THE EFFECT OF DIFLUNISAL ON HYALURONIC ACID PRODUCTION BY 'ACTIVATED' HUMAN SYNOVIAL FIBROBLASTS

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- 1 Cultured human synovial fibroblasts were 'activated' by leucocytes or poly (I)·poly (C) to overproduce hyaluronic acid.
- 2 Diflunisal in concentrations of 30, 15 and 10 $\mu\text{g/ml}$ completely abrogated the overproduction of hyaluronic acid induced by leucocytes.
- 3 Diflunisal (10 $\mu\text{g/ml}$), indomethacin (2 $\mu\text{g/ml}$) and aspirin (200 $\mu\text{g/ml}$) partially inhibited the overproduction of hyaluronic acid induced by poly (I)·poly (C), although none of them decreased it to control non 'activated' levels.
- 4 Diflunisal 25 $\mu\text{g/ml}$ completely abrogated hyaluronic acid overproduction induced by poly (I)·poly (C).
- 5 This model could be an *in vitro* indicator for *in vivo* pharmacological levels of anti-inflammatory drugs.

Introduction

Castor and we have shown that hyaluronic acid produced by human synovial fibroblasts is a sensitive parameter in the study of the inflammatory process: inflammatory cells induce overproduction of hyaluronic acid (Yaron & Castor, 1969) and anti-inflammatory drugs inhibit it (Yaron, Yaron & Allalouf, 1971). This may be an additional model for the study of anti-inflammatory drug function (Yaron, Yaron & Allalouf, 1972). Overproduction of hyaluronic acid is a major parameter of connective tissue 'activation' described by Castor (1975). The polynucleotide poly (I)·poly (C) can also 'activate' human synovial fibroblasts and stimulate their hyaluronic acid production (Yaron *et al.*, 1976). This stimulation is interrelated with that of prostaglandin E and inhibited by anti-inflammatory drugs (Yaron *et al.*, 1978).

The new aspirin derivative, diflunisal [5-(2,4-difluoro-phenyl) salicylic acid], has been shown to be effective in osteoarthritis (Andrew *et al.*, 1977) and in rheumatoid arthritis (Fishel, Weis & Yaron, 1980). We describe here its effect on hyaluronic acid production by 'activated' human synovial fibroblasts.

Methods

Fibroblasts derived from synovial membranes obtained during meniscus tear operations of other-

wise healthy persons were grown in tissue culture flasks (5 ml medium in each) by methods previously described by Castor *et al.* (1962). Cultures were nourished by medium CMRL 1066 (GIBCO) (80%), heat inactivated human serum (10%), and foetal calf serum (10%), supplemented with L-glutamine, penicillin and streptomycin.

Diflunisal supplied by Merck Sharp and Dohme in pure powder was dissolved in medium CMRL 1066 prior to addition to cultures in different concentrations. Leucocytes were separated from peripheral blood of healthy donors and frozen-thawed before addition to cultures as previously described (Yaron & Castor, 1969). Poly (I)·poly (C) was dissolved in buffered saline prior to addition to cultures. Experiments were performed when fibroblasts reached a stage of a complete monolayer, usually 5 or 6 days after last trypsinisation. Additives were added to experimental flasks (3–4 flasks for each additive) along with complete medium replacement. 'Interaction' time between different additives and experimental cultures was 48 h. At the end of this period the medium of all experimental flasks was separated for hyaluronic acid estimation and the fibroblasts were trypsinized and counted manually in a haemocytometer. Hyaluronic acid in the culture medium was measured by a method described by Castor, Wright & Buckingham (1968) whereby the mucopolysaccharide is isolated and its uronic acid

Figure 1 The effect of diflunisal on hyaluronic acid (HA) production by 'activated' human synovial fibroblasts. Vertical bars represent s.e. mean.

content determined. Statistical significance of experimental results was based on the ANOVA tables (Dunnett, 1964).

Results

Addition of leucocytes (10×10^6 /flask) induced a two fold increase in hyaluronic acid production by fibroblast cultures (Figure 1). This stimulation was completely abrogated by diflunisal in concentrations of 30, 15, and 10 $\mu\text{g/ml}$ ($P < 0.05$). Diflunisal (1 $\mu\text{g/ml}$) also significantly decreased overproduction of hyaluronic acid induced by leucocytes although it did not bring it down to control, non-'activated' cultures. The inhibition of overproduction of hyaluronic acid showed a tendency to direct correlation with diflunisal concentrations (Figure 1).

Poly (I)·poly (C) (100 $\mu\text{g/ml}$) induced a threefold increase in hyaluronic acid production by the fibroblast cultures (Figure 2). Aspirin 200 $\mu\text{g/ml}$, indomethacin 2 $\mu\text{g/ml}$, diflunisal 10 and 25 $\mu\text{g/ml}$, all significantly ($P < 0.05$) decreased the overproduction of hyaluronic acid. Indomethacin 2 $\mu\text{g/ml}$ and diflunisal in both concentrations were significantly more effective in this respect than aspirin 200 $\mu\text{g/ml}$. Only diflunisal 25 $\mu\text{g/ml}$ completely abrogated the 'activation' phenomenon.

Discussion

Diflunisal was shown to possess both analgesic (Van Winzum & Rodda, 1977) and anti-inflammatory properties (Stone *et al.*, 1977). We have recently found it effective in the treatment of rheumatoid arthritis (Fishel *et al.*, 1981).

After a single oral dose of 250 mg diflunisal, maximal mean concentrations of 25 $\mu\text{g/ml}$ were found in the plasma 3 h after ingestion (Tempero, Cirillo & Steelman, 1977). Steady state blood levels of diflunisal following twice daily oral doses of 125, 250, 375 and 500 mg were 13, 38, 60 and 85–90 $\mu\text{g/ml}$ respectively (Steeleman, Tempero & Cirillo, 1978).

Hyaluronic acid in human synovial effusions was found to be a sensitive indicator of altered connective tissue cell function during inflammation (Castor, Prince & Hazelton, 1966). Anti-inflammatory drugs were shown to decrease the stimulation of hyaluronic acid induced by leucocytes (Yaron *et al.*, 1971) or poly (I)·poly (C) (Yaron *et al.*, 1976). Different anti-inflammatory drugs (salicylates, phenylbutazone, indomethacin) were effective in our model in concentrations similar to therapeutic plasma levels (Yaron *et al.*, 1971). In the present study, concentrations of diflunisal effective in decreasing overproduction of hyaluronic acid induced by leucocytes or poly (I)·poly (C) were similar or smaller than

Figure 2 The effect of diflunisal, aspirin and indomethacin on hyaluronic acid (HA) production by 'activated' human synovial fibroblasts.

maximal pharmacological levels in the plasma mentioned above. The anti-inflammatory activity of diflunisal in the rat was reported to be up to 16 times more than that of aspirin (Steelman *et al.*, 1978). In our model the effect of diflunisal was stronger than that of doses 20 times bigger of aspirin and similar to doses five times smaller of indomethacin (Figure 2). The reliability of the results is supported by the fact that in both leucocyte and poly (I)-poly (C) 'activation' the inhibition by diflunisal was dose dependent. It might be difficult to compare *in vitro* drug concentrations to those in the plasma when

relating the concentrations to their pharmacological action. This is because many drugs are partially protein bound and it is possible that the degree of protein binding in the *in vitro* model and in the plasma are different. In addition, it may well be that the concentration of the drug in the synovial fluid rather than the plasma is important in the case of anti-rheumatic drugs. Nevertheless we suggest that the bioassay of anti-inflammatory drugs in our model could be an *in vitro* indicator for their *in vivo* pharmacological levels.

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(Revised March 3, 1981)