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1α-Hydroxyvitamin D_3 inhibits Type II collagen-induced arthritis in rats

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Abstract

The effects of 1α-hydroxyvitamin D_3 and 24,25-dihydroxyvitamin D_3 on Type II collagen-induced arthritis in rats, an experimental model of rheumatoid arthritis, were examined. Oral administration of 1α-hydroxyvitamin D_3 significantly suppressed the incidence of arthritis and inhibited hind paw swelling. The level of anti-Type II collagen antibodies was decreased in the 1α-hydroxyvitamin D_3 treated-group. In contrast, 24,25-dihydroxyvitamin D_3, indomethacin, and gold had no effect on either the incidence of arthritis or the antibody levels. These findings demonstrate a beneficial effect of 1α-hydroxyvitamin D_3 on Type II collagen-induced arthritis in rats and indicate that it has an antirheumatic effect.

Key words: 1α-Hydroxyvitamin D_3; Collagen-induced arthritis; Anti-Type II collagen antibody; Rheumatoid arthritis

1. Introduction

1α-OH-D_3 is a vitamin D_3 derivative that is known to be a regulator of calcium homeostasis. 1α-OH-D_3 is transported by a vitamin D-binding protein to the liver where it undergoes 25-hydroxylation to become 1α,25-$(OH)_2$-D_3, a biologically active vitamin D_3 metabolite [1–3]. Specific receptors for 1α,25-$(OH)_2$-D_3 (calcitriol) are expressed by peripheral blood monocytes [4] and mitogen-activated T lymphocytes [5], and calcitriol is also known to be potent in suppressing the proliferation and inducing the differentiation of cells in the immune system [6–9].

In 1977, Trentham et al. [10] reported Type II collagen-induced arthritis developed in rats, and antibodies against autologous Type II collagen were shown to be critically involved in the pathogenesis of this type of arthritis [11]. In addition, we recently found that RA patients have circulating antibodies against human Type II collagen, and that these antibodies frequently appear during the early phase of the disease [12,13]. These findings led us to a special interest in Type II collagen-induced arthritis as a model of RA.

In the present study, we examined the effects of 1α-OH-D_3 and 24,25-$(OH)_2$-D_3 on Type II collagen-induced arthritis in comparison with a nonsteroidal anti-inflammatory agent (indomethacin) and gold.

2. Materials and methods

2.1. Induction of arthritis

Bovine native Type II collagen (Cosmo Bio. Ltd.) was dissolved in 5 mM acetic acid at a concentration of 4 mg/ml and was mixed with an equal volume of 1 mg/ml muramyl dipeptide (Dai-ichi Pharmaceutical Co. Ltd.) in distilled water. The solution was emulsified in an equal volume of Freund's incomplete adjuvant (Difco Laboratories Co. Ltd.) at 4°C. Female Lewis rats weighing 150 g were immunized with 1 ml of the cold emulsion by intradermal injection into the back. Animals were weighed every 4 days after injection. The volumes of the two hind paws were measured by the water displacement method with a hind paw volume meter (Muromachi Co. Ltd.), and the mean value for both paws was calculated. Results were expressed as the percent increment in paw volume relative to that on day 0.

2.2. Histological observation

The affected feet were removed at post mortem, fixed in 10% buffered formalin, and decalcified in EDTA in buffered formalin (5.5% w/v). The feet were then embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

2.3. Assay for anti-Type II collagen antibodies

The anti-Type II collagen antibody level was measured by an ELISA using buffered normal rabbit serum as the blocking agent to diminish non-specific binding of IgG to the plastic plates as previously described [13]. Serum samples were diluted to 1:1000 for IgG, and 1:50 for IgM in buffered normal rabbit serum and were added to the plates. The second antibody consisted of peroxidase-conjugated rabbit anti-rat IgG or IgM (Cooper Biochemical, Cochranville, PA) was diluted to 1:2000 with 25% buffered normal rabbit serum. A positive standard consisting of pooled serum from arthritic Lewis rats was included in each plate. The reactivity of serum with Type II collagen was more than 80% inhibited by the antigen.

2.4. Drug treatment

Immunized rats were divided into 6 groups of 15 rats each, and drugs were given orally on a daily basis from the day of the first immunization.

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Abbreviations: 1α-OH-D_3, 1α-hydroxyvitamin D_3; 24,25-$(OH)_2$-D_3, 24,25-dihydroxyvitamin D_3; 1α,25-$(OH)_2$-D_3, 1α,25-dihydroxyvitamin D_3; CIA, collagen-induced arthritis; ELISA, enzyme-linked immunosorbent assay; RA, rheumatoid arthritis.

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2.5. Statistical analysis

For continuous variables (i.e. antibody levels), the mean values were compared between the groups by Student's *t*-test. For dichotomous variables (i.e. the incidence of arthritis), the percentages were compared between groups.

3. Results and discussion

Although some weight loss was observed in CIA rats at 3–5 weeks after immunization, there was no significant difference in body weight between the control and CIA rats after 7 weeks. This finding indicates that rats adapted to the inflammatory stress caused by Type II collagen injection. Tumor necrosis factor released from macrophages may be involved in the manifestation of such inflammatory stress, since similar effects of weight loss and adaptation were previously reported in rodents treated with this cytokine [14]. Drug treatment did not affect body weight of CIA rats.

The overall incidence of arthritis was calculated by histological examination of the feet of rats. All rats in the untreated group demonstrated inflammatory arthritis characterized by the proliferation of synovial lining cells, periarticular edema, infiltration of mononuclear cells and polymorphonuclear leukocytes, and the destruction of bone and articular cartilage by granulation tissue [10]. Administration of 1α-OH-D_3 reduced the incidence of arthritis to 13.3% (2/15 rats), while the incidence in the 24,25-$(OH)_2$-D_3-treated group was 86.7% (13/15 rats).

The hind paw volume of untreated CIA rats increased gradually and reached a maximum around 6 weeks after immunization (Fig. 1). 1α-OH-D_3 significantly inhibited

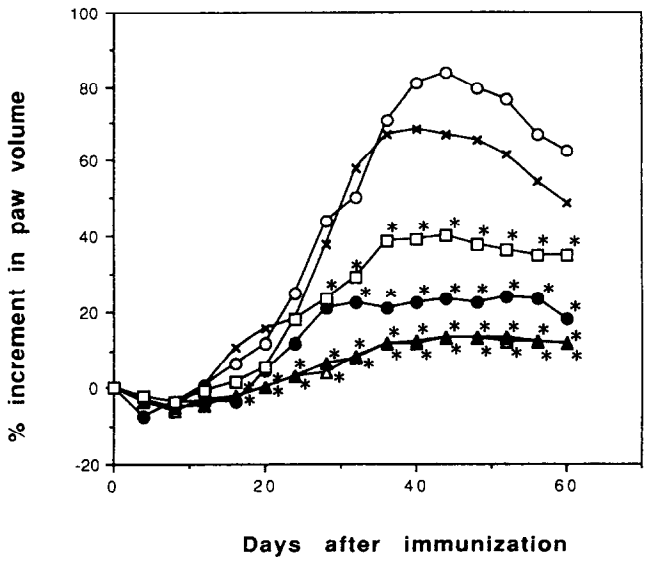

Fig. 1. Effects of 1α-OH-D_3 and 24,25-(OH)2-D_3 on hind paw swelling in rats with Type II collagen-induced arthritis. Each value represents the mean of 15 rats. Asterisks (*) indicate a significant difference relative to the untreated rats ($P < 0.01$). ○ = untreated; ● = indomethacin (5 mg/kg); △ = 1α-OH-D_3 (0.1 μg/kg); ★ = 1α-OH-D_3 (0.25 μg/kg); × = 24,25-(OH)2-D_3 (10 μg/kg); □ = gold (15 mg Au/kg).

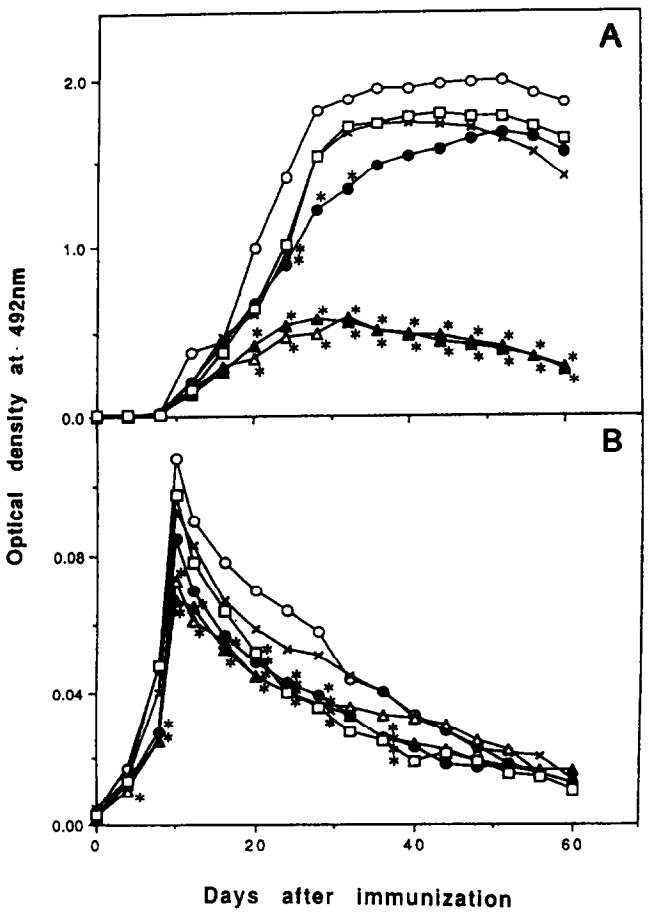

Fig. 2. Effects of 1α-OH-D_3 and 24,25-(OH)2-D_3 on anti-Type II collagen antibody levels. Each value represents the mean of 15 rats. Asterisks (*) indicate a significant difference relative to the untreated rats ($P < 0.01$). (A) Levels of IgG antibody directed against native Type II collagen. Serum samples were diluted to 1:1000 with buffered normal rabbit serum. (B) Levels of IgM antibody directed against native Type II collagen. Serum samples were diluted to 1:50 with buffered normal rabbit serum. ○ = untreated; ● = indomethacin (5 mg/kg); △ = 1α-OH-D_3 (0.1 μg/kg); ▲ = 1α-OH-D_3 (0.25 μg/kg); × = 24,25-(OH)2-D_3 (10 μg/kg); □ = gold (15 mg Au/kg).

paw swelling throughout the experiment at a dose of both 0.1 μg and 0.25 μg/kg. On the other hand, 24,25-$(OH)_2$-D_3 had almost no inhibitory effect. Gold and indomethacin were found to inhibit paw swelling to 50% and 35% of that in the untreated group, respectively.

1α-OH-D_3 significantly inhibited all the histopathological changes noted in CIA rats, including the proliferation of synovial lining cells, periarticular edema and destruction of bone and articular cartilage, although a small amount of granulation tissue was still observed.

The level of IgG antibody directed against native Type II collagen, which was quantified in sera diluted to 1:1000, began to increase just before the onset of arthritis at 10–14 days after immunization (Fig. 2). IgM antibody, which was quantified in sera diluted to 1:50, appeared earlier at 4–7 days after immunization (Fig. 2B). 1α-OH-D_3 (0.1 μg and 0.25 μg/kg) significantly decreased the

levels of IgG and IgM antibodies ($P < 0.01$) (Fig. 2A,B). In contrast, 24,25-$(OH)_2$-D_3, gold, and indomethacin showed no inhibitory effect on both the IgG and IgM antibodies.

In this study, 1α-OH-D_3 was found to inhibit the development of CIA by suppressing anti-Type II collagen antibody production, even though it has been reported that most of the slowly acting anti-rheumatic drugs (i.e. D-penicillamine, levamisole, sulfapyridine, and gold) have no beneficial effect on this type of arthritis [15]. Indomethacin also inhibited the hind paw swelling and histopathological changes, perhaps due to the suppression of prostaglandin E_2 production [16]. However, it had no effect on the anti-Type II collagen antibody level.

The difference between the effects of vitamin D_3, indomethacin, and gold on CIA was probably due to differences in their mechanism of action. Vitamin D_3 is a steroid hormone that binds to specific intracellular receptors expressed by numerous types of cells, including lymphoid cells [17]. Such receptors are not expressed by normal resting T and B lymphocytes, but they can be demonstrated following activation of these cells. The major effect of vitamin D_3 is on the early stages of T cell activation as well as the inhibition of interleukin-2 synthesis [18]. Therefore, the inhibition of CIA by 1α-OH-D_3 administration may be primarily due to the suppression of T cell proliferation and interleukin-2 synthesis.

We recently developed an improved ELISA for the detection of anti-collagen antibodies in human serum to diminish non-specific background interference and provide a higher sensitivity [13]. Using this assay system, IgG antibodies against native Type II collagen were detected in 22.7% of the serum samples from 480 patients with RA [12]. The antibodies were found to be collagen type-specific, showing no reactivity with human Type I and Type III collagens. In addition, these anti-Type II collagen antibodies appeared at a high incidence (75.4%) during the early phase of the disease.

Thus, the data obtained from the present study using CIA rats suggest that 1α-OH-D_3 may have a therapeutic value as an immunoregulatory agent in RA patients. There is a good possibility that calcitriol, a biologically active form of 1α-OH-D_3, has an antirheumatic effect not only through the suppression of inflammation, but also through inhibition of the autoimmune response to Type II collagen.

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