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Nimesulide improves the disease modifying anti-rheumatic profile of methotrexate in mice with collagen-induced arthritis

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ABSTRACT

Methotrexate is a disease modifying anti-rheumatic drug that is widely used for the treatment of rheumatoid arthritis. Nimesulide is a non-steroidal anti-inflammatory drug which is frequently used as adjuvant therapy for symptomatic alleviation of rheumatoid arthritis. In this study, we have evaluated the potential influence of nimesulide on the disease modifying anti-rheumatic properties of methotrexate using the collagen-induced arthritis model. Mice were immunized with collagen type II for the induction of arthritis and treated with methotrexate (2.5 mg/kg) twice a week, nimesulide (20 mg/kg) every other day or a combination of both drugs. Treatment started one week after the onset of arthritis until day 40. An arthritic index was used to compare the severity of arthritis between different treatments. In addition, articular hyperalgesia, joint stiffness, radiological deterioration and intra-articular leucocytic infiltration were evaluated. Methotrexate alone showed modest but significant analgesic and anti-inflammatory effects, and the effects of nimesulide were comparable. On the other hand, nimesulide significantly improved the disease modifying anti-rheumatic profile of methotrexate in terms of arthritic index and joint mobility. Furthermore, although nimesulide failed to show any radiological evidence of articular protection, it significantly improved methotrexate-induced joint protection as judged by X-ray analysis.

1. Introduction

Rheumatoid arthritis is a chronic and debilitating, autoimmune disease affecting 1–2% of the population (Sangha, 2000). Rheumatoid arthritis is characterized by symmetrical polyarthritis, algesia, joint stiffness, and progressive articular damage (Huskisson and Drury, 1994). The use of non-steroidal anti-inflammatory drugs partially alleviates the symptoms of rheumatoid arthritis but cannot prevent its long term disease progression (Weinblatt, 1996). The use of cytotoxic or disease modifying anti-rheumatic drugs (DMARDs) in the early stages of rheumatoid arthritis has been recommended by most health care authorities to prevent subsequent articular damage (Pincus et al., 1999). Recently, several novel treatments have been used for the treatment of rheumatoid arthritis, including TNFα blocking agents (Caramaschi et al., 2009; Christensen et al., 2009; Feher and Lengyel, 2009; Nasonov, 2009; Quartuccio et al., 2009; Wiens et al., 2009), lymphocyte co-stimulation-targeted therapy (Falgarone et al., 2009) and B-cell targeted therapy (Bracewell et al., 2009). Despite the clinical success of these novel agents, their high cost and need for repeated injections means that conventional DMARDs continue to be in widespread clinical use (Martinez Lopez et al., 2009; Smolen et al., 2005).

Methotrexate was originally designed as antiproliferative cytotoxic agent for the treatment of different types of malignancies via the inhibition of dihydrofolate reductase enzyme (DHFR) (Baugh et al., 1973; Chabner et al., 1985). Subsequently, methotrexate has become the leading DMARD for the treatment of rheumatoid arthritis and other autoimmune diseases for more than four decades in much smaller doses than those used in the treatment of neoplasia (Schroder and Stein, 2003). Apart from the interference with folate biosynthesis, methotrexate was found to influence adenosine level and interact with adenosine receptors on immune-effector leucocytes (Cronstein et al., 1993; Hasko and Cronstein, 2004). The use of combination therapy of methotrexate or other DMARDs for rheumatoid arthritis has been suggested to improve efficacy and minimize toxic side effects via decreasing the dose of individual agents (Alarcon, 2000; Pincus et al., 1999).

Nimesulide is a cycloxygenase-2 (COX-2) selective antagonist with COX-2/COX-1 selectivity ratio of about 90 (Masferrer et al., 1994;

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Sengupta, 1998). The role of NSAIDs, such as nimesulide, in the treatment of rheumatoid arthritis is to control pain, and other symptomatic manifestations of the disease (Pincus et al., 1999). Besides COX-2 enzyme inhibition, nimesulide inhibits the activity of myeloperoxidase (MPO) and other superoxide anion generating enzymes (Bevilacqua et al., 1994). It is also reported to down regulate the expression of platelet activation factor, TNFα and metalloproteinases (Pelletier and Martel-Pelletier, 1993). Nimesulide prevents the oxidative and proteolytic inactivation of α_1-proteinase inhibitor; and most interestingly, it is reported to inhibit phosphodiesterase IV enzyme with consequent elevation for leukocytic cAMP, and adenosine levels (Capecchi et al., 1993; Tool et al., 1996).

Rheumatoid arthritis can be studied experimentally using a wide variety of animal models, including collagen-induced arthritis (Williams, 1998). Collagen-induced arthritis accurately simulates the clinical, symptomatic, and pathological manifestations of rheumatoid arthritis (Holmdahl et al., 1989; Trentham, 1982). The current work was designed to investigate the potential enhancing effect of nimesulide on the disease modifying anti-rheumatic profile of methotrexate in collagen-induced arthritis.

2. Materials and methods

2.1. Chemicals and drugs

Collagen Type II was prepared from bovine cartilage (Miller and Rhodes, 1982). Complete Freund Adjuvant (CFA), hexadecyl trimethylammonium bromide (HTAB), and *o*-dianisidin were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Methotrexate powder was gifted from T3A Pharmaceutical Co. (Assiute, Egypt). Nimesulide was gifted from Alkan Pharmaceutical Co., (6th of October city, Egypt).

2.2. Arthritis induction

Male Swiss albino mice (7 weeks old, 30 g weight) were acclimatized in the animal house facility of the National Research Center (Dokki, Giza, Egypt) for at least one week prior to experimentation. Animals were kept at 20 ± 4 °C and 65 ± 10% relative humidity during the whole experiment. Standard food pellets and water were supplied *ad labium*.

Collagen type II solution (4 mg/ml) was prepared by dissolution overnight at −4 °C in 0.1 M acetic acid with frequent vigorous shaking. The final solution was emulsified with an equal volume of CFA. A volume of 100 μl of emulsion was injected intradermally. Animals showed positive signs of arthritis by day 14 were assigned randomly into 5 treatment groups ($n = 10$). Another booster dose (100 μg collagen) was injected intradermally at day 21 (Cook et al., 2001).Groups consisted of a normal non immunized group (injected only with the vehicle of collagen emulsion), a control group with collagen-induced arthritis that received no treatment; a group with collagen-induced arthritis that received methotrexate (2.5 mg/kg s.c. twice a week) (Urakawa et al., 2002); a group with collagen-induced arthritis that received nimesulide (20 mg/kg i.p. every other day); and a group with collagen-induced arthritis that received methotrexate (2.5 mg/kg) and nimesulide (20 mg/kg). The dose of nimesulide was calculated based on Pagat's conversion scale (Pagat and Barnes, 1964). All treatments started one week post onset of arthritis (day 21) until day 40. The treatment protocol was approved by the National Research Center Animal Rights Committee.

2.3. Recording arthritic index

To determine the arthritis severity, mice paws were inspected every other day and scored as follows: 0.5 = slight swelling and erythema in a single digit; 1 = slight swelling and erythema in two or more digits; 2 mild swelling and erythema of the whole limb; 3 = gross swelling and erythema of the whole limb; and 4 = gross deformity and limb disability. Arthritic index represents the sum of the animal's four paws (Urakawa et al., 2000). Arthritic index was measured blindly by 2 independent researchers from day −2 until the end of the experiment (day 40).

2.4. Assessment of articular hyperalgesia

Articular hyperalgesia was assessed using the Hargreaves method with minor modifications (Hargreaves et al., 1988); briefly animals were allowed to acclimatise in a 10 × 17 cm enclosure (7310-planter test, Ugo Basile, Comerio, Italy) for 30 min. Arthritic joints were challenged with an infrared beam (Halogen "Bellaphot" 64607 OSRAM, 8 V-50 W-IR movable bulb, Ugo Basile, Comerio, Italy) through the transparent bottom of the enclosure to induce pain. The time required for mice to lick the paw was recorded by photocell-linked digital timer and designated as withdrawal latency (WDL) (Chillingworth and Donaldson, 2003). Articular hyperalgesia was measured 2 days before induction (base line), day 10 (pre-arthritic point), day 27 (early phase of treatment) and at the end day of experiment (day 40).

2.5. Assessment of locomotor activity

Locomotor activity of mice was determined by activity cage. Mice were placed for 5 min in grid floor detecting activity cage (model-7430, Ugo Basile, Comerio, Italy) equipped with insulated horizontal stainless steel bars; the odd bars are grounded and the even ones are wired in four sets. The animals' make or break with their paws produce random configuration which is converted into pulses. Average number of movement per minute was recorded and compared between different treatment groups (Chillingworth and Donaldson, 2003). Locomotor activity was measured 2 days before induction (base line), day 10 (pre-arthritic point), day 27 (early phase of treatment) and at the end day of experiment (day 40).

2.6. Determination of myeloperoxidase enzyme activity

Intra-articular MPO activity was determined 40 days after induction according to the method of McVey and Vigna (McVey and Vigna, 2001). Briefly, animals were euthanized by cervical dislocation, joints were immediately extracted surgically, weighed and rapidly ice crushed in liquid nitrogen and then homogenized for 15 min in 0.5% hexadecyl trimethylammonuim bromide (HTAB) and potassium phosphate buffer (50 mM, pH 6). Joint homogenate (10% w/v) was centrifuged at 40,000 × *g* for 15 min. An aliquot of 100 μL of the clear supernatant was mixed with 2.9 ml *o*-dianisidin (0.167 mg/ml) and hydrogen peroxide (0.0005%), and measured at 460 nm for 2 min at 15 s interval (Helios γ-UV/Vis spectrophotometer equipped with Vision® software package for kinetic analysis). The average rate of change in absorbance was used to calculate the MPO activity per gram joint tissue (Molar absorbtivity 1.13×10^4). One unit of MPO is defined as the amount required for degrading 1 μmol of peroxide per min at 25 °C (Bradley et al., 1982).

2.7. X-ray analysis

Joint deformity and articular damage were radiologically evaluated by X-ray imaging 40 days after induction. Briefly, animals were euthanized by cervical dislocation, anterior and posterior paws were fixed in 10% buffered formalin solution for 24 h. Each appendage was placed on an X-ray cassette containing Veroplex Dental Film speed E (Medivance Instrument Limited, London, UK) and radiographs were taken with conventional X-ray unite (Pioneer road-S-240 with stator CGR and tube type RAD 13-Salt Lake city, UT, USA) at 40 KV, 0.04 mA s. Radiological evaluation was performed according to Clark et al. (1979). Simply, the X-ray films were scored from 0 to 4 (0 = normal,