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Correlation of Anti-Inflammatory Activity with Inhibition of Prostaglandin Synthesis Activity of Nonsteroidal Anti-Estrogens and Estrogens (38532)

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Uterine hyperemia and hypertrophy induced by estrogen administration and the inflammatory response share several biological aspects. In both instances, increased fluid inhibition and blood flow result in swelling of the tissue. A common mediator in both phenomena may be prostaglandins. Saksena and Harper (1) demonstrated that the level of $PGF_{2\alpha}$ in blood and in uterine tissue varied with the stage of the estrous cycle in the rat. Clark *et al.* (2) showed that PGE_1 caused uterine vasodilation similar to that induced by estrogen. Caldwell *et al.* (3) administered estrogen to castrated ewes and obtained elevated uterine secretion of prostaglandin. Ryan *et al.* (4) using a radioimmunoassay technique confirmed that estrogen increased the prostaglandin level in the rat uterus.

Prostaglandins have been implicated as mediators of the inflammatory response (5–8). Willis (9) identified a release of prostaglandin in tissue inflamed by carrageenan administration. Vane and coworkers (5–8) inhibited prostaglandin synthesis *in vitro* using several standard clinically used nonsteroidal anti-inflammatory agents. This effect has also been reported for newer nonsteroidal anti-inflammatory agents as well (10–12). Steroidal anti-inflammatory agents were found to reduce the amount of prostaglandin in inflamed rabbit eyes (13) and to inhibit synthesis of prostaglandin in skin (14). The anti-inflammatory corticoids also inhibit the uterotrophic response to estrogen (15–18). Nonsteroidal anti-inflammatory agents have been shown to be inhibitory for estrogen-induced hyperemia and to decrease the prostaglandin level in that tissue (4). A number of nonsteroidal anti-estrogens also are anti-inflammatory.

The present report relates studies in which nonsteroidal anti-estrogenic and estrogenic compounds were used to inhibit carrageenan-induced inflammation and *in vitro* synthesis of prostaglandin. Preliminary findings have been published (19).

Materials and Methods. Prostaglandin synthetase activity of bovine seminal vesicle microsomal preparations was determined by a direct spectrophotometric method modified (12) from that described by Takeguchi and Sih (11).

Anti-inflammatory activity was assessed using the carrageenan-inflamed rat paw method of Winter *et al.* (20), modified as follows: Adult male (150 g) Sprague–Dawley (Charles River) rats (7 per group) were treated, subcutaneously, 1 hr before administration of carrageenan or, orally, at 24 hr and 1 hr before the phlogistic agent was injected into the hind paw. The test compounds were dissolved or suspended in sesame oil. These included indomethacin (IM), Diethylstilbestrol (DES), clomiphene (CL), ethamoxytriphetol (MER-25) and triparanol (MER-29). Paw volumes were obtained at the time of the injection of carrageenan and 4 hr later, using a plethysmograph. The difference in volume between these two measurements indicated the degree of edema.

Results and Discussion. The chemical structures of the compounds employed in the studies are shown in Fig. 1. Indomethacin was selected as our reference standard since it is the most potent anti-inflammatory agent and is also the most potent inhibitor of prostaglandin synthetase. The results of our studies (Fig. 2, Table I and II) confirm the high activity of this compound. The other compounds employed were selected because of their varying degrees of estrogenic and/or anti-estrogenic activities. Diethylstilbestrol, a potent estrogen which can also compete with the natural estrogen, estradiol, for binding sites in the uterus (21), was found to be an inhibitor of prostaglandin synthesis with

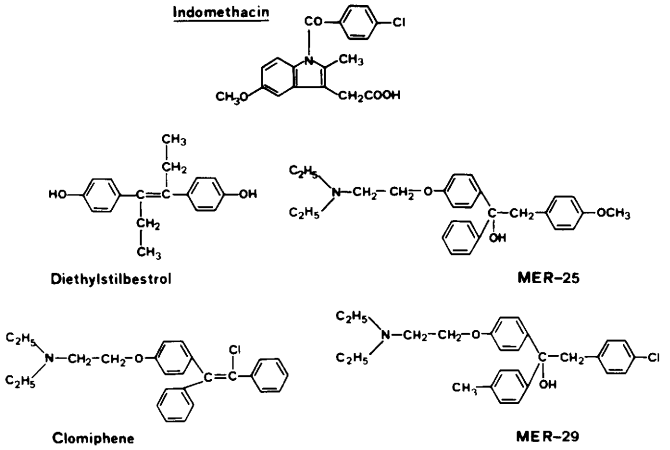

FIG. 1. Chemical structures of compounds employed in studies.

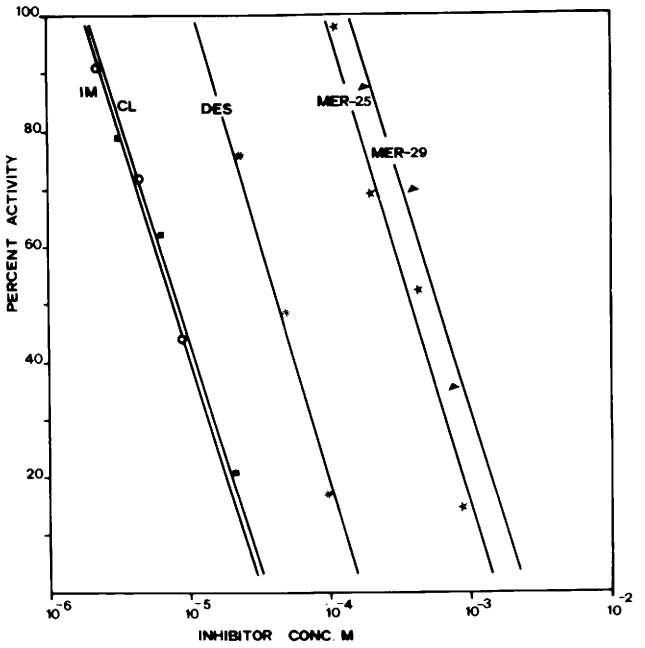

FIG. 2. Inhibition of prostaglandin synthesis by indomethacin (IM), clomiphene (CL), ethamoxytriphetol (MER-25), and triparanol (MER-29) in bovine seminal vesicle microsome preparations.

an ID_{50} of 0.18 that of IM and was also active in the anti-inflammatory assays when administered subcutaneously or orally. Clomiphene, a weak estrogen, an anti-estrogen and ovulation-inducer (22,23) was a very potent prostaglandin synthetase inhibitor, being equal in this parameter to IM. It was also anti-inflammatory in the carrageenan-inflamed rat paw test, although very much weaker than IM in this activity. MER-25, an anti-estrogen with little or no inherent estrogenic activity (23, 24), was an inhibitor of the synthesis of prostaglandin but weak compared to IM. This compound produced only marginal anti-inflammatory effects after oral administration and was inactive when injected subcutaneously. MER-29, a non-estrogenic inhibitor of cholesterol synthesis (25, 26) and a very weak anti-estrogen, was an extremely weak inhibitor of prostaglandin

synthetase but did have weak anti-inflammatory activity by both routes of administration.

These results indicate that nonsteroidal estrogens are effective inhibitors of the synthesis of prostaglandin in the bovine seminal vesicle microsome preparation *in vitro*. In addition, these compounds have some anti-inflammatory activity *in vivo*. However, in neither of these two experimental procedures is there a correlation of these two activities with the estrogenic or anti-estrogenic potencies. The most potent estrogen (DES), and the most potent of the anti-estrogens (CL), were the most potent inhibitors of the synthesis of prostaglandin and both had anti-inflammatory activity. The order of activity in the *in vitro* system, however, was not predictive of the magnitude of anti-inflammatory activity *in vivo*. MER-25 and MER-29 were relatively weak inhibitors compared to IM in the prostaglandin synthetase studies. Aspirin and phenylbutazone are also weak inhibitors of prostaglandin synthetase but good *in vivo* anti-inflammatory agents (11, 12, 29, 28). Therefore the *in vitro* prostaglandin synthetase system may be a good qualitative predictor for anti-inflammatory activity but is poor for predicting quantitative *in vivo* anti-inflammatory activity. The apparent discrepancy may be due to the difference in solubility of the compounds when used *in vitro*, and the availability of the compound when administered *in vivo*. It is also important to note that the synthetase systems for prostaglandin synthesized may be different in different tissues and that the pathological condition of inflammation may alter prostaglandin synthesis. The hyperemic response of the uterus to estrogen is a normal physiological response, whereas the response of tissue to an irritant is a pathological response. The difference of results between the two test systems employed is therefore not surprising.

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

Compound	ID_{50} (μM)[a]
Indomethacin	8.0
Clomiphene	8.5
Diethylstilbestrol	45.0
MER-25	380.0
MER-29	600.0

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

Prostaglandins are synthesized in many tissues and different tissues metabolize prostaglandins differently and at different rates. It is also known that these natural substances are involved in a number of physiological and biochemical activities as well as as in different diseases. Administration of prostaglandins or inhibitors of their synthesis have been shown to be useful in the treatment of several diseases, of which inflammation is only one type. In the study reported

TABLE II. COMPARISON OF VARIOUS COMPOUNDS FOR INHIBITION OF CARRAGEENAN-INDUCED EDEMA IN THE RAT PAW.

Compound	Dose mg/kg	Subcutaneous administration		Oral administration	
		Edema Vol ml ± SE	Inhibition of edema %	Edema Vol ml ± SE	Inhibition of edema %
Control	—	0.58 ± 0.03	—	0.72 ± 0.03	—
Indomethacin	2	0.42 ± 0.03	27.6	0.44 ± 0.03	38.9
Indomethacin	5	0.34 ± 0.02	41.4	0.35 ± 0.03	51.4
Indomethacin	10	0.24 ± 0.02	58.6	0.23 ± 0.01	68.1
Clomiphene	5	0.48 ± 0.03	17.2	0.61 ± 0.03	15.3
Clomiphene	25	0.45 ± 0.02	22.4	0.52 ± 0.02	27.8
Diethylstilbestrol	2	0.46 ± 0.03	20.7	—	—
Diethylstilbestrol	5	0.40 ± 0.02	31.0	0.50 ± 0.03	30.6
MER-25	100	0.56 ± 0.02	3.4	0.59 ± 0.05	18.1
MER-29	100	0.48 ± 0.02	17.2	0.55 ± 0.03	23.6

here, the three most potent inhibitors of prostaglandin synthesis, IM, CL, and DES, are representative of three different therapeutic classes of compounds. Only one of these (IM), was also potent as an anti-inflammatory agent. The prostaglandin synthetase inhibition system may serve as a relatively simple and rapid general screen for selection of potent compounds for study in various diseases where prostaglandin has been shown to be involved. Failure of therapy in one pathological condition does not exclude the efficacy of the compound in other therapeutic areas.

Summary. Diethylstilbestrol, clomiphene, ethamoxytriphetol and triparanol were 0.18, 1.0, 0.02 and 0.01 times as potent in the *in vitro* inhibition of prostaglandin synthetase, respectively as was indomethacin. In the *in vivo* carrageenan-induced rat paw edema studies, diethylstilbestrol was more potent as an anti-inflammatory agent than was clomiphene, and ethamoxytriphetol and triparanol were only marginally effective. The most potent of the compounds tested was indomethacin. The results reported demonstrate that the nonsteroidal anti-inflammatory agents and the nonsteroidal estrogens and anti-estrogens share the property of inhibition of prostaglandin synthetase.

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