AMLODIPINE: A NEW THERAPEUTIC STRATEGY IN RHEUMATOID ARTHRITIS

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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-1β, 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-1β, 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.

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anti-inflammatory effect of amlodipine in collagen 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-1β 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 μg/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

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The time course of the disease status of animals is shown in Figure (1A). When animals treated with 50 μg/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-1β in serum:
Amlodipine produced marked decrease in levels of TNF-α and IL-1β in comparison with control CIA groups (Table 1, Figure 3). Whereas the level of TNF-α and IL-1β 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-1β, NOx and MDA levels in collagen induced arthritis in rats (mean±SE)**

<table>
<thead>
<tr>
<th>groups</th>
<th>TNF-α (pg/ml)</th>
<th>IL-1β (pg/ml)</th>
<th>NOx (nmol/ml)</th>
<th>MDA (nmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>93±7.64</td>
<td>23.5±4.95</td>
<td>18±1.91</td>
<td>0.74±0.12</td>
</tr>
<tr>
<td>Collagen arthritis group</td>
<td>1290±122.2</td>
<td>369±38.8</td>
<td>130±17.78</td>
<td>3.4±0.39</td>
</tr>
<tr>
<td>(CIA) P₁</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Amlodipine treated group</td>
<td>695±64.33</td>
<td>152±21.97</td>
<td>78.5±8.95</td>
<td>1.5±0.19</td>
</tr>
<tr>
<td>P₂</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

P₁: statistical significance between control group and collagen induced arthritis group.
P₂: statistical significance between amlodipine treated group and collagen induced arthritis control group.

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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).
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-1B 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-1B is involved in the induction of catabolic process that affect cartilage matrix namely, it is reported that IL-1B 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-1 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-1B play a key role contributing to over production of NO via induction of inducible NOS in chondrocytes through activation of nuclear factor kappa- B (NF-κB) 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-1B 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


24- Kim HA and song YW. (1999) : Apoptotic chondrocyte


