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Evaluation of Anti-inflammatory Drugs Based upon the Inhibition of Matrix-Induced Ornithine Decarboxylase Activity during Connective Tissue Proliferation (40673)

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Subcutaneous implantation of demineralized bone matrix into allogeneic recipient rats initiates a series of interconnected cellular events resulting in differentiation of new bone (1–4). One of the early events in this sequential development is a local proliferation of mesenchymal cells in the vicinity of the implanted matrix (3). This is characterized by a sharp increase in the activity of ornithine decarboxylase (ODC; EC 4.1.1.17) 3 days after implantation (5). During the course of our studies on the effect of glucocorticoids and adrenalectomy on the process of endochondral bone differentiation we found that glucocorticoids either injected subcutaneously or implanted locally with the matrix powder inhibited the increase in ODC activity. The inhibitory effects were specific for glucocorticoids and were restricted to matrix-induced plaques (6, 7). Thus it seemed reasonable to explore the potential of this *in vivo* system of connective tissue proliferation as a method to evaluate the antiinflammatory potency of a variety of steroidal and nonsteroidal drugs. The present study describes the influence of various steroidal and nonsteroidal antiinflammatory agents on the matrix-induced ornithine decarboxylase activity.

Materials and methods. Demineralized bone matrix powder with a particle size between 74–420 μm was implanted subcutaneously in bilateral sites in the thoracic region of 50 to 80-g male rats of Long–Evans strain (1, 4). The drugs were mixed with matrix powder and implanted locally at doses ranging from 0.1 to 10 mg per rat as described in Table I. The day of implantation was designated as Day 0. Individual plaques were harvested on Day 3 after implantation and homogenized in ice-cold buffer containing 50 m*M* Tris–HCl, 5 m*M* dithiothreitol, and 1 m*M* EDTA at pH 7.2. The homogenates were centrifuged at 30,000 *g* for 30 min at 4° and ODC activity and protein were assayed in aliquots of the supernatant fractions according to Jänne and Williams-Ashman (8) and Lowry *et al.* (9), respectively, as previously described (5, 7). ODC activity was expressed as pmole of $^{14}CO_2$ released per hour per milligram of protein. The results were evaluated using the Student's *t* test.

L-[1-^{14}C]Ornithine was obtained from New England Nuclear. Corticosterone, hydrocortisone, triamcinolone, prednisolone, betamethasone, and acetyl salicylic acid (aspirin) were purchased from Sigma Chemical Company. The following compounds were generous gifts: dexamethasone and indomethacin (Merck, Sharp and Dohme), flucinolone acetonide and naproxane (Syntex Laboratories Inc.), meclofenamic acid (Warner-Lambert, Co.), chloroquine diphosphate (Sterling Winthrop Research Institute), ibuprofane (Upjohn Co.), and fenoprofane calcium (Lilly Laboratories).

Results. The results of the studies are summarized in Table I. Local implantation of steroidal antiinflammatory compounds produced different degrees of inhibition of ODC activity which varied with the structure of the compound. Synthetic fluorinated steroids such as dexamethasone, betamethasone, or flucinolone acetonide exhibited the highest inhibition of ODC activity at comparable doses. Dexamethasone also had an inhibitory effect at a much lower dose (0.1 mg). Hydrocortisone at a dose of 1 mg per rat had a minimal effect on ODC activity. Prednisolone and triamcinolone at the same dose were more potent inhibitors than either hydrocor-

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TABLE I. INFLUENCE OF STEROIDAL AND NONSTEROIDAL AGENTS ON MATRIX-INDUCED ORNITHINE DECARBOXYLASE ACTIVITY[a]

Drug	Dose (mg/rat)	ODC activity pmol $^{14}CO_2$/mg protein ± SE	*P* values	Percentage of activity
Control		550 ± 58	—	100
Steroids				
Hydrocortisone	1.0	381 ± 30	< 0.02	69
Corticosterone	1.0	346 ± 32	< 0.001	63
Prednisolone	1.0	305 ± 24	< 0.001	55
Triamcinolone	1.0	234 ± 32	< 0.001	42
Dexamethasone	0.1	193 ± 61	< 0.001	35
Dexamethasone	1.0	91 ± 32	< 0.001	16
Betamethasone	1.0	80 ± 8	< 0.001	15
Flucinolone acetonide	1.0	19 ± 8	< 0.001	3
Nonsteroids				
Indomethacin	1.0	22 ± 5	< 0.001	4
Meclofenamic acid	1.0	302 ± 21	< 0.001	55
Acetyl salicylic acid	1.0	573 ± 45	NS	104
Acetyl salicylic acid	10.0	561 ± 54	NS	102
Ibuprofane	1.0	673 ± 48	NS	122
Ibuprofane	5.0	431 ± 31	0.05	78
Fenoprofane	1.0	456 ± 33	NS	83
Fenoprofane	5.0	441 ± 79	NS	80
Naproxane	1.0	539 ± 22	NS	98
Chloroquine	1.0	541 ± 37	NS	98

[a] Six plaques from three rats were used in each experiment. NS, not significant at $P < 0.05$ level.

tisone or corticosterone. Among the nonsteroidal antiinflammatory agents examined indomethacin produced the highest inhibitory effect on ODC activity at a dose of 1 mg/rat, while meclofenamic acid produced only a 45% inhibition at the same dose. However, ibuprofane at this same dose had no significant effect on ODC activity whereas at a higher dose (5 mg) it exhibited a marginal inhibition ($P < 0.05$). On the other hand fenoprofane at two dose levels exhibited a small but insignificant inhibition of ODC activity. Chloroquine and naproxane at the dose levels tested did not influence ODC activity nor did acetyl salicylic acid at a dose of 1 or 10 mg/rat.

Discussion. The foregoing results suggest that different antiinflammatory drugs differ in their ability to inhibit ODC activity during matrix-induced mesenchymal cell proliferation. Connective tissue proliferation is one of the late events in the inflammatory reaction (10). Ornithine decarboxylase activity is a convenient marker for tissue proliferation and has been correlated with [^{3}H]thymidine incorporation (11–14).

Evaluation of antiinflammatory agents is usually limited to a few experimental models such as inhibition of erythema, edema, and granuloma confined to early phases of inflammatory reaction (10). In recent years several *in vitro* models have been developed which permit the evaluation of antiinflammatory drugs (10, 15–17). These include inhibition of prostaglandin synthetase activity (17), inhibition of *in vitro* release of lysosomal enzymes (18–19), and competitive binding of nonsteroidal antiinflammatory agents to glucocorticoid receptors (20). Since antiinflammatory drugs are widely used therapeutically in inflammatory connective tissue disorders (21, 22), it may be useful to have an *in vivo* method based on connective tissue proliferation as used in the present investigation. Gryglewski (10) has emphasized the importance of concurrent testing in several models, to predict clinical effectiveness. Therefore, the present system which simulates a late event in the inflammatory reaction such as tissue proliferation may be advantageous for the assay of antiinflammatory agents.

Although the precise mechanisms underlying the increase in ODC activity during mesenchymal cell proliferation are not known it is likely that antiinflammatory agents inhibit this response directly. Previous

experiments have eliminated the possible production of a putative inhibitor of ODC activity by glucocorticoids. Further the enzymatic activity could not be suppressed by *in vitro* addition of 5 m*M* dexamethasone (7). Recently glucocorticoids have also been shown to suppress polyamine biosynthesis and ornithine decarboxylase activity in other systems such as in psoriatic epidermis (30), spleen, and thymus (24). It is noteworthy that in the present experiment the magnitude of inhibition of ODC by steroidal antiinflammatory agents appears to be related to their potency in other assays (22, 25). In the present study dexamethasone, betamethasone, and flucinolone acetonide exhibited the greatest inhibition of ODC activity compared to equivalent doses of hydrocortisone or corticosterone. On the other hand, among nonsteroidal drugs indomethacin, meclofenamic acid, and to some extent ibuprofane were effective inhibitors of the increase in ODC activity. Aspirin, a potent inhibitor of prostaglandin synthetase (17) was ineffective even at a dose of 10 mg per rat which supports the concept that different antiinflammatory compounds may not be equally effective in every experimental system. Further, it should be emphasized that the effective potency of a drug at any time is dependent upon its solubility characteristics, possible metabolic activation, and its biological as well as plasma half-life (25). Thus a compound which may be highly effective immediately after *in vivo* administration may not be persistently as potent during the later phases. This may be the case with nonsteroidal antiinflammatory agents utilized in the present study. These considerations are of importance in the choice of drugs for long-term therapy of chronic inflammatory connective tissue disorders.

In conclusion, the bone matrix-induced elevation of ornithine decarboxylase activity during mesenchymal cell proliferation is potentially a useful model to evaluate the efficacy of steroidal and nonsteroidal antiinflammatory drugs.

Summary. The ability of several steroidal and nonsteroidal antiinflammatory agents to locally inhibit bone matrix-induced ornithine decarboxylase activity was examined. All of the glucocorticoids tested had an inhibitory effect on ODC activity. The synthetic glucocorticoids dexamethasone, flucinolone acetonide, and betamethasone were most effective in inhibiting ODC activity whereas cortisol and corticosterone had minimal inhibitory effects. The effects of the glucocorticoids on ODC activity was correlated with their known antiinflammatory potency in other systems. Nonsteroidal drugs had a varying response in this system. Indomethacin and meclofenamic acid were potent inhibitors of ODC activity whereas acetyl salicylic acid, fenoprofane, and noproxane were ineffective in doses examined.

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