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DICLOFENAC SODIUM (GP 45840, VOLTAREN), A POTENT INHIBITOR OF PROSTAGLANDIN SYNTHETASE

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Abstract—Diclofenac sodium, the sodium salt of *o*-(2,6-dichlorophenylamino)-phenylacetic acid (GP 45840, Voltaren), is a potent inhibitor of sheep seminal vesicle prostaglandin synthetase *in vitro*. Like indomethacin, aspirin and certain other nonsteroidal anti-inflammatory drugs, it acts as a competitive and irreversible inhibitor. It is suggested that diclofenac sodium exerts most of its pharmacological effects via inhibition of prostaglandin synthetase.

Diclofenac sodium, the sodium salt of *o*-(2,6-dichlorophenylamino)-phenylacetic acid (GP 45840, Voltaren), is a new, clinically effective anti-inflammatory agent [1–6]. In animal models of acute and chronic inflammation, it is distinctly more active than phenylbutazone and comparable in potency to indomethacin [1]. Its therapeutic index (the ratio of acutely toxic doses in mice or rats to effective doses in anti-inflammatory models) is higher than that of phenylbutazone or indomethacin [1]. It exhibits analgesic and antipyretic activity in appropriate animal tests and prevents bradykinin-induced bronchoconstriction in guinea pigs [1]. It also prevents platelet aggregation under various conditions [7, 8].

The pharmacological effects of diclofenac sodium are consistent with a mechanism of action involving inhibition of prostaglandin (PG) synthesis, a mechanism that appears to explain most of the biological effects of indomethacin, aspirin and other nonsteroidal anti-inflammatory agents [9–15]. It was of interest, therefore, to evaluate diclofenac sodium directly for its ability to inhibit PG synthetase. The results of studies *in vitro* with sheep seminal vesicle PG synthetase are presented in this report.

MATERIALS AND METHODS

Lyophilized sheep seminal vesicle microsomes [16] served as the source of PG synthetase. The enzyme was assayed by a modification of the procedure described by Takeguchi *et al.* [16]. Enzyme preparation (10–20 μg/ml), ^{14}C-arachidonic acid (0·4 to 6 μM, obtained from Applied Science Labs, Inc., sp. act. 14–58 mCi/mmole), epinephrine (1 mM), glutathione (1 mM) and the compound being tested were incubated at 25° in 0·1 M Tris buffer (pH 8·3, total volume usually 1 ml). Arachidonic acid was first dissolved in ethanol; the final concentration of ethanol was kept constant in each experiment and did not exceed 5% (v/v). This level of ethanol did not affect the enzyme assay. Compounds being tested were dissolved in ethanol or directly in buffer, in some cases with the aid of NaOH. Care was taken that the final pH of the incubation medium did not deviate from pH 8·3.

After an appropriate incubation period (10 min unless otherwise indicated), the enzyme reaction was stopped by the addition of one drop of concentrated HCl/ml of incubation medium. The acidified mixture was saturated with sodium sulfate and extracted with two 5-ml portions of ethyl acetate containing 7 μM arachidonic acid. The latter was added as a carrier and also to facilitate the subsequent thin-layer chromatographic separation of labeled compounds. The combined extracts were evaporated to dryness. The residue was dissolved in acetone and chromatographed on thin-layer plates in the solvent system described by Tomlinson *et al.* [17]. The plates were scanned with a Varian aerograph radio scanner; the radioactive zones corresponding to PGE_2 (the predominant product) were scraped off, transferred to liquid scintillation vials and counted.

RESULTS AND DISCUSSION

As shown in Fig. 1, concentrations of diclofenac sodium as low as 1–4 μM interfered markedly with the synthesis of PGE_2 from arachidonic acid. The rate of synthesis was depressed initially and remained so for the duration of the experiment. This is indicative of irreversible inhibition as occurs with indomethacin, aspirin or 5,8,11,14-eicosatetraynoic acid [18–21]. As is evident from the data presented in Fig. 2, diclofenac sodium inhibited the enzyme competitively with respect to substrate, arachidonic acid.

Inhibition constants (K_i values) for diclofenac sodium and several other PG synthetase inhibitors are presented in Table 1. All of the compounds listed, with

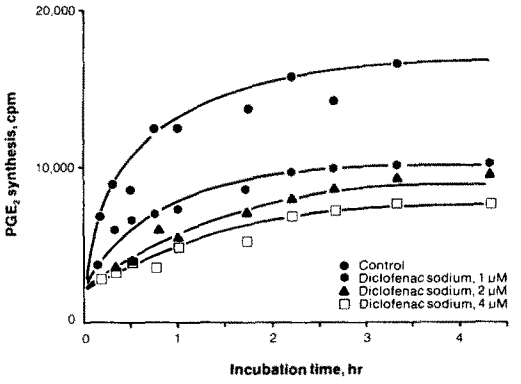

Fig. 1. Effect of diclofenac sodium on PGE_2 synthesis from arachidonic acid. The initial arachidonic acid concentration was 6 μM. PGE_2 synthesis is expressed as counts/min/0·5 ml of incubation medium. The results of a typical experiment are shown; each point represents a single measurement. The same pattern of inhibition was consistently noted in additional experiments.

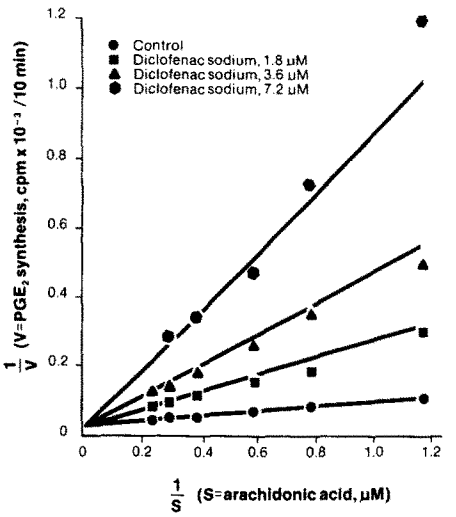

Fig. 2. Double reciprocal plot showing competitive inhibition of PGE_2 synthesis from arachidonic acid by diclofenac sodium. PGE_2 synthesis is expressed as counts/min ($\times 10^{-3}$)/0·5 ml of incubation medium during a 10-min incubation period. The results of a typical experiment are shown; each point represents a single measurement. The same pattern of inhibition was noted in two additional experiments.

the exception of 2,7-naphthalenediol, behaved as competitive inhibitors in these experiments. The competitive inhibitors acted irreversibly, although to varying extents.* Thus, with respect to the mode of inhibition, diclofenac sodium resembles indomethacin, aspirin and certain other nonsteroidal anti-inflammatory drugs. The low K_i of diclofenac sodium indicates that it is among the most potent compounds of the group.

A further point of resemblance between diclofenac sodium and indomethacin is the failure of either agent to cause complete irreversible inhibition of sheep seminal vesicle PG synthetase under our experimental conditions. Concentrations of diclofenac sodium higher than those shown in Fig. 1 did not further reduce the yield of PGE_2. This behavior was exhibited by indomethacin also. However, aspirin was capable of producing complete irreversible inhibition. Published studies with 5,8,11,14-eicosatetraynoic acid suggest that it too causes total irreversible inhibition [20].

A likely explanation for the behavior of diclofenac sodium and the other competitive, irreversible inhibitors is that they induce or enhance inactivation of the enzyme subsequent to formation of the enzyme–inhibitor complex [18–21]. We cannot satisfactorily explain the difference between diclofenac sodium and indomethacin on the one hand, and aspirin and 5,8,11,14-eicosatetraynoic acid on the other, with respect to the

* Reversibility was assessed by determining the net synthesis of PGE_2 during a 16-hr incubation in the presence of excess arachidonic acid (initial concentration of 2–6 μM) and varying inhibitor concentrations (multiples of the K_i). Under such conditions, PGE_2 synthesis was markedly reduced by some drugs at concentrations near their K_i value. For example, indomethacin and diclofenac sodium inhibited synthesis to the extent of about 50 per cent at concentrations 1–2 times the K_i. On the other hand, concentrations of the potent inhibitor Su-21524 [19] up to 20 times the K_i did not affect the net production of PGE_2. We have, therefore, classified the latter compound as a reversible inhibitor and the former as irreversible inhibitors. Some drugs fall between the two extremes. For example, concentrations of phenylbutazone and oxyphenbutazone 7–10 times the K_i are required to inhibit net PGE_2 synthesis to the extent of 50 per cent under the specified conditions. They are only weakly irreversible in comparison to indomethacin or diclofenac sodium; yet they are not reversible in comparison to Su-21524. Oxyphenbutazone has previously been characterized as a reversible inhibitor in a study under somewhat different experimental conditions [18, 23]. Indomethacin behaved as an irreversible inhibitor in the same study; phenylbutazone was not evaluated.

Table 1. K_i values for diclofenac sodium and other inhibitors of PGE_2 synthesis*

Inhibitor	K_i (μM)
Diclofenac sodium	2·4
Meclofenamic acid	2·6
Mefenamic acid	3·2
2,7-Naphthalenediol	3·4
5,8,11,14-Eicosatetraynoic acid	4·9
Indomethacin	6·5
Oxyphenbutazone	730
Phenylbutazone	98
Aspirin	5500

* These values were determined at substrate concentrations ranging from 0·4 to 5 μM and a constant inhibitor concentration [22]. Each value represents the mean of 2–4 separate determinations.

extent of enzyme inactivation. It is conceivable that the crude enzyme system contains multiple forms of PG synthetase with different susceptibilities to inactivation when bound to particular inhibitors.

In summary, diclofenac sodium is a potent inhibitor of sheep seminal vesicle PG synthetase *in vitro*. Like indomethacin, aspirin and certain other nonsteroidal anti-inflammatory drugs, it acts as a competitive and irreversible inhibitor. In view of these properties and the mounting evidence that inhibition of PG synthesis is the main mechanism of action of nonsteroidal anti-inflammatory agents [9–15], we consider it likely that diclofenac sodium exerts most of its pharmacological effects via inhibition of PG synthetase.*

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* After this manuscript was submitted, it was brought to our attention that two papers describing diclofenac sodium as a potent inhibitor of bovine seminal vesicle PG synthetase are in press [R. Ziel and P. Krupp, *Int. J. clin. Pharmac. Ther. Toxicol.*, in press; R. Ziel and P. Krupp, in *Temperature Regulation and Drug Action, IInd Symp., Paris* 1974 (Eds. J. Jacob, P. Lomax and E. Schönbaum). S. Karger, Basel, in press].