Inhibition of tissue damage by the arachidonate lipoxygenase inhibitor BW755C
(leukocyte accumulation/prostaglandins/leukotrienes/anti-inflammatory drugs)

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ABSTRACT The effects of three anti-inflammatory drugs, which interfere with arachidonic acid transformation by three different enzymes, have been compared by using a simple model of tissue damage and foreign body rejection. In groups of control rats, subcutaneously implanted polyester sponges were rejected after a mean of 12 days. Indomethacin, which selectively inhibits prostaglandin synthesis, did not significantly change time to rejection but BW755C (3-amino-1-[m-(trifluoromethyl)phenyl]-2-pyrazoline), which is a dual inhibitor of prostaglandin and leukotriene synthesis, prolonged time to rejection to a mean of 22 days. The anti-inflammatory steroid dexamethasone, which reduces arachidonic acid metabolism by stimulating the formation of a phospholipase inhibitor, prolonged time to sponge rejection as BW755C did. Treatment with BW755C or dexamethasone was also accompanied by a reduction in total leukocyte numbers in inflammatory exudates collected at 1–14 days, whereas indomethacin increased leukocyte migration on days 1 and 2 and had no effect at later times. These results suggest that the inhibition of the leukotriene-forming lipoxygenase suppresses leukocyte activation and that this leads to a reduced rate of tissue damage in experimental inflammation.

The inhibition of prostaglandin synthesis accounts for the anti-inflammatory and analgesic properties of aspirin-like drugs (1). These drugs are much less effective in reducing the accumulation of inflammatory leukocytes (2), and this may explain why they do not alter the course of chronic arthritis or prevent tissue damage and incapacitation (3).

The pathogenesis of chronic inflammatory conditions is closely associated with accumulation of phagocytic leukocytes. The appearance of first polymorphonuclear leukocytes (PMNs) and then mononuclear leukocytes in injured tissues is characteristic of the chronic inflammatory response. Release of lysosomal enzymes from these cells, after specific stimuli or cell death, contributes to tissue damage and necrosis (4). A putative mediator of leukocyte activation is leukotriene B4, which is a potent stimulator of chemotaxis and degranulation (5, 6) and has been detected in experimental inflammation (7) and human joint disease (8, 9).

In common with the prostaglandins, leukotrienes are products of arachidonic acid peroxidation (10). The cyclo-oxygenase that catalyzes prostaglandin formation is selectively inhibited by aspirin-like drugs, but the lipoxygenase that initiates leukotriene synthesis is resistant to these drugs (11). The experimental compound BW755C (3-amino-1-[m-(trifluoromethyl)phenyl]-2-pyrazoline) (12) is a dual cyclo-oxygenase and lipoxygenase inhibitor that prevents prostaglandin and leukotriene synthesis in vitro (13) and in vivo (14). Anti-inflammatory steroids do not inhibit either of these enzymes, but by stimulating the production of a phospholipase inhibitor (15, 16) they reduce the availability of arachidonic acid for conversion to both prostaglandins and leukotrienes. This suppression of leukotriene as well as prostaglandin synthesis may explain why steroids provide a more comprehensive anti-inflammatory activity than aspirin-like drugs. We have now compared the effects of BW755C with a selective cyclooxygenase inhibitor, indomethacin, and with the steroid dexamethasone in a simple model of tissue damage and foreign body rejection.

METHODS AND MATERIALS
Polyester sponges were soaked in 2% carrageenin (wt/vol in sterile saline) and implanted subcutaneously in male Wistar rats (=200 g) (12). Drugs were administered orally twice daily in an aqueous vehicle. In some experiments animals were observed on a daily basis until the sponges were rejected. Each animal was inspected twice a day and a subjective assessment of tissue damage was made on a randomized blind basis. Tissue damage was scored on a 0–5 scale according to the following criteria: 0, no change from time of implantation; 1, first signs of hair loss; 2, hair loss and edema; 3, extensive hair loss, edema, and necrosis (blackening of the skin); 4, skin split and sponge visible; 5, sponge rejected. Each animal was scored twice a day, giving a maximum possible score of 10 when the sponge was rejected.

In other experiments animals were killed 1–14 days after implantation, inflammatory exudates were collected from the sponges for leukocyte counts, and samples of tissue were taken for histology. Smears of exudates were examined for bacterial contamination and exudates were cultured on blood agar plates for 24 hr in aerobic and anaerobic conditions.

BW755C was synthesized by F. C. Copp at the Wellcome Research Laboratories.

RESULTS
Inflammatory exudates collected from the sponges of control rats 2 days after sponge implantation contained approximately 50 × 10⁶ leukocytes per ml and these cells were predominantly (>95%) PMNs. The skin above the implant became edematous and also heavily infiltrated with PMNs. After 4 days, macrophages appeared in the lower dermis and by 6 days mononuclear leukocytes were present in exudates in approximately equal numbers to the PMNs. There was evidence of fibrin deposition around the sponge as early as 24 hr after implantation and by 6 days there was extensive deposition of fibrin and collagen in the area of the sponge. Fibroblasts appeared in association with the collagen. In exudates collected after 6 days, mononuclear leukocytes were predominant over PMNs and total leukocyte numbers increased to approximately 100 × 10⁶ per ml. Externally, these...

Abbreviations: BW755C, 3-amino-1-[m-(trifluoromethyl)phenyl]-2-pyrazoline; PMNs, polymorphonuclear leukocytes.
changes were associated with swelling, hair loss, and blackening of the skin. At 8 days, more than 90% of the epidermis above the sponge was undergoing necrosis, progressing to the formation of a dry scab by days 10–14, during which period the sponges were usually rejected. Normal hair growth quickly resumed at the implantation site.

Indomethacin (0.5 mg/kg, twice per day) significantly increased total leukocyte numbers in 1- and 2-day exudates, but this effect was not maintained after 4 days (Fig. 1). Also, in indomethacin-treated animals the external signs of tissue damage were accelerated (Fig. 2). Sponges were rejected from indomethacin-treated rats 1.5 days before the controls, but this difference was not significant (Fig. 3). BW755C (10 mg/kg, twice per day) caused a 45–65% decrease in total leukocyte numbers in exudates collected 1–8 days after implantation (Fig. 1). Dexamethasone (0.1 mg/kg, twice per day) also reduced cell numbers by up to 80% on days 2–8 (Fig. 1). Both BW755C and dexamethasone significantly delayed the onset of visible tissue damage (Fig. 2), and this was accompanied by a significant increase in rejection time (Fig. 3). Skin samples taken from animals treated with BW755C at day 14 showed signs of acute inflammation with edema and leukocyte infiltration but epidermal necrosis was markedly reduced compared with control samples taken at 10 days. None of the drug treatments altered the relative proportion of PMNs and mononuclear leukocytes in sponge exudates.

Microscopic examination of exudates did not reveal substantial bacterial contamination, but cultures of exudates produced a mixed population of organisms in which small Gram-negative rods were predominant. Bacterial contamination in exudates from animals treated with BW755C did not differ significantly from that in exudates from control animals. In vitro, BW755C (1 mM) did not prevent the growth of

BW755C, and dexamethasone caused similar effects on 8- to 14-day exudates but exudates could not be collected reliably from indomethacin-treated animals after day 8 due to sponge rejection.

**Fig. 2.** Effects of drugs on tissue damage and necrosis around subcutaneous sponge implants. During a period of 14 days after implantation, each animal was observed twice a day and a subjective assessment of tissue damage was made on a randomized blind basis. Tissue damage was scored on a 0–5 scale as described in the text. Each animal was scored twice a day, giving a maximum possible score of 10. Each point is the mean of daily scores from 8–62 animals and the bars represent ± 1 SEM. Drugs were administered orally twice daily and control animals received vehicle alone. Dosages were as follows: indomethacin, 0.5 mg/kg; dexamethasone, 0.1 mg/kg; and BW755C, 10 mg/kg.

**Staphylococcus aureus or Escherichia coli.**

Indomethacin, at the dose used in this study, acts as a selective inhibitor of prostaglandin synthesis, but higher doses also inhibit leukocyte migration (2). In such high amounts, indomethacin (4–16 mg/kg) caused a dose-dependent reduc-

**Fig. 3.** Effect of indomethacin, BW755C, and dexamethasone on the rejection of subcutaneously implanted polyester sponges in rats. Each histogram represents the mean from at least 10 animals and the bars represent ± 1 SEM. Drugs were administered orally twice daily: indomethacin, 0.5 mg/kg; BW755C, 10 mg/kg; and dexamethasone, 0.1 mg/kg.
tion in leukocyte numbers at 24 hr, but the drug at these doses was not well tolerated after repeated administration for more than 3 days. BW755C was well tolerated in this study, with no significant changes in weight gain from control animals. Effective doses of dexamethasone resulted in a loss of weight compared with controls but there were no other clinical signs of toxicity.

DISCUSSION

These results support the theory that inhibition of leukocyte migration reduces tissue inflammation, necrosis, and damage. Corticosteroids and immunosuppressive agents such as azathioprine and methotrexate are known to reduce leukocyte migration into damaged tissues, and this could be an important component of their anti-inflammatory activity (17, 18). BW755C also suppresses leukocyte migration, and this may be directly linked to the inhibition of leukotriene B₄ synthesis (14). The reduction of tissue damage and delay in sponge rejection caused by BW755C cannot be explained by an anti-bacterial effect.

In some types of chronic degenerative disease such as rheumatoid arthritis the control of leukocyte accumulation could be an important factor in limiting tissue damage. Similarly, inhibition of leukocyte accumulation has been shown to reduce tissue damage after myocardial infarction (19, 20). The results reported here indicate that conventional nonsteroid anti-inflammatory drugs such as indomethacin do not reduce leukocyte accumulation or leukocyte-mediated tissue damage at anti-prostaglandin doses.

In common with other chemoattractant factors, leukotriene B₄ induces lysosomal enzyme release from leukocytes (6). Inhibition of lipoxygenase could, therefore, result in a cytoprotective effect at the site of leukocyte activation. This may explain why there is reduced necrosis in tissues from animals treated with BW755C. The dual inhibition of arachidonate cyclo-oxygenase and lipoxygenase may represent an improved anti-inflammatory mechanism for certain chronic diseases, giving a steroid-like therapeutic effect while avoiding the toxicity normally associated with steroid treatment. It is also possible that a drug which selectively inhibits lipid peroxidation could be a useful clinical tool in situations in which tissue rejection is a problem.

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