2 synthesis than is its enantiomer. This difference must be attributed to the configuration about the single asymmetric center in these compounds (Figure 2), which provides

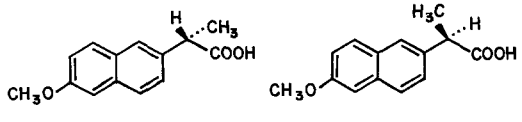

FIGURE 2 - Enantiomers of 2-(6'-methoxy-2'-naphthyl)-propionic acid

TABLE II

COMPARISON OF THE RELATIVE ANTI-INFLAMMATORY, ANTI-PYRETIC AND ANALGESIC POTENCIES OF INDOMETHACIN, NAPROXEN, ITS l-ENANTIOMER AND ASPIRIN AND THEIR ABILITY TO INHIBIT PGE_2 SYNTHESIS

Compound	Animal Models				BSVM System
	Anti-inflammatory		Anti-pyretic[13]	Analgesic[10]	Inhibitor Activity
	Adjuvant Induced Arthritis Activity[11]***	Carrageenin[9]			Anti-PGE_2 Synthesis
Indomethacin	2000**	48[12,13]	18[12,13]	58[12,13]	2140
Naproxen	200**	33[12,13,14]	22[12,13,14]	7[12,13,14]	150
Naproxen enantiomer	--	$\geq$1.5[14]	1.5[14]	$\geq$0.5[14]	2
Aspirin	1*	1[12,13,14]	1[12,13,14]	1[12,13,14]	1

*Relative activity of aspirin arbitrarily set at 1.0.
**Unpublished data.
***As determined on days 14-28 of the adjuvant induced arthritis.

an ideal test of the proposed relationship between inhibition of prostaglandin synthesis and anti-inflammatory, anti-pyretic properties Table II shows that indeed naproxen also possesses 10-20-fold higher activity than the enantiomer as an anti-inflammatory or anti-pyretic agent which strongly suggests that these two sets of activities are intimately related, and subject to the same steric requirements. In fact, despite variations of the levels of activities in the different animal models the overall correlation between the ability to block PGE_2 synthesis and anti-inflammatory, analgesic and anti-pyretic activities is in the proper order for all four compounds, thus supporting the current proposals with respect to the mode of action of non-steroidal anti-inflammatory drugs. The apparent absence of intermediates between arachidonic acid and PGE_2 during incubation in the presence of the inhibitors strongly suggests that the block in synthesis is at an early step and most likely at the level of arachidonate. The possibility that a simple competition exists between arachidonic and the inhibiting acid is currently being investigated.

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