J. Pharm. Pharmacol. 1984, 36: 709–710
Communicated January 13, 1984

The effect of therapeutic agents on cartilage degradation in-vivo

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Implantation of minced autologous cartilage into inflamed air pouches in mice allows the study of therapeutic agents on both the inflammatory process and cartilage degradation. Non-steroidal anti-inflammatory agents were found to reduce cell accumulation in response to carrageenan, but were unable to prevent proteoglycan loss from cartilage. In contrast, D-penicillamine had no effect on the inflammatory process but significantly reduced proteoglycan loss. Our findings suggest that the autologous cartilage transplantation model in the mouse may be useful for studying novel anti-arthritic agents.

Arthritis is a disease which causes synovial membranes of joints to become chronically inflamed. As the disease progresses the inflammation destroys tissue and is accompanied by destruction of joint cartilage and bone.

The problem for the pharmacologist is to find therapeutic agents that can reduce both the inflammation and articular tissue damage. Drugs have now been developed which are very good at reducing inflammation. Many of the agents with anti-inflammatory activity act via the blockade of the cyclo-oxygenase pathway of arachadonic acid (Vane et al 1982). The development of these agents has largely come about from the use of animal models of acute inflammation such as paw oedema (Winter et al 1962), experimental pleurisy (Willoughby 1975) or sponge implants (Higgs et al 1979). Although these models are very useful for the detection of agents which reduce leucocyte migration and vascular permeability, they are unable to correlate anti-inflammatory activity with the ability to protect cartilage from damage. The models that are relied upon for finding the disease modifying agents, are adjuvant arthritis or antigen induced arthritis (Billingham 1983). These systems resemble human arthritis in respect of the erosion of bone and cartilage in joints, however the periostitis and bone involvement clinically parallels Reiter's disease (Pearson 1963; Muirdon & Peace 1969). Furthermore, such models are difficult to quantify in that they require either X-ray or post mortem examination of the affected joints.

The present study describes a novel system for studying pharmacological agents both on the inflammatory process and cartilage damage.

Methods

Animals: Outbred SAS/4 female mice, ca 30 g, were kept in a temperature-controlled environment with free access to food and water. Ten mice were used for each experiment.

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Air pouch: The air pouch as described by Edwards et al (1981) was used as the site of cartilage implantation. Mice were injected with 5 ml of air into the subcutaneous tissues of the back. Five days after induction carrageenan was injected into the pouch, followed 24 h later by cartilage implantation.

Irritant: carrageenan, Viscarin 402 (lot 272500) was kindly provided by Marine Colloids, Springfield, USA. Each mouse received 1 ml of a 1% solution.

Cartilage implantation: The method described by Sin et al (1984) was used. Briefly, the method involved the sterile removal of autologous xiphisternum. This was cleared of adipose tissue and attached bone before being weighed. Each piece of cartilage was cut into six pieces and implanted into the inflamed air pouch. After 7 days the cartilage was removed and the concentration of proteoglycan assayed by the method of Farndale et al (1982). Calculations were made to take into account the quantity of proteoglycan per unit weight of tissue, and expressed as a percentage of normal xiphisternum. Total leucocytes present in the cavity 7 days after carrageenan injection were measured using a Coulter counter. Statistical analysis was by Student's *t*-test.

Drugs: aspirin, benoxaprofen, piroxicam, indomethacin and D-penicillamine were administered to animals orally for 7 days, starting one day after carrageenan injection.

Results and discussion

The production of an air pouch in the mouse, followed by an inflammatory irritant, allows the activity of anti-inflammatory drugs to be tested. It was found, however, that the system was not as reproducible as a similar system used in rats; fluctuations in cell numbers between individual mice producing high standard errors.

Nevertheless, the results (Table 1) obtained show clearly that there was no correlation between reduced inflammatory responses and protection of cartilage. All four non-steroidal agents tested had anti-inflammatory activity in this system. Indomethacin, benoxaprofen and aspirin reduced cell infiltration at the highest concentration administered. Piroxicam had activity at both concentrations used. The reduced inflammatory infiltrate observed in these groups of animals did not appear to protect the cartilage from degradation, i.e. following treatment with these agents, proteoglycan loss was the same as in control animals. D-Penicillamine, in contrast, was found to have no anti-inflammatory activity. In fact, the cell number in the air pouch increased modestly with

Table 1. Effect of daily treatment with drugs on numbers of cells migrating into the air pouch and loss of proteoglycan from implanted cartilage.

Drug	Dose mg kg^{-1}	Inflammatory cell number $\times 10^6$	Loss of proteoglycan as % of normal xiphisternum
Control	—	5·5 ± 1·1	−28·8 ± 5·0
Indomethacin	2·5	2·9 ± 0·6*	−28·2 ± 5·4
	1·0	3·5 ± 0·8	−27·9 ± 4·0
Benoxaprofen	25·0	2·8 ± 0·5*	−29·95 ± 4·3
	10·0	3·7 ± 1·0	−25·9 ± 5·7
Aspirin	200·0	3·0 ± 0·3*	−31·6 ± 4·3
	100·0	3·5 ± 0·7	−28·2 ± 4·2
Piroxicam	10·0	3·0 ± 0·9*	−27·3 ± 2·8
	5·0	2·9 ± 0·6*	−27·9 ± 4·6
D-Penicillamine	50·0	7·0 ± 0·1	−16·7 ± 2·3**
	25·0	4·2 ± 0·1	−22·4 ± 3·1

*$P < 0·01$, **$P < 0·05$ ± s.e.m.

the highest dose (50 mg kg^{-1}) administered. The concentration of proteoglycan in cartilage fragments removed from D-penicillamine-treated animals was found to be higher than the control value indicating that this compound at a dose of 50 mg kg^{-1} could inhibit cartilage degradation.

The breakdown of cartilage in-vivo is a complex process. Synovium or inflammatory cells can secrete substances into synovial fluid such as lymphokines, lysosomal enzymes, prostaglandins and free radicals all of which have been implicated in the destructive process (Mitrovic 1982). Clinical studies have shown that non-steroidal anti-inflammatory agents which would be expected to reduce cell numbers and mediators postulated to be involved in cartilage degradation are capable of giving symptomatic relief but have little disease modifying activity (Huskisson 1980). Our present findings support this concept and indicate that blockade of either the cyclo-oxygenase pathway or lipoxygenase pathway of arachadonic acid metabolism is not effective in preventing cartilage degradation.

D-Penicillamine, an agent which can enhance the inflammatory response (Dieppe et al 1976; Sedgwick et al 1983) was found to inhibit cartilage degradation. This supports the idea that D-penicillamine is a disease-modifying agent and that its mechanism of action is independent of the inflammatory process.

It has long been a problem to bring together in-vivo data acquired using the classical models of inflammation, such as pleurisy or sponge implants, with in-vitro data on therapeutic agents tested, for example, on cartilage synovium co-cultures (Sheppeard et al 1982). The air pouch model is unique in having some of the characteristics of synovium (Edwards et al 1981). With the introduction of irritants, an environment can be produced containing all the cells and mediators which have been postulated to cause cartilage breakdown. Implantation of cartilage produces an in-vivo culture system which although being still remote from the human disease allows the study of therapeutic agents on the living process.

In conclusion, the present study presents a new system which can be used to search for drugs to be used in the treatment of the arthropathies. The use of this system has supported our previous findings that the inflammatory process is not the initial trigger for joint destruction (Sin et al 1984) and suggests that anti-inflammatory drugs may have no effect on cartilage erosion.

The authors wish to acknowledge the Arthritis and Rheumatism council and the Knights Bachelor Fund for financial support.

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