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Inhibition of prostaglandin synthetase by F-776, 5-(4-chlorophenyl)-β-hydroxy-2-furanpropanoic acid, a new anti-inflammatory/analgesic compound

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F-776 [5-(4-chlorophenyl)-β-hydroxy-2-furanpropanoic acid] (Fig. 1) has been shown to exhibit anti-inflammatory, analgesic, and antipyretic activities in various rat models. The anti-inflammatory effect of F-776 is not affected by previous bilateral adrenalectomy in rats. In addition, it is not an immunosuppressant, and it is uniquely non-ulcerogenic [1].

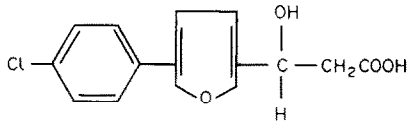

Fig. 1. Structure of F-776.

Prostaglandins (PG) have been reported to evoke inflammatory and pyretic responses [2, 3], and the observed inhibition of PG synthesis by indomethacin, aspirin, and other nonsteroidal anti-inflammatory drugs has been proposed as the mechanism of their anti-inflammatory and antipyretic activities [4, 5]. This mechanism of action was subsequently confirmed by others in studies with several other nonsteroidal anti-inflammatory agents [6–9]. The inhibitory effect of F-776 on PG biosynthesis reported here is proposed as the basis of its pharmacologic actions.

Materials and methods

Arachidonic acid and [^{3}H]arachidonic acid (61–98 Ci/mmole) were purchased from the New England Nuclear Corp. (Boston, MA). PGE_2, $PGF_{2\alpha}$, and reduced glutathione (GSH) were obtained from the Sigma Chemical Co. (St. Louis, MO). L-Epinephrine bitartrate was obtained from CalBiochem (San Diego, CA). Indomethacin was supplied by Merck, Sharp & Dohme Research Laboratories (Rahway, NJ). Aspirin and F-776 were supplied by Norwich-Eaton Pharmaceuticals (Norwich, NY). Silica gel G thin-layer chromatographic plates were obtained from Brinkman Instruments (Westbury, NY). All the other chemicals used were of reagent grade. Bull seminal vesicles were purchased from Pel Freeze Biologicals, Inc. (Rogers, AR).

[^{3}H]Arachidonic acid was diluted with cold arachidonic acid in ethanol to a specific activity of 1.5 Ci/mole and a concentration of 66 mM and stored at −5°under nitrogen until used. The purity of the [^{3}H]arachidonic acid was checked frequently by thin-layer chromatography using an ethyl acetate—acetic acid (100:2) solvent system. PGE_2 and $PGF_{2\alpha}$ were dissolved separately in 95% (v/v) ethanol to give a concentration of 2 mg/ml and stored at −5° under nitrogen until used. GSH and L-epinephrine were dissolved freshly in 50 mM Tris–HCl buffer, pH 8.3, at 21°.

Preparation of PG synthetase. A microsomal PG synthetase was prepared from bull seminal vesicles [10]. Frozen bull seminal vesicles (150 g) were thawed, trimmed free of excess fat and connective tissue, diced, and homogenized in a Waring Blendor for 2 min at 5° with 300 ml of 100 mM Tris–HCl buffer (pH 8.3 at 5°). The homogenate was centrifuged at 12,000 *g* for 10 min in a refrigerated ICE-B-20 or Sorvall 2B centrifuge (4°), and this supernatant fraction was filtered through a double layer of cheesecloth. The filtered supernatant fraction was then centrifuged at 105,000 *g* for 1 hr in a Beckman L3-40 ultracentrifuge using a Type 30 rotor. The pelleted microsomes were resuspended in 4–5 ml of 10 mM Tris–HCl buffer (pH 8.3 at 21°) and lyophilized. The lyophilized powder was pulverized, weighed, and stored at −5° until assay.

Assay of PG synthetase. Radioactive arachidonic acid was used to measure the rate of formation of PGE_2 and $PGF_{2\alpha}$ by PG synthetase [9, 11]. The standard incubation mixture (1.0 ml total volume in a 10-ml polypropylene tube) contained 0.1 ml of 30 mM GSH, 0.1 ml of 30 mM L-epinephrine, 0.005 ml of 66 mM arachidonic acid containing [^{3}H]arachidonic acid (0.15 μCi) and 0.695 ml of 50 mM Tris–HCl buffer (pH 8.3). After addition of 0.1 ml of microsomal PG synthetase (3.3 mg protein), the mixture was incubated with constant shaking at 37° in a Dubnoff metabolic shaking incubator. The control mixture (omitting microsomal PG synthetase) was prepared and incubated in the same manner as described above. It was found that PG formation was increased linearly with incubation time for at least 5 min. Therefore, a 5-min incubation time was chosen, and the incubation reaction was terminated by the addition of 0.1 ml of 3 N HCl.

After adding 10 μg of PGE_2 and $PGF_{2\alpha}$ to the reaction mixture as carriers, the mixture was extracted twice with 3 ml ethyl acetate. Both ethyl acetate extracts were pooled and dried at 37° under nitrogen gas. The dried substances were redissolved in 0.1 ml of 95% pure methanol and an aliquot (0.02 ml) was spotted on a silica gel G thin-layer chromatographic plate and developed in an ethyl acetate–acetic acid (100:2) solvent system for 1 hr at room temperature. In this solvent system, the R_f values of $PGF_{2\alpha}$, PGE_2, and arachidonic acid were 0.17, 0.33, and 0.88 respectively. These compounds (PGE_2, $PGF_{2\alpha}$, and arachidonic acid) were located by visualization in iodine vapor. The spots corresponding to PGE_2, $PGF_{2\alpha}$, and arachidonic acid were scraped and placed in counting vials containing 15 ml of scintillation counting solution. Radioactivity was determined using a β-liquid scintillation counter for 10 min per sample. The silica gel remaining on the plate was also scraped and counted. The radioactivity recovery rate from ethyl acetate extractions and from thin-layer chromatographic plates was about 60–90 and 75–100 per cent respectively. The nmoles of PGE_2 and $PGF_{2\alpha}$ synthesized under this condition were calculated from the fraction of total plate radioactivity per PGE_2 or $PGF_{2\alpha}$ spot and the initial arachidonic acid concentration. These values were calculated after correction for the radioactivity loss.

Action of F-776 and other nonsteroidal anti-inflammatory drugs on PG biosynthesis. F-776, aspirin, and indomethacin were dissolved in 95% (v/v) ethanol to give an initial concentration of 0.01 M (indomethacin) or 1 M (the rest). The stock solutions of these compounds were diluted further with 50 mM Tris-HCl buffer (pH 8.3) to give the concentrations that inhibited PG synthetase activity by 20–80 per cent. The concentration of the compounds producing 50

per cent inhibition of PG synthetase activity, i.e. IC_{50}, was estimated from the least squares straight line fitted to a log concentration–per cent inhibition curve based on four concentrations with duplicate assays in two or more experiments. The final concentration of ethanol in the reaction mixture was less than 0.1% (v/v) which was without effect on the PG synthetase activity.

Results and discussion

The specific activity of microsomal PG synthetase as calculated from the formation of PGE_2 and $PGF_{2\alpha}$ was found to be 4.74 ± 3.49 (mean ± S.D.) nmoles · mg^{-1} · min^{-1} and 0.62 ± 1.16 nmoles · mg^{-1} · min^{-1} respectively. This difference was probably related to the different proportions of isomerases for PGE_2 and $PGF_{2\alpha}$ in microsomal PG synthetase powder. Therefore, the inhibitory activity against $PGF_{2\alpha}$ formation in comparison with PGE_2 formation of F-776 and other reference compounds in the present study may become less important.

The effects of F-776 and other anti-inflammatory drugs on PG synthetase activity are summarized in Table 1. F-776 was found to be an effective inhibitor of microsomal PG synthetase from bull seminal vesicles *in vitro*. F-776 gave an IC_{50} of 1.91 mM and 2.35 mM for inhibition of PGE_2 and $PGF_{2\alpha}$ synthesis respectively. The IC_{50} values for indomethacin (PGE_2 = 0.024 mM; $PGF_{2\alpha}$ = 0.020 mM) and aspirin (PGE_2 = 17.6 mM; $PGF_{2\alpha}$ = 15.3 mM) in this study are generally in agreement with other reports [8, 9, 11]. F-776 was found to be 9-fold more potent than aspirin but only 1/80 as active as indomethacin in inhibition of PGE_2 synthesis.

Table 1. Inhibition of prostaglandin (PG) synthesis in bovine seminal vesicles by F-776 and other nonsteroidal anti-inflammatory drugs and related anti-inflammatory activity in the rat

Compound	Inhibition of PG synthesis IC_{50} (mM)		Anti-inflammatory activity* ED_{50} (mg/kg, p.o.)
	PGE_2	$PGF_{2\alpha}$	
Aspirin	17.6	15.3	155.3
F-776	1.91	2.35	60.7
Indomethacin	0.024	0.020	9.0
Correlation coefficient and *t*-test	0.97†, P < 0.2	0.98†, P < 0.2	

* Data were obtained from Ref. 1.

† Coefficient of correlation between the inhibition, by the compounds, of PG synthesis and anti-inflammatory activity in rats.

According to other investigators [5], the inhibitory actions against PG synthetase from bovine and sheep seminal vesicles by nonsteroidal anti-inflammatory drugs do not necessarily correlate with the pharmacological actions of drugs in the mouse, rat and rabbit. When we compare the *in vitro* data on PG inhibition in bovine seminal vesicles with the anti-inflammatory activity data obtained from rats [1], positive, but not significant, correlations (P < 0.2) existed between IC_{50} values for PGE_2 and $PGF_{2\alpha}$ formation and ED_{50} values for anti-inflammatory activity (Table 1).

In summary, F-776 was found to be a potent inhibitor of microsomal PG synthetase from bovine seminal vesicles. A comparison of the potency of F-776 with the reference standards shows that it is possible to deduce the following order of (decreasing) potency for these compounds: indomethacin > F-776 > aspirin. It is proposed that the anti-inflammatory activity of F-776 results from its inhibition of PG synthesis.

Scientific Affairs Department
Norwich-Eaton Pharmaceuticals
Division of Morton-Norwich Products, Inc.
Norwich, NY 13815, U.S.A.

CHAU TING HUANG*
KEITH O. ELLIS

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* Address correspondence to: Chau Ting Huang, Ph.D., Norwich-Eaton Pharmaceuticals, P.O. Box 191, Norwich, NY 13815, U.S.A.