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Pharmacological studies of diacerein in animal models of inflammation, arthritis and bone resorption

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Abstract

Diacerein has proved to be effective in the treatment of osteoarthritis. We investigated the effects of diacerein in animal models of carrageenin-, zymosan-, or dextran-induced paw edema and adjuvant-induced arthritis and in ovariectomized rats. In acute inflammatory models, unlike classical nonsteroidal anti-inflammatory drugs such as naproxen and ibuprofen, diacerein inhibited the rat paw edema induced by various agents. In the adjuvant-induced arthritic rats, diacerein at 100 mg/kg/day significantly suppressed the paw edema and the increase in serum mucoprotein. Addition of 3 mg/kg/day naproxen to each diacerein (3, 10, 30 mg/kg/day) dose resulted in significantly greater anti-inflammatory activity than with naproxen alone. In the ovariectomized rats, diacerein (10, 100 mg/kg/day) also significantly prevented bone loss and reduced the serum alkaline phosphatase and decreased the excretion of urinary hydroxyproline. In addition, rhein (10, 30 μM) inhibited calcium release from mouse calvaria induced by interleukin-1β, prostaglandin E_2 and parathyroid hormone 1–34 human fragment. These findings indicate that diacerein is a novel anti-inflammatory drug with pharmacological properties different from those of classical nonsteroidal anti-inflammatory drugs and support the clinical investigation of the use of combination therapy with diacerein and nonsteroidal anti-inflammatory drugs in patients with not only osteoarthritis but also rheumatoid arthritis.

Keywords: Diacerein; Rhein; Inflammation; Arthritis; Bone

1. Introduction

A large number of nonsteroidal anti-inflammatory drugs are currently used for the treatment of osteoarthritis as first-line therapy; however, several adverse effects limit their clinical usefulness. Nonsteroidal anti-inflammatory drugs do not slow the damage to the joints or change the course of the disease (Paulus and Furst, 1985). Osteoarthritis is a degenerative process of the joints characterized by the progressive destruction and erosion of cartilage. Moreover, osteoarthritis is regarded as an age-associated disease, and occurs in a significantly increasing number of patients, but the underlying mechanisms of osteoarthritis are not yet clear. Although destruction of bone and cartilage is a hallmark of osteoarthritis and rheumatoid arthritis, and one of the most critical problems clinically, the precise mechanism of this destruction remains unknown. Numerous studies have shown a relationship between bone changes and articular cartilage damage in arthritis. Bones are remodelled as a result of bone resorption and new bone formation. Many inflammatory factors including interleukin-1, interleukin-6, tumor necrosis factor-α, nitric oxide, and prostaglandin are known to affect bone formation and resorption (Stashenko et al., 1987; Kawaguchi et al., 1995; Ralston and Grabowski, 1996). Recently, considerable interest has arisen in drugs that are not primarily aimed at the palliation of symptoms but which affect the basic pathogenetic mechanism underlying tissue damage in the joint. These disease-modifying drugs of osteoarthritis are aimed at modifying the pathobiologic and pathoanatomic changes in articular cartilage, in particular, by inhibiting matrix metalloproteinases or by stimulating anabolic activity.

Clinical studies have suggested that diacerein is an effective drug for the symptomatic treatment of osteoarthritis (Nguyen et al., 1994; Pelletier et al., 2000). It is also known to inhibit the production of interleukin-1 and

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to prevent cartilage breakdown in a mouse granuloma model (Moore et al., 1998), and to slow the progression of cartilage lesions in a canine model of osteoarthritis (Brandt et al., 1997). Diacerein is completely metabolized by animals and humans to rhein, an active metabolite of diacerein (Debord et al., 1994), and rhein reduces the production of superoxide anion in human neutrophils (Mian et al., 1987; Tamura et al., 2001b). Rhein down-regulates the gene expression and production of matrix metalloproteinases and up-regulates the production of tissue inhibitors of metalloproteinase-1 in rabbit articular chondrocytes (Tamura et al., 2001a). Diacerein and rhein have no effect on cyclooxygenase (Pelletier et al., 1998). However, nonsteroidal anti-inflammatory drugs have anti-inflammatory effects that are mainly due to the inhibition of cyclooxygenase. Therefore, the mechanism of action of diacerein has not yet been fully clarified. We investigated the anti-inflammatory activity of diacerein in a model of acute inflammation and in a model of chronic inflammation, adjuvant-induced arthritis in rats. We also investigated the effects of diacerein on bone turnover to obtain further insight into the clinical application of this agent.

2. Materials and methods

2.1. Animals

Male Wistar as well as female Lewis and female Sprague–Dawley rats were purchased from Charles River Japan (Kanagawa, Japan). Male ddy mice were purchased from SLC (Shizuoka, Japan). The animals were kept at the specific pathogen-free animal facility where a temperature of 22–24 °C, a humidity of 50–60%, and a 12-h day/night cycle were constantly maintained.

2.2. Drugs

Diacerein was provided by Proter (Milan, Italy). Rhein was synthesized in our laboratories. Naproxen and ibuprofen were purchased from Sigma (St. Louis, MO, USA).

2.3. Induction of acute paw edema in rats

Male Wistar rats in groups of eight were injected with 0.1 ml of 1% λ-carrageenin (Picnin A, Zushi Chemical, Kanagawa, Japan), 1% dextran (Sigma) or 1% zymosan (Sigma) in saline into the right hind paw under light ether anesthesia. The volume of the injected hind paw was measured before and 3 h after the carrageenin, 45 min after the dextran or 4 h after the zymosan injection, using a plethysmometer (TK-101; Unicom, Chiba, Japan). Doses of diacerein, naproxen and ibuprofen were administered orally in 5% arabic gum solution (as 10 ml/kg) 30 min before the injection of irritants. Vehicle (5% arabic gum solution) was administered as control.

2.4. Induction of adjuvant arthritis in rats and drug administration

Adjuvant-induced arthritis was produced by an injection of 0.6 mg of *Mycobacterium butylicum* (Difco Laboratories, Detroit, MI, USA) suspended in paraffin oil into the right footpad of 8-week-old female Lewis rats under light ether anesthesia on day 0. Hindpaw volumes were measured using the plethysmometer on day 21 and the measured volumes are given as a percentage of the day 0 volume. Doses of diacerein, naproxen or vehicle were administered orally once daily starting on day 0 and up to day 20. Animals were killed on day 22 after general anesthesia by inhalation of ether. Blood was collected from the abdominal aorta, and the thymus and the spleen were dissected and weighed. The serum concentration of mucoprotein was measured with a commercial kit (Ostuka, Tokyo, Japan).

2.5. The ovariectomized rat model

An ovariectomy or sham operation was performed on female Sprague–Dawley rats (13 weeks old) under sodium pentobarbital anesthesia. Diacerein was orally administered once a day for 6 weeks. After the final administration of diacerein, the animals were placed in metabolic cages to collect their urine over a 24-h period. Thereafter, the animals were anesthetized with ether and blood was collected from the abdominal aorta. The serum osteocalcin level was measured with a commercial radioimmunoassay kit (Biomedical Technologies, Stoughton, USA), using rat osteocalcin as the standard. The serum alkaline phosphatase and urinary creatinine levels were measured using commercially available kits (Wako Pure Chemical, Osaka, Japan). The urinary hydroxyproline level was assayed by the method of Jamall et al. (1981). Urinary hydroxyproline excretion is reported as the ratio to the urinary creatinine. Bone mineral

Table 1
Effects of diacerein and reference compounds on carrageenin-, dextran- or zymosan-induced paw edema

Compound	Dose (mg/kg)	Swelling (%)		
		Carrageenin	Dextran	Zymosan
Control	–	69.8 ± 4.3	53.6 ± 5.0	51.8 ± 4.8
Diacerein	10	NT	44.5 ± 2.3	38.5 ± 2.8[a]
	30	72.0 ± 3.0	36.6 ± 3.7[b]	39.8 ± 3.5[a]
	100	54.3 ± 4.9[a]	33.6 ± 3.2[b]	35.5 ± 2.3[b]
	200	47.7 ± 3.0	27.9 ± 3.8[b]	33.3 ± 2.5[b]
Control	–	69.8 ± 4.3	52.3 ± 1.7	46.0 ± 1.5
Naproxen	3	23.5 ± 1.9[b]	48.8 ± 3.2	38.7 ± 2.0
Ibuprofen	30	23.9 ± 2.3[b]	46.5 ± 2.8	40.7 ± 1.6

The values represent the means ± S.E.M. for eight rats per group.
NT: not tested.
[a] $P<0.05$: significantly different from the respective control value by the Aspin–Welch or Dunnett test.
[b] $P<0.01$: significantly different from the respective control value by the Aspin–Welch or Dunnett test.

Table 2
Effects of diacerein and naproxen on rat adjuvant-induced arthritis on the 21st day

Treatment	Dose (mg/kg/day)	Swelling (%)		Serum mucoprotein (mg/dl)
		Treated	Untreated	
Normal	–	–	–	343.3 ± 6.7[a]
Control	–	162.6 ± 7.2	106.2 ± 8.7	725.6 ± 11.2
Diacerein	10	129.8 ± 10.0[b]	82.0 ± 8.0	658.6 ± 14.0
	100	91.9 ± 7.7[c]	51.3 ± 8.6[c]	561.0 ± 28.9[c]
Naproxen	3	81.5 ± 6.3[a]	45.6 ± 3.7[a]	660.7 ± 20.4

Diacerein or naproxen was orally administered once a day, starting on day 0 up day 20. The values represent the means ± S.E.M. for 10 rats per group.
[a] $P<0.01$: significantly different from the control value by the Aspin–Welch test.
[b] $P<0.05$: significantly different from the control value by Dunnett test.
[c] $P<0.01$: significantly different from the control value by Dunnett test.

measurements were performed on the femoral bone using a dual-energy X-ray absorptiometer (DCS-600; Aloka, Tokyo, Japan). The values of the total bone mineral content (mg) and bone mineral density (mg/cm^2) were obtained for the tibia specimen.

2.6. Culture of mouse calvaria

The calvaria was aseptically removed from 5- to 6-day-old mice and cut along the sagittal suture to divide it into the left and right halves, which were cut to obtain two pieces of equal area. Each half calvaria was incubated in a 6-well plate for 6 h at 37 °C under 5% CO_2–95% air in Dulbecco's modified Eagle's medium (Life Technologies, Rockville, MD, USA) with 15% horse serum, 2.5% fetal bovine serum (Life Technologies) and antibiotics (100 units/ml penicillin, 100 μg/ml streptomycin and 250 ng/ml fungizone®, Life Technologies). After preculture, each half calvaria was transferred to 1.5 ml of fresh medium containing rhein with or without stimulator, recombinant human interleukin-1β (30 pg/ml; Genzyme, Cambridge, MA, USA), prostaglandin E_2 (1 μM; Sigma) and parathyroid hormone 1–34 human fragment (10 nM; Sigma), for 4 days. The medium was changed every 2 days and fresh treatments were added. Rhein was dissolved in dimethyl sulfoxide. The final concentration of dimethyl sulfoxide was 0.1%. The concentrations of rhein used were chosen to reflect levels that are below, at, or above the established therapeutic anti-inflammatory serum values described in clinical practice (Nicolas et al., 1998). To determine the bone-resorbing activity of rhein, the medium calcium (Ca) level was measured on the last 2 days of the culture using a commercial calcium kit (Calcium *C*-test, Wako).

2.7. Data analysis

The data were analyzed by the *F*-test followed by the Aspin–Welch test or the Wilcoxon test. Multiple comparisons between treatment groups were assessed by a one-way analysis of variance test, followed by the Dunnett test. A P value of less than 0.05 was considered to be statistically significant. The data are presented as the means ± S.E.M.

3. Results

3.1. Effect of diacerein on acute paw edema in rats

The effect on rat paw edema induced by various agents was investigated. Table 1 shows the effect of diacerein on carrageenin-, dextran- or zymosan-induced paw edema. Diacerein significantly inhibited the carrageenin-induced paw edema at doses of 100 and 200 mg/kg. Diacerein at doses of 10–200 mg/kg also inhibited the dextran- or zymosan-induced paw edema. Naproxen (3 mg/kg) and ibuprofen (30 mg/kg) significantly inhibited the carrageenin-induced paw edema but did not inhibit the dextran- or zymosan-induced paw edema.

3.2. Effects of diacerein on rat adjuvant-induced arthritis

The daily administration of diacerein (10, 100 mg/kg/day) or naproxen (3 mg/kg/day) from the day of adjuvant injection to day 21 reduced the volume of both the adjuvant-treated and untreated foot (on treated foot, $P<0.05$ at 10

Table 3
Effects of diacerein concomitant with naproxen on rat adjuvant-induced arthritis on the 21st day

Treatment (mg/kg/day)	Swelling (%)		Tissue weight (mg/100g BW)		Serum mucoprotein (mg/dl)
	Treated	Untreated	Thymus	Spleen	
Normal	–	–	233.8 ± 6.8[a]	234.9 ± 7.1[a]	394.2 ± 6.7[a]
Control	252.6 ± 14.1	97.4 ± 11.2	130.4 ± 10.5	396.8 ± 20.1	1129.4 ± 20.9
Naproxen 3	125.0 ± 8.2[b]	31.4 ± 3.0[b]	167.0 ± 11.0	379.8 ± 24.6	998.7 ± 47.4
Naproxen 3 + Diacerein 3	99.6 ± 7.3[b]	29.8 ± 3.9[b]	167.3 ± 6.0	368.7 ± 8.0	590.3 ± 89.7[b]
Naproxen 3 + Diacerein 10	88.0 ± 6.5[b]	20.4 ± 5.1[b]	189.8 ± 11.9[b]	324.0 ± 9.0[c]	587.2 ± 74.5[b]
Naproxen 3 + Diacerein 30	66.7 ± 9.0[b]	15.1 ± 2.1[b]	191.7 ± 5.6[b]	300.6 ± 4.8[b]	582.3 ± 44.8[b]

Diacerein and naproxen were orally administered once a day starting on day 0 up day 20. The values represent the means ± S.E.M. for 6–8 rats per group.
[a] $P<0.01$: significantly different from the control value by the Aspin–Welch test.
[b] $P<0.01$: significantly different from the control value by Dunnett test.
[c] $P<0.05$: significantly different from the control value by Dunnett test.

Table 4
Effects of diacerein on body weight, uterine weight, femoral bone mineral content density of rats at 6 weeks after ovariectomy

Treatment	Dose (mg/kg/day)	Body weight (g)	Uterine weight (mg)	Bone mineral content (mg)	Bone mineral density (mg/cm^2)
Sham	–	325 ± 6	97.1 ± 9.7	435.3 ± 11.3	209.0 ± 3.3
Control	–	402 ± 10[a]	14.3 ± 0.7[a]	393.2 ± 9.1[b]	183.3 ± 2.4[a]
Diacerein	10	394 ± 11	15.2 ± 0.7	432.4 ± 8.0[c]	193.0 ± 1.9[c]
	100	389 ± 7	14.9 ± 0.7	439.0 ± 6.6[c]	194.1 ± 1.8[c]

Diacerein was orally administered once a day for 6 weeks. The values represent the means ± S.E.M. for 10 rats per group.
[a] $P<0.01$: significantly different from sham group by Wilcoxon test.
[b] $P<0.05$: significantly different from sham group by Wilcoxon test.
[c] $P<0.01$: significantly different from ovariectomized control group by Williams test.

mg/kg/day and $P<0.01$ at 100 mg/kg/day, on untreated foot, $P<0.01$ at 100 mg/kg/day) (Table 2).

Blood was collected at the end of the experiment on day 21 and serum was prepared for the determination of mucoprotein, as an acute-phase reactant. During the inflammatory conditions, the serum level of mucoprotein increased and diacerein significantly decreased the serum level at 100 mg/kg/day ($P<0.01$), although naproxen did not inhibit it (Table 2).

Addition of 3 mg/kg naproxen to each diacerein dose resulted in a significantly greater inhibition of swelling and reduction of serum mucoprotein than that produced by naproxen alone at doses of 3, 10 and 30 mg/kg. Furthermore, the ratio of thymus weight-to-body weight of rats with adjuvant-induced arthritis receiving combination treatment was significantly greater than that of control rats ($P<0.01$), and spleen-to-body weight ratios of rats treated with combination were lower than those of control rats ($P<0.01$) (Table 3).

3.3. Effects of diacerein on bone mineral content and density of femur in ovariectomized rats

We examined the effect of diacerein on bone turnover in ovariectomized rats. Ovariectomy increased body weight and decreased bone mineral content, bone mineral density and uterine weight (Table 4). Bone mineral content and bone mineral density were significantly decreased by 9.7% and 12.3% from those of the sham-treated group, respectively. Diacerein significantly prevented the ovariectomy-induced loss of bone mineral content (0.7% and 0% loss at 10 and 100 mg/kg/day, respectively) and bone mineral density (7.7% and 7.1% loss at 10 and 100 mg/kg/day, respectively). However, diacerein had no effect on the body and uterine weights compared with those of the ovariectomized controls. The levels of serum osteocalcin and alkaline phosphatase, markers of bone formation, were significantly increased in the ovariectomized rats, and ovariectomy increased the rate of bone turnover (Table 5). Diacerein had no effect on the serum osteocalcin level but significantly inhibited the serum alkaline phosphatase level. The level of urinary hydroxyproline, a marker of bone resorption, was significantly increased in the ovariectomized rats while diacerein significantly inhibited it (Table 5).

3.4. Effect of rhein on the bone-resorbing

We measured the Ca concentration in media containing a bone resorption stimulant. The addition of interleukin-1β, prostaglandin E_2 and parathyroid hormone 1–34 human fragment resulted in significant dose-dependent increases in the Ca concentration in the medium (data not shown). Therefore, we used the following concentration of these bone resorption stimulants: 30 pg/ml of interleukin-1β, 1 μM prostaglandin E_2, and 10 nM of parathyroid hormone 1–34 human fragment. Fig. 1 shows that interleukin-1β, prostaglandin E_2 and the parathyroid hormone 1–34 human fragment significantly increased Ca release by 1.24-, 1.41- or 1.42-fold over basal values. Rhein inhibited the interleukin-1β or prostaglandin E_2-induced Ca release. There were significant reductions in stimulated Ca release with 10 μM

Table 5
Effects of diacerein on concentration of serum osteocalcin, alkaline phosphatase and urinary hydroxyproline in rats at 6 weeks after ovariectomy

Treatment	Dose (mg/kg/day)	Serum		Urinary
		Osteocalcin (ng/ml)	Alkaline phosphatase (IU/l)	Hydroxyproline/creatinine (μM/mM)
Sham	–	208.4 ± 9.6	60.5 ± 3.2	54.4 ± 9.0
Control	–	293.6 ± 11.4[a]	79.8 ± 4.4[a]	77.9 ± 5.0[b]
Diacerein	10	290.4 ± 9.3	61.0 ± 2.5[b]	61.7 ± 5.0[a]
	100	285.0 ± 14.3	52.8 ± 2.1[b]	65.8 ± 2.9[c]

Diacerein was orally administered once a day for 6 weeks. The values represent the mean ± S.E.M. for 9–10 rats per group. Levels of serum osteocalcin, alkaline phosphatase and urinary creatinine were measured with commercial kits. Urinary hydroxyproline was assayed by the method of Jamall et al. (1981) and values were corrected for the concentration of urinary Cre.
[a] $P<0.01$: significantly different from sham group by Wilcoxon test.
[b] $P<0.01$: significantly different from ovariectomized control group by Williams test.
[c] $P<0.05$: significantly different from ovariectomized control group by Williams test.

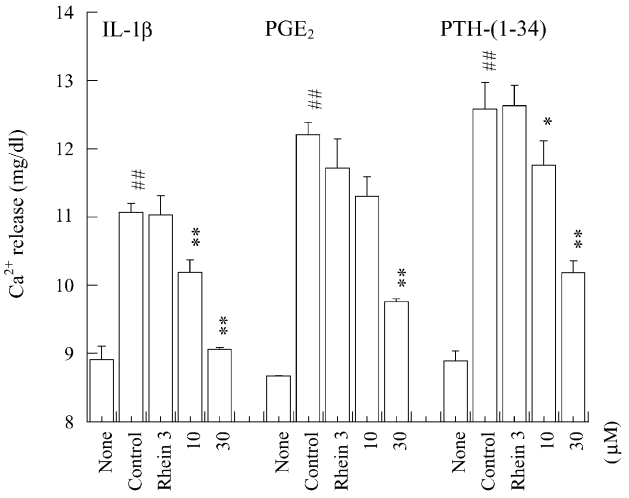

Fig. 1. Effect of rhein on bone resorption stimulated by interleukin-1β (IL-1β), prostaglandin E_2 (PGE_2) or parathyroid hormone 1–34 human fragment (PTH-(1–34)). Calvaria were treated continuously for 4 days, with a change of medium at 2 days. The values represent the means ± S.E.M. for groups of 6 bones per group in three independent experiments. $^{\#\#}P<0.01$: significantly different from nontreated group by Wilcoxon test. $^{*}P<0.05$ and $^{**}P<0.01$: significantly different from each stimulated control group by Williams test.

rhein, with a maximum of 94 and 69% inhibition at 30 μM, respectively. Rhein also significantly inhibited the parathyroid hormone 1–34 human fragment-induced Ca release by 64% at 30 μM (Fig. 1).

4. Discussion

Osteoarthritis is not merely a disease of cartilage—all tissues of the joint, including the synovium, subchondral bone, periarticular muscle, and supporting ligaments, are affected in this disease. Changes in bone in osteoarthritis include the development of osteophytes, activation of the zone of calcified cartilage with endochondral calcification at that site, and thickening of the subchondral plate (Li and Aspden, 1997; Fazzalari and Parkinson, 1997). Bone turnover in the osteoarthritic joint is also increased (Seibel et al., 1989; Mansell and Bailey, 1998). Cartilage destruction is a crucial feature of osteoarthritis and is generally considered irreversible. Accordingly, drugs that block the destruction of cartilage will be of therapeutic value. Currently, the primary approach in the clinical treatment of osteoarthritis involves the use of nonsteroidal anti-inflammatory drugs, analgesics and hyaluronan, which provide symptomatic relief but exert no apparent disease-modifying effect (Bollet, 1981; Pelletier and Martel-Pelletier, 1989).

Diacerein is an effective agent for the treatment of osteoarthritis in patients (Pelletier et al., 2000) and in animal models (Brandt et al., 1997). The anti-inflammatory effect of diacerein is linked to mechanisms that have not yet been completely clarified. To obtain a further insight into the effects of diacerein, various experimental animal models of acute (carrageenin-, zymosan-, or dextran-induced paw edema) and chronic inflammation (adjuvant-induced arthritis) and ovariectomized rats were used to investigate the articular protective activity of diacerein.

The results of these investigations clearly demonstrate that diacerein has a different spectrum of anti-inflammatory activity to that of the classical nonsteroidal anti-inflammatory drug, naproxen and ibuprofen. While nonsteroidal anti-inflammatory drugs inhibit cyclooxygenase, diacerein does not inhibit prostaglandin synthesis (Pelletier et al., 1998). The absence of a cyclooxygenase inhibitory action of diacerein also offers an explanation for the low potency of the drug in carrageenin-induced paw edema, which is highly susceptible to inhibition by nonsteroidal anti-inflammatory drugs. It has been demonstrated with other irritants that, compared to carrageenin-induced paw edema, the inflammation induced by dextran or zymosan is relatively resistant to the action of nonsteroidal anti-inflammatory drugs. Dextran-induced paw edema causes histamine, serotonin or bradykinin release, and it has been reported that nonsteroidal anti-inflammatory drugs have no effect on this edema (Merlos et al., 1990). Zymosan is well-known as a potent releaser of arachidonic metabolites. Our findings with naproxen and ibuprofen suggest that zymosan-induced paw edema is not only cyclooxygenase-dependent. Furthermore, zymosan induces numerous other inflammatory mediators such as free radicals (Forrest et al., 1986) and cytokines like interleukin-1 (Fantuzzi et al., 1997). Diacerein inhibited the dextran- or zymosan-induced paw edema at doses lower than those effective against carrageenin, but nonsteroidal anti-inflammatory drugs are rather ineffective against dextran- or zymosan-induced paw edema in contrast to carrageenin-induced paw edema. Rhein inhibits reactive oxygen species production by human activated neutrophils (Tamura et al., 2001b). Its anti-inflammatory effect may be partially due to inhibition of the production of reactive oxygen species, based on its inhibition of neutrophil activation. Moreover, diacerein is also known to inhibit the production of interleukin-1 (Moore et al., 1998). Taken together, it is likely that diacerein modulates the inflammatory response via interference with multiple signaling pathways. Further studies are needed to elucidate the precise mechanism of anti-inflammatory action of diacerein.

The rat adjuvant-induced arthritis model is widely used for the evaluation of nonsteroidal anti-inflammatory drugs and anti-rheumatic drugs. This chronic polyarthritis has many features that are common to rheumatoid arthritis (Newbould, 1963) and, therefore, the anti-inflammatory activity of diacerein against chronic inflammation was assessed in this model. C-reactive protein is a useful biochemical measure for the clinical assessment of patients with rheumatoid arthritis. A number of acute-phase proteins have been studied in rat adjuvant-induced arthritis. Cyclosporin A, methotrexate and auranofin lowered the plasma C-reactive protein concentrations in parallel with reduced paw swelling, while nonsteroidal anti-inflamma-

tory drugs inhibited paw swelling, but did not lower the C-reactive protein concentrations (Connolly et al., 1988; Otterness et al., 1991). Nonsteroidal anti-inflammatory drugs potently inhibited edema and diacerein also had an inhibitory effect. Furthermore, diacerein alone reduced the serum concentration of mucoprotein, a parameter of the systemic response. Diacerein was given to arthritic rats to determine if it could reverse the abnormal plasma concentrations of mucoprotein which were unaffected by treatment with nonsteroidal anti-inflammatory drugs. When diacerein was orally administered for 3 weeks to rats with adjuvant-induced arthritis, it significantly inhibited swelling of the injected and non-injected paws at doses of 100 mg/kg. The level of mucoprotein, which was raised approximately 2- to 3-fold above normal in arthritic rats, was reduced by 23% after treatment of arthritic rats with diacerein at 100 mg/kg. Addition of naproxen to each diacerein dose resulted in a significantly greater inhibition of swelling and reduction of serum mucoprotein level than that obtained with naproxen alone. Diacerein, in addition to inhibiting chronic systemic paw inflammation, also altered abnormal concentrations of mucoprotein in the adjuvant arthritic rat, thus distinguishing diacerein from standard NSAIDs. Although the mechanism of action of diacerein has not been clarified, diacerein might inhibit the occurrence and progress of adjuvant arthritis in a more pathogenetic manner. Since diacerein significantly suppresses the increase in plasma NO in rat adjuvant-induced arthritis (Tamura and Ohmori, 2001), one of the mechanisms of the inhibitory effect of diacerein on adjuvant-induced arthritis may be partly related to its reduction of the NO production induced by adjuvant-induced arthritis.

Osteoarthritis is characterized by the progressive erosion of articular cartilage. Osteoporosis and a reduction of bone mass are often observed in osteoarthritis and rheumatoid arthritis. To obtain further insight into the effects of diacerein, ovariectomized rats and mouse calvaria cultures were used to investigate the effect of diacerein on bone. We demonstrated that diacerein prevented the decrease in bone mineral content and bone mineral density in ovariectomized rats. Moreover, diacerein inhibited the increase in the hydroxyproline excretion in urine and the serum alkaline phosphatase concentration. From these results, diacerein reverses the change in bone metabolism seen in ovariectomized rats, and can be considered to maintain bone mineral density by improving the balance of bone formation and bone absorption. Rhein, an active metabolite of diacerein, inhibited interleukin-1β, prostaglandin E_2 or the parathyroid hormone 1–34 human fragment-induced bone resorption in the mouse calvaria culture. This may be valuable information for the therapeutic effects of diacerein on osteoarthritis or rheumatoid arthritis-induced bone destruction.

Bone mineral measurements have confirmed significant bone loss at 8 weeks after ovariectomy in the femoral bone in rats. Ovariectomy-induced bone loss is caused by an increase in bone resorption, which is accompanied by an increase in bone formation (bone turnover). In contrast to intact rats where bone formation equals bone resorption, the resorption of bone by osteoclasts is not completely compensated for by increased bone formation by osteoblasts in ovariectomized rats. The capacity of a compound to reduce ovariectomy-induced bone loss may be due to a reduction in bone resorption, a stimulation of bone formation, or a combination of these two processes. Human osteoarthritis subchondral osteoblasts were reported to have abnormal phenotypes, elevated alkaline phosphatase, and increased release of osteocalcin (Lajeunesse et al., 1999). The levels of urinary hydroxyproline, a marker of bone resorption, and the levels of serum alkaline phosphatase, a marker of bone formation, were both increased in the ovariectomized rats. Our finding that diacerein significantly reduced them points to a diacerein-induced inhibition of the rate of bone turnover. Whether diacerein reduces bone resorption more than bone formation, and therefore has an additional stimulatory effect on the bone-forming capacity of the osteoblasts, needs further investigation.

Bone resorption is mediated by several processes, including osteoclast differentiation and activation, and matrix metalloproteinases-dependent matrix degradation. Prostaglandin E_2 is known to be a critical factor in bone formation and resorption, dependent on their levels (Dietrich et al., 1975; Akamine et al., 1992). Cytokines such as interleukin-1 and interleukin-6 have bone-resorbing activity and are likely involved in the pathogenesis of osteoporosis (Miyaura et al., 1995; Kimble et al., 1994). Everts et al. (1999) reported that osteoclastic bone resorption depends on the activity of both cysteine proteinases, such as cathepsin K, and matrix metalloproteinases in calvaria. Cytokines such as interleukin-1 significantly induce the expression of matrix metalloproteinases in mouse calvarial cultures, and the potency of various cytokines to induce matrix metalloproteinases is closely correlated to their bone-resorbing activity, which involves the degradation of the bone matrix (Kusano et al., 1998). Hill et al. (1994, 1995) also reported that matrix metalloproteinase inhibitors prevent interleukin-1 or parathyroid hormone-induced bone resorption. Although rhein down-regulates the interleukin-1α-induced production of pro matrix metalloproteinases in chondrocytes (Tamura et al., 2001a), it is not clear whether matrix metalloproteinase production in the mouse calvarial cultures is inhibited.

In conclusion, diacerein is different from nonsteroidal anti-inflammatory drugs with respect to several pharmacological properties. The data obtained in this study suggested that diacerein may be a promising drug for not only osteoarthritis but also rheumatoid arthritis.

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