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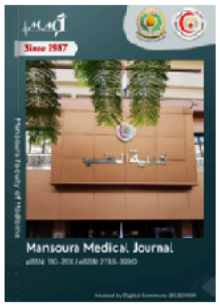

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AMLODIPINE; A NEW THERAPEUTIC STRATEGY IN RHEUMATOID ARTHRITIS

Shalaby A

'Department of clinical pharmacology Faculty of Medicine, Mansoura University

ElHawary A

'Department of pathology Faculty of Medicine, Mansoura University

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By

Shalaby A.* and El Hawary A. **

From

**Department of clinical pharmacology and ** Department of pathology, Faculty of Medicine, Mansoura University*

ABSTRACT

This experimental study was carried to evaluate the possible therapeutic effects of amlodipine on collagen-induced arthritis in rats. Collagen arthritis were induced in a sprague dawley rats by intradermal injection of total volume of 0.1 ml of cold emulsion consisting of native type II collagen and complete freund's adjuvant with a second immunization was given after 21 days. Rats were divided into two main groups: group A, received 0.5 ml saline solution and served as normal control, group B, arthritic group, this group was subdivided into two equal subgroups each compromised 10 rats as follows, group I, arthritic control which treated with saline and group 2 amlodipine treated arthritic group (50 ug/kg). For all groups, drugs were given as a single daily intramuscular injection for 18 days next day after the second immunization. Paws were examined macroscopically for redness, swelling and deformities. The severity of arthritis was evaluated by histopathological scoring of the knee joints and biochemically by measuring serum levels of TNF-α, IL-Iβ, nitric oxide and serum malondialdehyde. It was found that administration of amlodipine significantly suppressed the progression of arthritis and decreased the production of TNF-α, IL- Iβ, nitric oxide as well as malondialdehyde levels in the serum.

In conclusion, these findings indicate that administration of amlodipine may have significant therapeutic effect on the rat model of rheumatoid arthritis which was probably due to antioxidant effect and inhibition of pro-inflammatory cytokines as well as nitric oxide production.

Key word : RA, CIA, adjuvant arthritis, amlodipine, TNF-α, IL-1β

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease involving multiple joints. The main pathology of the affected synovial tissue consists of hyperplasia and subintimal infiltration of T and B lymphocytes. In chronic untreated arthritis synovial tissue hyperplasia forms the pannus tissue that irreversibly destroys the cartilage and bone in the affected joint. RA progression is associated with elevated levels of tumour necrosis factor-alpha (TNF-α) and interleukin Iβ produced by macrophages [1,2]. On the other hand over production of nitric oxide and oxidative stress have been proposed to play a key role on the pathogenesis of RA [3, 4].Thus down regulation of TNF-α and IL- Iβ as well as decreased production of free radical and nitric oxide may be a rational strategy for treatment of RA. Important issues concerning RA therapy are the ability to control symptoms and signs of the disease for prolonged period as well as the capacity to retard the damaging effect of rheumatoid inflammation on articular cartilage and bone. For the remedy of RA, disease modifying antirheumatic drugs, NSAIDs and steroids are clinically common therapeutic agents and recently TNF-α inhibitor therapy in combination with methotrexate provided sustaind clinical therapeutic benefit [5]. However, the validity for long term treatment with these medicines has not yet been proven and further adverse events were reported with extremely high frequency which limit their use early in the disease process and interfere with prolonged administration. Taking into consideration the state of RA therapy and the intricate pathogenesis of RA, the combination therapy using plural therapeutic agents or the therapy using agent containing plural therapeutic effects may be useful to suppress the inflammatory process [6, 7]. Based on these evidence, we were interested in amlodipine (one of the long acting dihydropyridine Ca-antagonists). Amlodipine was found to suppress TNF-α as well as IL -Iβ in lipopolysacchride stimulated macrophages cell line [8]. In addition, amlodipine, nifedipine and other dihydropyridine derivatives have been shown to cause an inhibition of inducible nitric oxide synthase in macrophages and murine models of congestive heart failure [9]. Thus, in this study we investigated the possible

anti-inflammatory effect of amlodipine in collogen II- adjuvant arthritis an experimental model of RA in rats.

MATERIALS AND METHODS

Reagents :

Amlodipine was purchased from Global Napi pharmaceutical Egypt. Complete freund's adjuvant was obtained from sigma (St. Louis. Mo. USA). Nitric oxide detection kits from R & D system Inc. (USA) TNF-α and IL-Iβ were obtained from genzyme (Cambridge, MA, USA). Other reagents used in this study were from analytical grade.

Animals :

All procedures were conducted in conform to the (Guide for the care and use of laboratory animals) published by the US National institute of health.

Sprague Dawley rats of both sexes (6 weeks old B.W. 130-170gm) were used. They kept on the same housing condition and all had free access to food and water.

Induction of collagen II-adjuvant arthritis :

Rats were randomly separated into 2 main groups, normal non-immunized group and arthritic group, in this group collagen induced arthritis (CIA) was induced and evaluated as described previously [10]. In brief, the rats received an intradermal injection of cold emulsion consisting of equal volume of complete freund's adjuvant and collagen II which dissolved overnight in 0.1 mol acetic acid at concentration of 4 mg/ml. Each rat was injected intradermally with 0.1 ml of this cold emulsion into the tail base with booster dose given after 21 days. The next day after the booster dose, the rats that had no macroscopic signs of arthritis were selected and divided into two groups, each contained 10 rats, the control CIA group which treated with saline and the amlodipine treated CIA group that treated with amlodipine (50 ug/kg) once daily I.M for 18 days [8]. The gradual onset of arthritis starts approximately 4 weeks after initial immunization. The progression of CIA was evaluated by measuring paw thickness using the paw oedemameter [11] and macroscopic scoring of the paws every 3 days as well as histological analysis of the knee joint on day 18.

Macroscopic scoring of CIA :

The severity of arthritis was evaluated for each paw by scoring method

according to the degree of erythema and swelling with a total score of 4 for each paw, where grade 0, normal. Grade I, swelling of one finger, grade 2, swelling of more than two fingers Grade 3, swelling of the heel, and grade 4, joint deformity with ankylosis (12).

Histological processing and analysis of the knee joints :

AT the end of 18 days after the second immunization rats were killed by cervical dislocation, the knee joints were dissected, fixed in 10% phosphate buffered formalin for 2 days and decalcified in 10% EDTA for 7 days, then embedded in paraffin. Standard section of 7 um were prepared and stained with Haematoxylin and eosin.

Histological changes were scored as described previously (13), using the following parameters. 0: normal, 1: infiltration of inflammatory cells, 2: synovial hyperplasia, 3: pannus formation, 4: bone erosion, 5: bone destruction.

Measurement of serum TNF-α and IL-IB :

At the end of 18 days after the second immunization. The serum were collected for evaluation of TNF-α and IL-IB using commercially available enzyme immune assay kits according to the manufacturer's recommendation.

Measurement of serum nitrate & nitrite level :

Serum level of nitric oxide was measured through its stable metabolites nitrate and nitrite using the Griess reaction as described by Green et al (14).

Measurement of serum malondialdehyde (MDA) :

MDA synthesis was used as a marker of lipid peroxidation and was measured by using the corresponding kits as described by Devi et al (15) and was read spectrophotometrically at 532 nm.

Statistical analysis :

Data were expressed as the mean + S.E.M. all results were analysed by one-way ANOVA followed by a multiple comparison test (Scheffe test). A P value less than 0.05 was statistically significant.

RESULTS

Suppressive effect of amlodipine on CIA :

The time course of the disease status of animals is shown in Figure (1A). When animals treated with 50 ug/kg of amlodipine daily, the progression of arthritis was markedly inhibited in rats treated with amlodipine compared with control rats. The increase in paw thickness was significantly decreased in rats treated with amlodipine than in control rats Figure (1B).

Effect of amlodipine on histopathological changes :

Sections of the Knee joints stained with haematoxylin and eosin showed inflammatory cells infiltration, erosion of the articular cartilage with cysts formation in control CIA group(figure 2 B,C) in comparison to normal control group (Figure 2 A). In amlodipine treated CIA group the severity of arthritis was markedly ameliorated (Figure 2 D).When the inflammation was assessed by histological scoring as described in Materials and Methods, amlodipine decreased the histological score significantly relative to control CIA group (Figure 2 E).

Effect of amlodipine on TNF-α and IL -IB in serum :

Amlodipine produced marked decrease in levels of TNF-α and IL -IB in comparison with control CIA groups (Table 1,Figure 3). Whereas the level of TNF-α and IL -IB were very low in serum of normal rats.

Effects of amlodipine on serum nitric oxide and Malondialdehyde levels (MDA) :

In comparison to control CIA group, amlodipine treated group showed significant decrease in both serum nitrite and MDA levels. (Table 1,Figure 4).

Table (1): **Effect of amlodipine on serum TNF-α ,IL-1B, NOx and MDA levels in collagen induced arthritis in rats (mean±SE)**

groups	TNF-α Pg/ml	IL-IB Pg/ml	NOx nmol/ml	MDA nmol/ml
Control group	93±7.64	23.5±4.95	18±1.91	0.74±0.12
Collagen arthritis group (CIA)	1290±122.2	369±38.8	130±17.78	3.4±0.39
P_1	<0.001	<0.001	<0.001	<0.001
Amlodipine treated group	695±64.33	152±21.07	78.5±8.95	1.5±0.19
P_2	<0.01	<0.01	<0.01	<0.01

P_1:statistical significance between control group and collagen induced arthritis group.
P_2:statistical significance between amlodipine treated group and collagen induced arthritis control group.

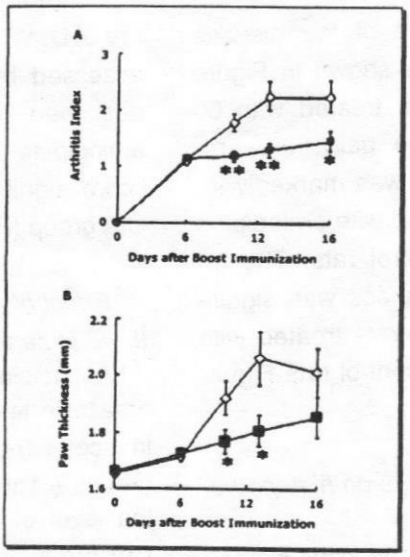

Fig. 1 : Time course of suppressive effect of amlodipine on arthritis in CIA in rats. From the day 21 boost immunization, amlodipine (Filled squares) and CIA control (open diagon). The arthritis index (A) and paw thickness (B) were measured at 3 days intervats. Data are mean ± SE (10 rats in each group).
*P < 0.05; **P <0.01 compared with that in the CIA-treated group alone.

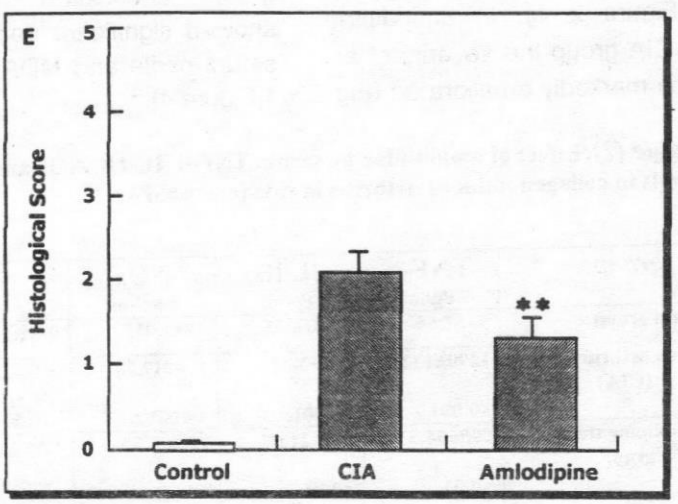

Fig. 2 (E) : Histological changes were scored on scale of 0-5 according to the method described previously. Values are Mean ± S.E.M. P < 0.05 Vs. control CIA group only.

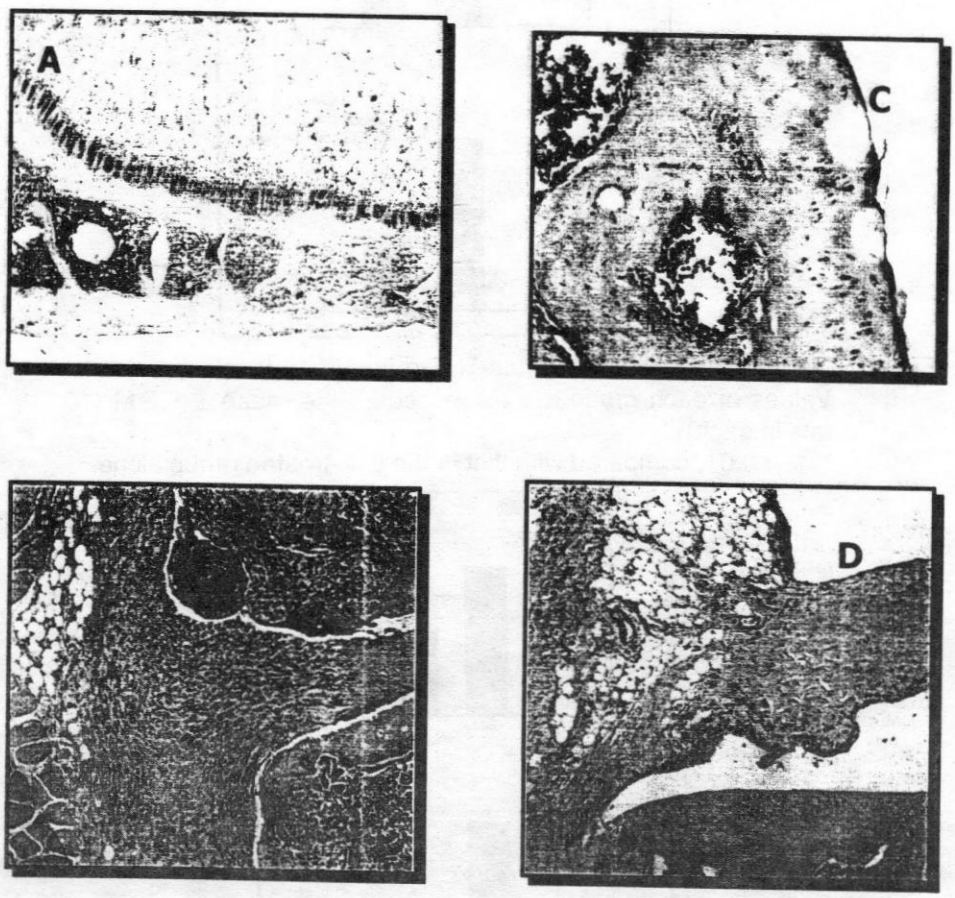

Fig. 2 : The effect of amlodipine on articular cartilage in CIA. H & E staining of the knee joints of normal control (A), CIA control (B & C) and amlodipine treated CIA group (D). data represent 10 samples for each group the degree of pannus formation, erosion and cyst formation in the articular cartilage were markedly lower in the knee of rats treated with amlodipine (magnification x 100).

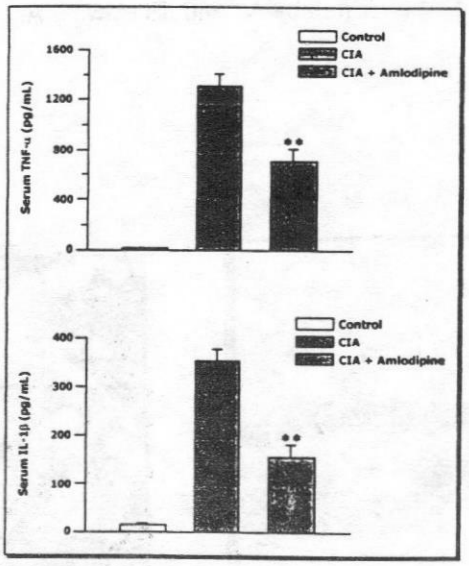

Fig. 3 : Effect of amlodipine on serum TNF-α and IL-1b levels in CIA rats. Values of each group are expressed as the mean ± S.E.M (10 rats in each).
** P < 0.01, compared with that in the CIA-treated group alone.

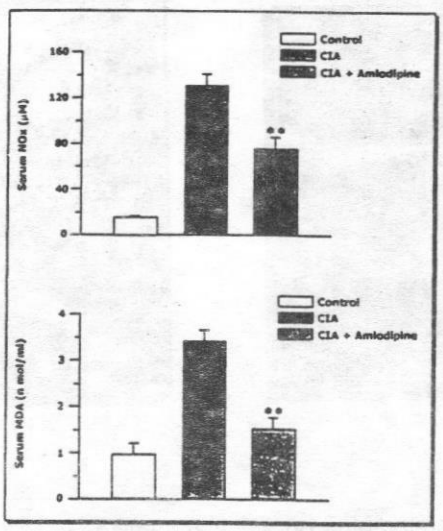

Fig. 4 : Effect of amlodipine on serum Nox and MDA levels in CIA treated rats. Values of each group are expressed as mean ± S.E.M (10 rats in each group).
** P < 0.01, compared with that in the CIA-treated group alone.

DISCUSSION

Rat adjuvant arthritis is often used as an animal model of RA in the evaluation of antirheumatic drugs.

The present study, clearly demonstrates the marked effectiveness of amlodipine in protecting CIA rats against joint destruction and histological analysis reveals marked protection against cartilage and bone erosion. This protective effect of amlodipine appears to result from its control of key components of RA pathogenesis including the down regulation of TNF-α and IL -IB as well as its suppressive effect on nitric oxide and free radial productions. Moreover, TNF-α plays a major role in the inflammatory process [16], while IL-IB is involved in the induction of catabolic process that affect cartilage matrix namely, it is reported that IL- IB not only stimulates the release of degenerative enzymes including matrix metalloproteinase from synoviocytes, but also inhibit the synthesis of extracellular matrix protein by chondrocytes [17]. It has also been suggested that IL-I is involved in the osteoclastogenesis and bone resorption that is increased in RA joints [18].

Furthermore, over production of nitric oxide (NO) produced via induction of inducible nitric oxide synthase (iNOS) has also been implicated as a causal or contributing factor to pathological changes that occur in RA [19-22]. It has also been reported that NO takes part in the induction of apoptosis in chondrocytes in vitro (23), in vivo [24] and ex vivo [25].

Pro-inflammatory cytokines especially TNF-α and IL -IB play a key role contributing to over production of NO via induction of inducible NOS in chondrocytes through activation of nuclear factor kappa- B(NK- KB) an important nuclear transcription factor for inducible nitric oxide synthase(iNOS) and many other pro-inflammatory cytokines [26, 27]. Moreover, it was demonstrated that the iNOS expression in chondrocytes is induced by a single stimulation of IL-IB or TNF-α [28], while multiple cytokines are required for the induction of iNOS in most of the other types of cells.

It was found that the induction of Nuclear factor kappa(NF-KB) and iNOS occurs via a Ca+_ calmodulin dependent protein kinase pathway [29]. These observation indicated that the increase of intracellular Ca+ has

an important role on iNOS expression. Our finding, inhibition by amlodipine of NO production is in agreement with other dihydropyridine Ca+_ antagonists (30, 31).

In addition, oxidative stress has been proposed to play a key role on the pathogenesis of RA by activating NF-KB and iNOS (32). In this study, amlodipine significantly inhibited the MDA production (a marker of oxidative stress in tissues) which is consistent with other DHP, calcium antagonists such as felodipine (33). Ca antagonists have also been shown to prevent glutathione loss (34). Intra cellular thiols regulate NF-KB activation and high intracellular thiol levels could liberate the activated NF-KB (35). These results suggest the possibility that Ca+_ antagonist inhibit NF-KB activation. It was found that nifedipine and amlodipine inhibit activation of NF-KB both in human epithelium-like lung carcinoma cell line and in macrophage cell line (36).

These observation support our finding that amlodipine exerts an antioxidant effect as well as anti-inflammatory action and is a potent inhibitor of nitric oxide production.

In conclusion, this study demonstrates that attenuation of pro-inflammatory cytokines and free radical production by amlodipine may be involved in the attenuation of iNOS and NO over production and postulate some of the potential new use of amlodipine as anti-inflammatory and antioxidant in inflammatory disease associated with over production of NO such as RA.

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الأميلوديبين كعلاج استراتيجى جديد للروماتويد المفصلى

أجرى هذا البحث التجريبى لدراسة فاعلية الأميلوديبين فى تثبيط الالتهابات فى الفئران المصابة بالالتهاب المفصلى وقد تم تقسيم الفئران إلى ثلاث مجموعات متساوية حيث أعطى الأميلوديبين كجرعة واحدة يومياً ٥٠ ميكروجرام/كج عن طريق الحقن بالعضل ولمدة ١٨ يوماً وقد لوحظ أن إعطاء الأميلوديبين للفئران المصابة بالإلتهاب المفصلى قد أحدث تحسن ذو دلالة إحصائية فى تورم المفاصل تابعة تحسن ذو دلالة إحصائية على مستوى الباثولوچى صاحبه نقص ذو دلالة إحصائية على مستوى الشقوق الحرة، سيرم أوكسيد النيتريك - معامل نخر العظام ألفا ومستوى الأنترلوكين ١-ب وفى ضوء هذه الدراسة يمكن استنتاج أن الأميلوديبين له تأثير قوى على الالتهابات المفصلية والمحدثة معملياً فى الفئران وذلك لأنه مضاد قوى للأكسدة ومثبط لسيتوكينات الالتهابات.