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Dual effect of nitric oxide donor on adjuvant arthritis

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ABSTRACT

The effect of medical use of NO donors on the pathogenesis of arthritis is still yet unclear. We investigated the effects of the NO donor, sodium nitroprusside (SNP), on the pathogenesis of adjuvant-induced arthritis in rats. Rats were given SNP intraperitoneally either from day 5 to day 14 (as a prophylactic protocol) or from day 16 to day 25 (as a therapeutic protocol) after inoculation of adjuvant. SNP administration, whether prophylactic or therapeutic, in doses of 0.1 and 1 mg/kg/d significantly aggravated pathogenesis of adjuvant arthritis in rats. SNP-treated rats showed significant ($P<0.05$) increase in arthritis index, hind paw volume, ankle joint diameter and hyperalgesia compared with control adjuvant arthritic rats. However, in adjuvant rats given the smallest dose of SNP (0.01 mg/kg/d), arthritis index, volume of hind paws, ankle joint diameter, body weight loss, and hyperalgesia were significantly lower than that of control adjuvant rats. After 30 d of the induction of adjuvant arthritis, TNF alpha levels exhibited insignificant changes either in control adjuvant rats or in rats given SNP compared with control non adjuvant rats. IL-10 levels in adjuvant control rats and adjuvant rats given 1 mg or 0.1 mg/kg/d from day 15 to day 25 were significantly lower than that of control non adjuvant rats. Histopathology examination of ankle joint showed that large doses of SNP (1 mg or 0.1 mg/kg/d) increased the mononuclear cells infiltration and erosion of cartilage induced by adjuvant while the infiltration of the inflammatory cells in the synovium of adjuvant rats treated with 0.01 mg/kg/d was minimal and the pannus was inhibited with alleviation of erosion of articular cartilage. Prophylactic small dose of SNP improved the histological status more than the therapeutic small dose. The present work reveals that SNP administration, either prophylactic or therapeutic, was deleterious in higher doses. However, the smallest dose used 0.01 mg/kg/d attenuates joint inflammation, hyperalgesia and body weight loss in adjuvant arthritic rats. These results suggest that small dose of NO donor may exert partial protective effects while the safety of the clinical use of NO donors, in higher doses, in patients with rheumatoid arthritis is questioned.

1. Introduction

Cardiovascular diseases have become the main cause of excessive mortality of patients with rheumatoid arthritis [1]. Nitric oxide donors have been used for many years as a vasodilator and symptomatic treatment for cardiovascular diseases as angina pectoris, hypertension and congestive heart failure. Therefore, the nitrate medication may be needed for treatment of cardiovascular diseases in patients with rheumatoid arthritis. On basis of epidemiological studies, NO donor medication may accentuate bone sclerosis and contribute to disease progression if used in the presence of osteoarthritis [2]. In rheumatoid arthritis (RA), it has been demonstrated that serum and synovial fluid nitrite concentrations were significantly higher than control [3]. It has been suggested that endogenous production of NO is enhanced in proportion to the degree of inflammation in patients with RA owing to enhanced iNOS activity [4]. Also, in osteoarthritis, NO production was found to be higher [5]. It mediates many of the destructive effects of interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-alpha) in the cartilage and inhibitors of NO synthesis have demonstrated retardation of clinical and histological signs and symptoms in experimentally induced osteoarthritis and other forms of arthritis [6].

It appears likely that the increase in NO associated with arthritis can be caused by pro-inflammatory cytokines and mechanical stress and molecular oxygen is required for production of NO that is associated with osteoarthritis and RA [7]. Further evidence of the deleterious effects of NO comes from the study of Nagy et al. who supported the NO inhibiting therapeutic strategies for the treatment of chronic inflammatory diseases such as RA and concluded that local inhibition of NO synthesis at the site of synovial inflammation may provide better therapeutic tool than systemic inhibition [8]. Their prior study revealed that overproduction of NO may perturb T cell activation, differentiation and effector response which may contribute in different ways to the pathogenesis of autoimmune diseases [9].

Contrary to the above studies, there also exists the conflicting notion that NO may be protective during an inflammatory process. It has been shown that NO prevents apoptosis in rheumatoid synovial cells by directly inhibiting caspase-3-activation [10] and the local

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production of NO may be protective by the virtue of its ability to regulate the release of pro-inflammatory mediators [11]. In addition, nitric oxide donors were found to increase the production of hyaluronic acid by synovial cells from patients with RA [12].

Other studies found that NO does not mediate the chronic inflammation and joint destruction which occur during the latter phase and the therapeutic administration of a selective inhibitor of iNOS does not ameliorate the chronic inflammation and tissue damage associated with adjuvant arthritis in rats [13]. Furthermore, it has been shown that NO has limited modulating effects in cartilage metabolism with evidence for both protective and deleterious effects [14] and no fundamental relationship between magnitude of NO production and arthritis susceptibility and severity suggesting that NO has no effector role in arthritis [15]. Similarly, it has been found that the relationships between measures of arthritis disease activity and urinary and serum nitric oxide levels were not significant in rheumatoid patients [16].

Numerous studies have unanimously shown an association between RA and impaired endothelium function [17]. Endothelial dysfunction is defined as loss of NO bioavailability in patients with chronic inflammatory conditions [18]. Some investigators have reported that activation of iNOS may lead to endothelial dysfunction by depleting the bioavailability of tetrahydrobiopterin from endothelial nitric oxide synthase (e NOS) and subsequently uncouple eNOS resulting in production of superoxide anion rather than NO [19,20]. More recently, Maki-Petaja et al. demonstrated an endothelial dysfunction and increased iNOS activity in rheumatoid patients [21]. They suggested that inflammation is a key mediator in the process of endothelial dysfunction possibly via activation of iNOS and increased production of myeloperoxidase enzyme.

Interesting studies have implicated that NO has dual effects. It has been reported that relatively low concentration of NO plays a defensive role in the immune system [22] and exerts anti-apoptotic effects via cGMP [23] while higher concentration causes numerous pathological processes including inflammation [24], vascular damage [25] and apoptosis in various cell types [26]. Additionally, Kwak et al. showed that low concentration of SNP suppresses subsequent high concentration SNP-induced apoptosis by inhibiting p38 kinase [27].

It is clear from the aforementioned reports that there is conflicting data about the effect of NO donors and the effect of systemic use of NO donors on pathogenesis of adjuvant-induced arthritis needs to be identified. Therefore, we examine the effect of SNP as a representative of nitric oxide donors on signs, symptoms, histopathology and cytokines in adjuvant-induced arthritis in rats.

2. Materials and methods

2.1. Animals

The experimental study was carried out using adult female albino rats of the Sprague–Dawley strain weighing between 160 and 200 g. The animals were acclimatized in a light- and temperature-controlled room with a 12–12 h dark–light cycle. The rats were fed with commercial pelleted rat feed and water was given *ad libitum*. Food was placed on the floor of the cage to facilitate access, as the pain which accompanies adjuvant-induced arthritis renders the rats immobile and unable to use their hind limbs to obtain food from the cover mesh of the cage. The experimental protocol was approved by the local ethical committee.

2.2. Reagents and drugs

Complete Freund's Adjuvant (CFA) was purchased from Difco Laboratories, Detroit, Michigan, USA. Squalene was purchased from MP Biomedicals, Inc. Sodium nitroprusside (SNP) was purchased from Sigma chemical, St. Louis, USA. SNP was freely dissolved in water.

2.3. Experimental induction of arthritis

In this study, adjuvant arthritis was induced in rats according to previously described methods for the evaluation of rheumatoid arthritis. Based on preliminary experiments, the method of Trentham et al. was modified by intradermal injection of 0.1 ml squalene before inoculation of CFA into a different site in the subplanter surface of right hind paw to increase the sensitivity of rats used to CFA [28]. Rats were divided into 8 groups (6 animals each). The first group (group I) served as normal control which received only 0.1 ml kg^{-1} saline. Each rat in the other 7 groups received 0.1 ml of CFA and 0.1 ml of squalene. Rats in group II received intraperitoneally 0.1 ml of distilled water, the vehicle in which SNP was dissolved (Adjuvant arthritic control group). Treatment was initiated on day 5 to day 14 in three groups III, IV and V with SNP, given intraperitoneally, in doses of 1, 0.1 and 0.01 mg/kg/d respectively (prophylactic protocol). In groups VI, VII and VIII, SNP was given i.p. as therapeutic protocol in doses of 1, 0.1 and 0.01 mg/kg/d respectively from day 16 to day 25. The day of inoculation was regarded as day 0 while day 16 was the day in which oedema in the contralateral, non-injected, hind paw was observed. Arthritis index, hind paw height, volume of paw oedema, body weight, rectal temperature and pain threshold to pressure on hind paws, were measured daily from day 0 until day 30 after adjuvant inoculation. At the end of the study, the animals were sacrificed and blood was collected. Blood samples were immediately centrifuged at 3000 rpm for 10 min and serum samples were stored at −80 °C until assayed for TNF-alpha and IL-10. Specimens of ankle joints' tissues were also examined for histopathology.

2.4. Arthritis index

Rats were evaluated daily for arthritis. The physical symptoms of arthritis were judged by the following grading system [29]: 0 = normal paws; 1 = erythema of toes; 2 = erythema and swelling of paws; 3 = swelling of ankles; 4 = complete swelling of the whole leg and inability to bend it. The maximum achievable score is thus 16. Arthritis index for each rat was calculated by adding the four scores of individual paws. A sensitized animal was considered to have arthritis when at least one non-injected paw was inflamed [30].

2.5. Measurement of body weight and temperature in arthritic rats

Body weight for each rat was recorded before and daily after adjuvant inoculation to assess food intake and weight gain throughout the period of arthritis. The difference between body weight in each day and that of day 0 was calculated to determine the change in body weight in arthritic rats.

Body temperature, as an index of inflammation, was monitored for rats, before and daily after disease induction between 9:00 AM and 11:00 AM, using a rectal thermometer.

2.6. Measurement of ankle diameter and paw volume changes

Changes in the ankle diameter of both ipsilateral (injected) and contralateral (non-injected) hind paws, from the height on day 0, were daily assessed using a Vernier scale [31].

Volumes of hind paws were measured before and daily after adjuvant inoculation by using water displacement plethysmometry [32]. The changes of volumes of hind paws, from those of day 0, were calculated.

2.7. Analgesimetry

Using a Ugo basile analgesimeter (Ugo Basile Biological Research Apparatus, Italy), a crescent pressure (in grams) was applied separately to the posterior paws until the animal displayed a reaction