Possible disease-modifying effects of naproxen in the adjuvant-arthritis rat

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Abstract
Naproxen was evaluated for possible disease-modifying effects in the Freund's adjuvant injected rat (AIR). Oral administration of the clinical dose, 7 mg/kg/day, lead to an almost complete inhibition of hindpaw swelling and cartilage and bone erosion. This was noted in animals maintained on drug as well as those in which therapy was discontinued. AIR, comparable to arthritic patients, demonstrate a reduced lymphocytic response to T cell mitogens. This response was normalized in naproxen-treated rats. These results suggest that naproxen has a disease modifying effect in the AIR.

Drugs used in the treatment of rheumatoid arthritis (RA) are generally classified as either palliative or disease-remitting. Among the former are the anti-inflammatory analgesics such as aspirin, indomethacin and naproxen; the latter include gold, D-penicillamine and levamisole. The former share the property of inhibiting the cyclooxygenase enzyme thereby preventing prostaglandin biosynthesis while the latter have multiple immunologic activities which may be either stimulatory or inhibitory [1]. Recently the prostaglandins, especially of the E series, have been shown to have a number of immune suppressing activities, e.g. they inhibit antibody formation, mitogen stimulation of lymphocytes and delayed hypersensitivity reactions [2-6]. Based on these observations, an inhibition of prostaglandin biosynthesis might be expected to enhance immune responses. Two lines of evidence suggest this would be of benefit to patients with RA. Firstly, these individuals have a reduced delayed hypersensitivity reaction and a diminished mitogen-stimulated lymphoblastic transformation [7-9]. Secondly, treatment with the immunostimulatory drug levamisole leads to clinical improvement [10]. Treatment with a cyclooxygenase inhibitor, then, may be of benefit by virtue of its immunostimulatory activity, and its action might be considered 'disease-modifying' as opposed to palliative.

The Freund's adjuvant injected rat (AIR) may be a reasonable model for the testing of a disease-modifying drug. The animal responds with hindpaw swelling, pannus formation, and connective tissue erosion [11]. In addition, the severity of the delayed-type hypersensitivity reaction to Mycobacterium (i.e., the inducing agent) correlated negatively with the degree of hindpaw swelling [12]. A comparable negative correlation between a delayed hypersensitivity reaction and disease activity has been noted clinically [8, 13]. Recently the AIR has been shown to have a decreased lymphocyte blast response to T-cell mitogens, and this inhibition was mediated by a glass-adherent (macrophage-type) cell. Treatment with corticosteroids and methotrexate inhibited the disease and increased the splenic lymphoblast response [14-16].

Treatment of the AIR with cyclooxygenase inhibitors reduced the swelling of the hindpaw [17]. Rats dosed with naproxen likewise demonstrated an inhibition of cartilage and bone erosion [18]. These indices are usually cited as evidence of disease modification. In unpublished studies we observed that following cessation of dosing with naproxen, the degree of hindpaw swelling and joint erosion were not significantly different from that observed in animals maintained on drug. If the drug were acting solely as
an anti-inflammatory, one might predict a 'rebound' effect and an initiation of paw swelling. To further investigate the possibility that a cyclooxygenase inhibitor may have a disease-modifying effect in this model, the following experiments were undertaken:

(1) The effects of naproxen on the blast response of rat splenocytes to T-cell mitogens in vitro were determined.

(2) The effects of treatment with naproxen on the blast response of rat splenocytes removed from adjuvant-injected rats were determined.

(3) The effects of cessation of drug treatment on subsequent paw swelling was investigated.

Materials and methods

Animals

Female Sprague Dawley derived rats (Hilltop Lab Animals), 160–180 g, were used throughout these studies.

Preparation of cell suspensions for mitogen assays

Spleens were removed aseptically and placed individually into 15 x 100 mm Petri dishes (Falcon) containing Hank’s Balanced Salt Solution (HBSS) plus 20% fetal calf serum (GIBCO), Penicillin-Streptomycin, 50 U/ml (GIBCO), and Gentamycin, 4 mM (Schering; Bloomfield, N.J.). The spleens were minced finely and passed through a 40 mesh steel screen. The cells were then drawn repeatedly into a 10 ml syringe, and passed through a nylon fiber filter. The cells were centrifuged at 500 g for 15 min, resuspended and brought to a concentration of 2.5 x 10⁶/ml.

Mitogen assay

A 96-well tissue culture plate (Costar) was used for incubation. Two hundred microliters of the cell suspension plus 50 μl containing the mitogen were added to each well, and cultured at 37°C under 5% CO₂ for 3 days. The Medium 199 contained fetal calf serum (20%), Pen-Strep (50 U/ml), Gentamycin (20 μg/ml), L. glutamine (4 mM), Hepes buffer (10 mM) and mercaptoethanol (5 x 10⁻⁵ M). Tritiated thymidine (New England Nuclear), 1 μCi, was added to each well, and 18 h later the cells were harvested into a Tubinglass cell harvester. The cell samples were added to 10 ml of Oxifluor and the tritiated thymidine counted using a Beckman liquid scintillation spectrometer.

Adjuvant arthritis

Complete Freund’s adjuvant (CFA) was prepared by suspending heat-killed Mycobacterium butyricum (Difco) in mineral oil at a concentration of 10 mg/ml. Two 0.05 ml aliquots were injected intradermally into the proximal quarter of the tail. On the seventeenth day after adjuvant injection the degree of swelling of the paw and tail were scored for all animals. A subjective score of 0–4 was used to grade the degree of swelling in each paw, and 0–3 in the tail. A score of 0 indicates a negative or normal, 1 indicates mild, 2 indicates moderate, 3 indicates severe and 4 indicates very severe. A maximum score of 19 was possible for each animal. At autopsy the hindpaws were removed by cutting at the tibiotarsal junction and weighed. Spleens were removed and cell suspensions prepared as described above. Drug-treated animals received naproxen, 3.5 mg/kg, B.I.D. (7 mg/kg/day) orally for 17 days beginning on the day of adjuvant injection. Naproxen was dissolved in a vehicle consisting of 0.5% carboxymethylcellulose, 0.4% polysorbate 80, 0.9% benzyl alcohol, 0.9% NaCl and 97.3% distilled water. Control animals received vehicle only. Animals were divided into one of three groups: (1) a control group receiving vehicle only; (2) a CFA-injected group receiving vehicle; (3) a CFA-injected group receiving naproxen. At autopsy the tail and paw swelling were determined subjectively, and the hindpaws were weighed. The mitogen response of splenocytes was determined as described above.

In a second experiment, drug was administered from the day of CFA injection for either 45 or 73 days, and autopsy performed on day 74. Two additional groups, one receiving drug and one receiving vehicle, were reinjected with CFA on the 56th day following the initial CFA injection. Radiographs were taken at two-week intervals beginning on day 18; hindpaw swelling was monitored subjectively at the same time. At autopsy hindpaw weights were determined.

Results

Blast response of rat splenocytes to Con A and PHA

Rat splenocytes responded with a marked proliferation to Con A following a three-day exposure period. In preliminary studies a requirement for 2-mercaptoethanol was demonstrated, and this was included in all cultures. An optimal response was obtained using Con A, 20 μg/ml (Fig. 1 and Table 1). Consistent with the increased blast response following Con A exposure, the incorporation of labeled amino acids and the total number of cells noted at the end of four days were increased (data not presented). Concomitant exposure to naproxen, 10⁻⁶ M, lead to a 30% decrease (p < 0.05) in the blast response induced by Con A, 20 μg/ml. At the suboptimal Con A concentration of 10 μg/ml, cells exposed to naproxen had the blast response inhibited by nearly 50%. As the concentration of naproxen was increased to 10⁻⁵ and 10⁻⁴ M, the blast response was normalized. A slight, but statistically insignificant increase in thymidine incorporation was observed following exposure to naproxen, 10⁻⁴ M. No evidence of toxicity, based on an inhibition of amino acid incorporation or cell number, was noted following exposure to naproxen, 10⁻⁴ M.

Rat splenocytes responded with a blastogenic response following exposure to PHA, 50 and 100 μg/ml. Both concentrations were equivalently active in stimulating thymidine