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# Effect of Diftalone and other Nonsteroidal Anti-inflammatory Agents on Synthesis of Prostaglandins (38560)

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Numerous publications have appeared in the last few years indicating that nonsteroidal anti-inflammatory compounds inhibit synthesis of prostaglandins. It has also been postulated by Vane and his coworkers that prostaglandins are mediators of the inflammatory response (1–4). Eakins *et al.* (5) have demonstrated that steroidal anti-inflammatory agents reduce the level of prostaglandins in inflamed eyes of rabbits and Greaves and McDonald-Gibson (6) have demonstrated that corticosteroids can inhibit prostaglandin synthesis in skin. Steroids however have been shown to be poor inhibitors of prostaglandin synthesis in seminal vesicle microsome preparations (7), as well as in microsomal preparation of dog spleen (3). This apparent discrepancy may be due to low solubility of the steroids or need for cellular integrity for steroid action. The seminal vesicle preparation, however, has proven to be a valuable tool for predicting anti-inflammatory action of nonsteroidal compounds (8–12).

Another source for variation in results is the concentration of arachidonic acid in various tissues. Certainly the quantity of arachidonic acid added as substrate in *in vitro* studies can influence the results (11).

Recently a new nonsteroidal agent, Diftalone (Phthalazino [2,3-b] phthalazine-5,12 (7H, 14H)-dione) has been shown to be an active anti-inflammatory agent in experimental animals (13–16) and in humans (17, 18). It was of interest therefore to study this compound and its metabolites for effects in the *in vitro* seminal vesicle prostaglandin synthetase system and to compare it with other anti-inflammatory agents. The present communication describes this work and the influence of arachidonic acid concentration on the results.

*Materials and Methods.* Prostaglandin synthetase activity was determined using a direct spectrophotometric assay technique suitably adapted from that described by Takeguchi and Sih (9). Bovine seminal vesicles obtained from adult animals and stored at 4°, were utilized within one and 0.5 hr after dissection. These were homogenized with 2 vol of 0.1 *M* phosphate buffer, pH 8.0, the homogenate was centrifuged at 12,000*g* in a Servall SS-4 centrifuge for 10 min. The supernatant fluid was taken up and centrifuged again at 100,000*g* for 60 min in a Beckman Model L3-50 ultracentrifuge. The pellet, a crude microsomal fraction, was lyophyllized and stored at −25°. Determination of adrenochrome formation from L-adrenaline was performed using the microsome preparations in a medium containing the following composition: Arachidonic acid 0.3 m*M* (Sigma Chemical Co.) and L-adrenaline tartrate 2.3 m*M* (BDH Chemical Co.) in 2.6 ml of 0.05 *M* Tris–HCl buffer, pH 8.4, containing 0.1% Tipol (Schell Co.). To this medium was added the test compounds dissolved in 0.1 ml ethanol. The reaction at 37° was followed for a period of time against a blank containing no arachidonic acid, in a Beckman Model DB-GT spectrophotometer connected to an automatic recording device. The rate of change in absorption at 310 nm, during the period of linear velocity was taken as the measure of prostaglandin synthetase activity.

*Results and Discussion.* Indomethacin (IM), phenylbutazone (PB) and aspirin (AS) produced a concentration-dependent inhibition of prostaglandin synthesis (Fig. 1). Similarly Diftalone and its metabolites (19, 20), 7,14-Dihydroxyphthalazino [2,3-b] phthalazine-5,12 (7H, 14H)-dione (L-8441) and 2 [1(2H)-oxo-2-phthalazinyl]methylbenzoic acid (L-7201) produced inhibition of prostaglandin synthetase. The $ID_{50}$ (50% inhibitory molar concentration) for these compounds is shown in Table 1. The relative potencies for IM, PB and AS were found to be similar to those reported by



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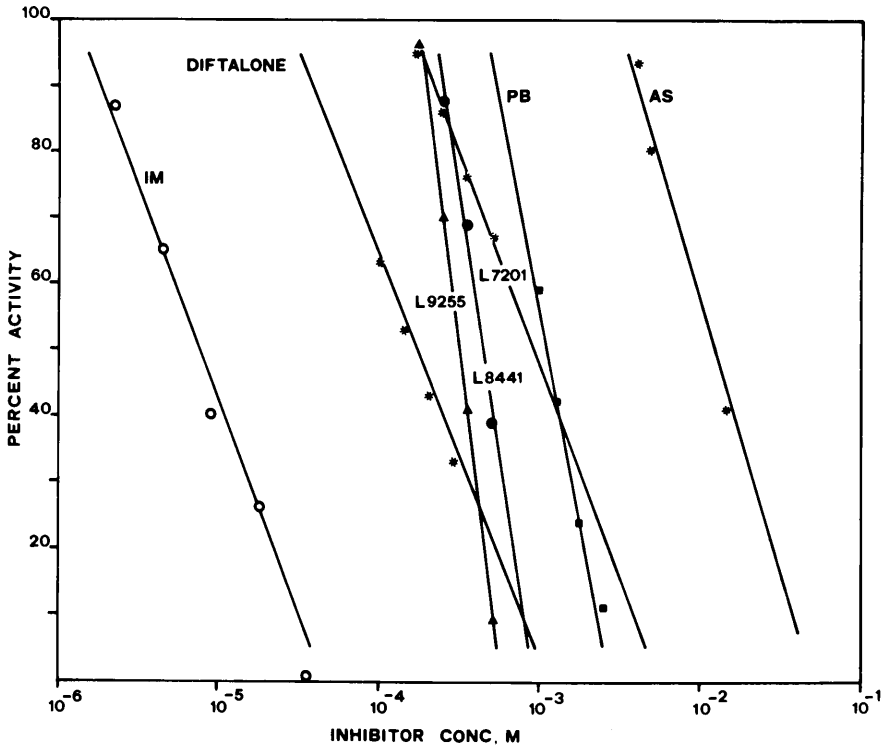


FIG. 1. Inhibition of prostaglandin synthesis by Diftalone, Diftalone metabolites and various standard nonsteroidal anti-inflammatory agents.

other laboratories (8, 9). These results however were somewhat at variance with those reported by Flower *et al.* (12) where the potency for IM was only 7–25% that reported by the other investigators. This discrepancy however may be due to the higher concentration of arachidonic acid used by Flower *et al.* (12) in their reaction mixture as pointed out by these authors. Rate of reaction generally is dependent upon concentration of the substrate; however, Fig. 2 shows that increasing the concentration of arachidonic acid from 0.05 to 2.53 m*M* produces an inhibitory effect on this rate. We therefore selected the more linear portion of the curve for determining the concentration of arachidonic acid to be used in our studies.

Comparison of IM and Diftalone (Fig. 3) in a Lineweaver–Burk plot shows that both compounds are inhibitory of prostaglandin synthesis; however whereas Diftalone produces competitive inhibition, IM does not. This finding of the noncompetitive action of IM is in confirmation of the work of Ho and Esterman (11) even though we employed a different method for prostaglandin synthesis determination and used a different range of arachidonic acid concentration, 0.002–0.020 m*M* vs 0.079–0.316 m*M* employed in our studies.

TABLE I. INHIBITION OF PROSTAGLANDIN SYNTHESIS FROM ARACHIDONIC ACID BY BOVINE SEMINAL VESICLE MICROSOMES.

| Compound | $ID_{50}$ ($\mu M$)[a] |
|---|---|
| Indomethacin | 7.5 |
| Diftalone | 170 |
| L-9255 | 310 |
| L-8441 | 440 |
| L-7201 | 900 |
| Phenylbutazone | 1100 |
| Aspirin | 12,000 |

[a] $ID_{50}$ = Concentration of inhibitor resulting in 50% inhibition of prostaglandin synthesis.

It is interesting that IM, AS, Diftalone and L-7201 produced parallel response curves (Fig. 1) whereas PB, L-9255 and L-8441

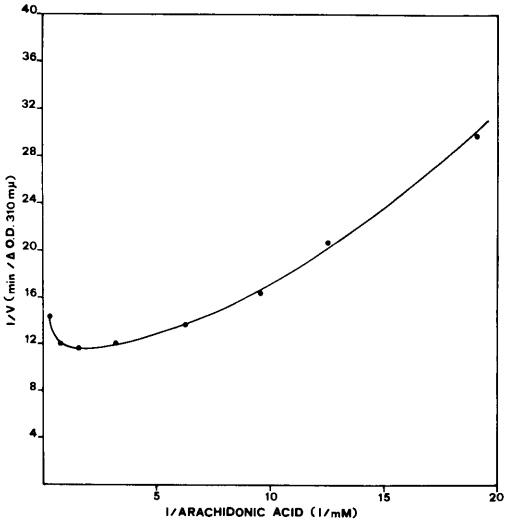


FIG. 2. Effect of concentration of arachidonic acid on prostaglandin synthesis.

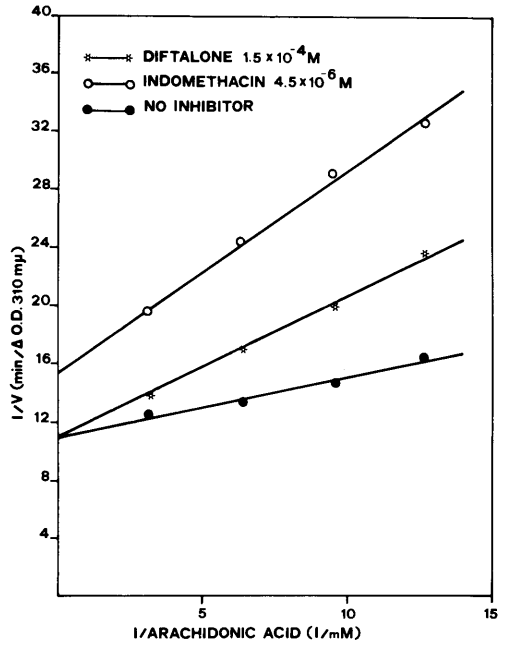


FIG. 3. Comparison of Diftalone and indomethacin on prostaglandin synthesis using a Lineweaver–Burk plot.

produced response curves parallel to each other but non-parallel to the first group of compounds. The reason for such different slopes is unknown but Flower *et al.* (12) found that PB was a good inhibitor of $PGE_2$ and $PGF_{2\alpha}$ but only a weak inhibitor of $PGD_2$ in a different experimental system; therefore it may be that L-9255 and L-8441 are also more selective inhibitors of synthesis of prostaglandins.

The inhibition of prostaglandin synthetase data (Table I) indicates that the most potent inhibitor was IM, it being approximately 23 times as potent as Diftalone; PB and AS, however were only 0.15 and 0.014 times as active respectively as Diftalone. The Diftalone metabolites were less active than the parent compound but more active than PB in these studies. Comparing the results of these studies to those obtained in the oral *in vivo* carrageenan edema anti-inflammatory investigations (15, 16), it was found that on a molar basis IM, PB and AS were 13, 0.6 and 0.25 times as active respectively as Diftalone in the Wistar strain rat, and 60, 2.25 and 0.6 times as active respectively in the Sprague–Dawley strain rat. There appears to be better agreement in the results obtained for Diftalone, IM and PB in the *in vivo* studies in the Wistar rat with those obtained in the *in vitro* prostaglandin synthetase inhibition studies. The metabolites of Diftalone were inactive orally. L-7201 and L-8441 were active and L-9255 was inactive when administered intraperitoneally (16). The reason for this discrepancy is unknown.

*Summary.* Diftalone and its metabolites 7,14-Dihydroxyphthalazino [2,3-b]phthalazine-5,12 (7H, 14H)-dione, 7-Hydroxyphthalazino [2,3-b]phthalazine-5,12 (7H, 14H)-dione and 12 [1(2H)-oxo-2-phthalazinyl]methylbenzoic acid inhibited prostaglandin synthesis in bovine seminal vesicle microsome preparations. Diftalone was the most active of these compounds but less active than indomethacin although more active than phenylbutazone or aspirin. The magnitude of the concentration of arachidonic acid influenced the velocity of the reaction in the synthesis of prostaglandins; the highest concentrations inhibited the rate of reaction.

The results of the *in vitro* inhibition of prostaglandin synthetase studies correlated well with those obtained in the *in vivo* carrageenan edema inhibition studies in the rat. The relative potencies for Diftalone, indomethacin and phenylbutazone were similar with both experimental procedures.

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