International Immunopharmacology 10 (2010) 1406–1414

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Influence of dipyridamole and its combination with NO donor or NO synthase inhibitor on adjuvant arthritis

Adel Gomaa [a,*], Mohsen Elshenawy [b], Noha Afifi [c], Eman Mohammed [d], Romany Thabit [b]

[a] Dept. of Pharmacology & Toxicology, College of Pharmacy, Taibah University, Al-madinah Almunawwarah, KSA
[b] Dept. of Pharmacology, Faculty of Medicine, Assiut University, Assiut, Egypt
[c] Dept. of Microbiology and Immunity, Faculty of Medicine, Assiut University, Assiut, Egypt
[d] Dept. of Pathology, Faculty of Medicine, Assiut University, Assiut, Egypt

ARTICLE INFO

Article history:
Received 15 May 2010
Received in revised form 9 August 2010
Accepted 9 August 2010

Keywords:
Phosphodiesterase inhibitor
Adjuvant arthritis
NO synthase inhibitor
NO donor
Synergism

ABSTRACT

The anti-arthritic and anti-inflammatory effects of dipyridamole and the possible involvement of NO in the dipyridamole action are not yet clear. The aim of this study was to evaluate the effects of dipyridamole alone and in combination with either the nitric oxide donor, sodium nitroprusside (SNP) or the non-selective nitric oxide synthase inhibitor, L-NG- monomethyl arginine (L-NMMA), on pathogenesis of adjuvant-induced arthritis model in rats. The results of the present work showed that prophylactic administration of dipyridamole alone and dipyridamole administration in combination with either low dose of SNP or L-NMMA significantly ameliorated pathogenesis of adjuvant arthritis in rats as evidenced by significant decrease in arthritis index, hind paws volume, loss of body weight, hyperalgesia compared with control vehicle (1% DMSO) treated adjuvant arthritic rats. Inflammatory cellular infiltrate in synovium of ankle joint and pannus formation were also markedly inhibited. Interleukin-10(IL-10) levels were significantly increased in these groups of animals. In contrast, a high dose of SNP counteracted the anti-inflammatory and anti-arthritic effects of dipyridamole. The inhibitory effect of therapeutic administration of dipyridamole alone on adjuvant arthritis syndrome was not significantly different from that of vehicle administration. In conclusion, dipyridamole has prophylactic but not therapeutic anti-arthritic and anti-inflammatory effects that appear to be dependent on inhibition of NO synthase. A synergistic combination between dipyridamole and NO synthase inhibitor or low dose of NO donor may have prophylactic and therapeutic values in autoimmune diseases like RA.

1. Introduction

Dipyridamole is a widely established drug for treatment of angina pectoris and cerebrovascular diseases. Its main mechanism of action is to inhibit the cellular uptake and metabolism of adenosine with resulting vasodilatory and anti-aggregatory effects. Through the combination of its antiplatelet and vasodilator function, dipyridamole probably improves tissue perfusion [1]. Dipyridamole is also a non-selective phosphodiesterase (PDE) inhibitor which inhibits degradation of cAMP and cGMP. The rank order of the inhibition of phosphodiesterase by dipyridamole is PDE5>PDE4>PDE2>PDE1 and 3 [2].

Increasing evidence has indicated that many phosphodiesterase inhibitors possess anti-inflammatory effects. Dipyridamole has been shown to have selective anti-inflammatory properties that may contribute to its action in the secondary prevention of stroke. Weyrich et al. found that dipyridamole differentially inhibited the expression of several gene products in human models of cellular inflammation [3]. In addition, it has shown that dipyridamole exerts its anti-inflammatory effect via activation of MKP-1, which dephosphorylates and inactivates p38 MARK. Inactivation of p38 MARK in turn inhibits 1KK-β activation and subsequently the NF-κβ signaling pathway that mediates lipopolysaccharide-induced cyclooxygenase-2 expression in RAW 264.7 cells [4].

It has also been reported that dipyridamole possesses antioxidant properties and attenuates the reactive oxygen species (ROS) formation in platelets and endothelial cells [5]. The antioxidant effects of dipyridamole in endothelial cells are at least partially mediated via suppression of inflammatory NF-κβ signaling [6]. Furthermore, it has shown that dipyridamole has another beneficial effect; it inhibits NO production by adenosine-independent processes [7]. Therefore, it is possible that inhibition of NO production by dipyridamole contributes to evoke the anti-inflammatory and antioxidant effects of dipyridamole.

Recently, it has been investigated that combination of a very low dose of prednisolone and dipyridamole has exhibited statistically significant synergistic effects in human clinical trials [8]. The synergistic effect of prednisolone with dipyridamole was also

* Corresponding author.
E-mail address: a.gomma@aun.edu.eg (A. Gomaa).

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doi:10.1016/j.intimp.2010.08.006

observed in collagen and adjuvant arthritic animal based on inhibition of additional inflammatory chemokine [9]. On the contrary, dipyridamole did not modify the symptoms of rheumatoid arthritis in other clinical trial described by Forrest et al. [10]. However, in animal models of rheumatoid, it is not clear whether or not dipyridamole has anti-inflammatory and immunomodulating effects and the role played by NO in dipyridamole actions remains to be determined. Therefore, in the present study, we examine whether dipyridamole possesses anti-inflammatory and anti-arthritic effects in adjuvant arthritic rats and whether the suppression of NO production or addition of exogenous NO can modify the dipyridamole action. We also examined the change in serum cytokines and histopathology after administration of dipyridamole alone or in combination with either NO synthase inhibitor, LNMMA or NO donor, sodium nitroprusside using prophylactic and therapeutic protocol.

2. Materials and methods

2.1. Animals

Adult female albino rats of the Sprague–Dawely strain weighing between 160 and 200 g were used. 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 that 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 of Faculty of Medicine, Assiut University.

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. Dipyridamole was obtained from Boehringer Ingelheim Pharmaceuticals, Inc. N^G monomethyl-L-arginine (L-NMMA) and sodium nitroprusside (SNP) were purchased from Sigma chemical, (St.Louis, USA). Dipyridamole was dissolved in 1% DMSO in distilled water.SNP and L-NMMA were freely dissolved in water.

2.3. Experimental induction of arthritis and drug treatment

In this study, adjuvant arthritis was induced in rats according to the previously described methods for evaluation of rheumatoid arthritis. Based on preliminary experiments, to increase the sensitivity of rats used to CFA, the method of Trentham et al. [11] was modified by intradermally injecting 0.1 ml of squalene before inoculation of CFA into a different site in the subplantar surface of right hind paw. Each animal in all groups, except those in the control non-adjuvant group, was injected with 0.1 ml squalene and 0.1 ml CFA.

Ninety six rats were used in this study. Two groups (I & II) of six animals each served as controls; these non-adjuvant and untreated adjuvant arthritic rats received saline orally. Other animals were randomly allocated to two treatment protocols (prophylactic or therapeutic). Each treatment protocol contains seven groups of six animals. Drug or vehicle treatment was started on day 5 till day 14 in prophylactic protocol and on day 16 till day 25 for the therapeutic protocol. The first four groups (III, IV, V and VI) in each protocol received oral dipyridamole alone in doses of 0, 270, 180 and 90 mg/kg/day respectively. Group III served as vehicle (1% DMSO) treated group. The other three groups (VII, VIII and IX) were treated with 180 mg/kg/day dipyridamole orally combined with 1 mg/kg/day SNP, 0.01 mg/kg/d SNP or 30 mg/kg/d L-NMMA respectively.

The day of inoculation was regarded as day 0 whereas day 16 was the day in which oedema in the contralateral, non-injected, hind paw was observed. This prophylactic schedule of treatment was selected to evaluate the inhibitory effect of dipyridamole on the development of arthritis in contralateral hind paws. This protocol demonstrates the immunomodulator effect of dipyridamole. However, a therapeutic protocol was used to assess the anti-inflammatory effect of dipyridamole on the development of arthritis

Arthritis index, volume of oedema in paws, 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 killed and the 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 joint 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 [12]: 0 = normal paws; 1 = erythema of toes; 2 = erythema and swelling of paws; 3 = swelling of ankles; and 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 [13].

2.5. Measurement of body weight and temperature in arthritic rats

The 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 on a given day and that on day 0 was calculated to determine the change in body weight in arthritic rats.

The 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 paw volume changes

Volumes of both ipsilateral (injected) and contralateral (non-injected) hind paws were measured before and daily after adjuvant inoculation by using water displacement plethysmometry [14]. The changes in volumes of hind paws compared with those on 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 that consisted of withdrawing the paw and/or vocalizing [15]. The slide of the device moved at the speed of 16 mm per second. The force on the paw was at rate of 16 g per second, so a distance of 11.5 mm is equivalent 115 g. The pain threshold to pressure on hind paws of rats was measured. The following formula was used to calculate the percentage change in pressure to the hind paws needed to elicit response on day x for each animal:

$$\frac{(\text{Pressure on day } x - \text{pressure before adjuvant injection on day 0}) \times 100}{\text{Pressure before adjuvant injection on day 0}}$$