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Dietary xylitol protects against the imbalance in bone metabolism during the early phase of collagen type II–induced arthritis in dark agouti rats

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Abstract

The aim of the present study was to evaluate the changes in bone metabolism during the early phase of type II collagen–induced arthritis in rats and to evaluate whether a 10% dietary xylitol supplementation is able to protect against these changes. Arthritis was induced in female dark agouti rats by injections of type II homologous rat collagen emulsified with an equal volume of incomplete Freund adjuvant. In one group, the diet was supplemented with 10% xylitol. After 17 days, the rats were killed. Serum osteocalcin, as a marker of bone formation, and serum tartrate-resistant acid phosphatase, as a marker of bone resorption, were measured. Histologic measurements were made from Masson-Goldner trichrome–stained sections of distal tibiae. All the collagen-injected rats had arthritic symptoms at the end of the experiment. Serum osteocalcin was significantly higher in the collagen-injected rats fed a xylitol-supplemented diet (CI-X) than in the collagen-injected rats not fed xylitol (CI) and in the controls. Serum tartrate-resistant acid phosphatase was significantly higher in the CI and CI-X groups than in the controls. Trabecular bone volume was significantly lower in the CI group as compared with the CI-X and control groups. These results suggest that, at the time of the appearance of arthritic symptoms, bone resorption activity is high, but bone formation is not severely affected. Furthermore, dietary xylitol seems to protect against the imbalance of bone metabolism during the early phase of collagen type II–induced arthritis.

1. Introduction

Arthritis is accompanied by osteoporosis-type changes in bone and cartilage [1]. Osteopenia results from the imbalance between bone resorption and bone formation, but data are conflicting regarding contributions of these 2 processes. Osteoporotic changes during arthritis have been attributed to increased bone resorption [2,3], decreased bone formation [4], or both [5]. Hanyu et al [6] found a decreased trabecular bone volume in rats 6 weeks after the induction of arthritis. Bone loss was reflected by a significant increase in osteoclast activity and by a temporary decrease in bone formation. Bonnet et al [7] found a decrease in bone formation 2 weeks after a single injection of Freund complete adjuvant in rats. On the other hand, Österman et al [8] reported increased bone resorption at 4 weeks after the induction of arthritis.

Signs of arthritis, including swelling of the hind limbs and unwillingness to use them, usually appear within 14 to 18 days after immunization. In the present study, bone measurements were made 17 days after the induction of the disease, when the early signs of arthritis were already detectable. Collagen type II–induced arthritis is a widely used model for human rheumatoid arthritis [9]. Dark agouti (DA) is a rat strain highly susceptible to develop experimental arthritis [10]. The first aim of the present study was to evaluate the changes in bone resorption and bone formation during the early phase of type II homologous rat collagen–induced arthritis in DA rats.

Xylitol is a 5-carbon polyalcohol that is found in most fruits and vegetables [11]. It is also an intermediate of mammalian carbohydrate metabolism. In the human body,

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5 to 15 g of xylitol is formed daily [12]. Our recent studies have shown that dietary xylitol protects effectively against ovariectomy-induced imbalance in bone metabolism [13]. As a result, xylitol protects effectively against the decrease of trabecular bone volume and against the weakening of bone biomechanical properties during experimental osteoporosis [14]. Although arthritis is also accompanied by osteoporosis, our hypothesis is that dietary xylitol may be able to protect against changes in bone metabolism during experimental arthritis. Thus, the second aim of the present study was to evaluate whether a 10% dietary xylitol supplementation is able to protect against the imbalance of bone metabolism during the early phase of type II collagen–induced arthritis in DA rats.

2. Materials and methods

2.1. Animals

Sixty female DA rats (Ola Hsd, Harlan, Netherlands) were used in the study. At the beginning of the study, the rats were 6 weeks old and their mean body weight was 116 g. The animals were fed a basal powder diet (RM1; Special Diet Services, Witham, Essex, United Kingdom). A total of 1 kg of this diet contains 885 g of cereal products (wheat, barley, and wheat feed), 60 g of vegetable proteins, 25 g of animal proteins (whey powder), 5 g of soybean oil, 7.1 g of calcium, 2.9 g of phosphorus, and 15 μg of cholecalciferol. The list of the other minor components of the diet is shown in the manufacturer's brochure. The rats had free access to tap water. They were housed in cages (Makrolon III; Tecniplast, Buguggiate, Italy) on a bed of European aspen shavings in a temperature- and light-controlled room (21°C-23°C, 12-hour light-dark cycle). They were weighed weekly, and their food intake was measured. The study protocol was approved by the Ethical Committee on Animal Experiments of the University of Oulu.

2.2. Induction of arthritis

Arthritis was induced in 2 experimental groups by 2 separate injections of lyophilized, type II homologous rat collagen (Chondrex, Redmond, WA), which was first suspended in 0.01 N acetic acid (2 mg/mL) by constant, gentle stirring overnight at 4°C. The suspension was then emulsified with an equal volume of incomplete Freund adjuvant (Difco, Detroit, MI) in a homogenizer for 3 minutes. The resulting stiff emulsion was kept cold in an ice bath. At the beginning of the study, a single injection of 0.1 mL of cold emulsion (100 μg collagen) was given intradermally at the base of the tail. The rats in the control group were given a similar injection with equal volume of physiological saline. The same procedure was repeated in each group 7 days later as a booster. The joint dimensions in the hind limbs of the rats were measured at the beginning and at the end of the experiment using a constant-tension Vernier caliper.

2.3. Xylitol administration

The rats in the control group and the rats in the first experimental group, collagen-injected rats not fed xylitol (CI), were fed continuously the RM1 basal powder diet. The rats in the second experimental group, collagen-injected rats fed a xylitol-supplemented diet (CI-X), were fed the same diet supplemented with 10% xylitol (wt/wt) (Cultor, Espoo, Finland) since the induction of arthritis.

2.4. Sample collection

Seventeen days after the first injections, blood samples were collected by a heart puncture under anesthesia using a 1:1:2 mixture of Hypnorm (Janssen Pharmaceutica, Beerse, Belgium), Dormicum (F Hoffmann–La Roche, Basel, Switzerland), and water (0.3 mL/100 g body weight, intraperitoneally). Serum was separated by centrifugation and stored at −80°C. After taking the blood samples, the rats were killed with an overdose of CO_2 followed by decapitation. Their right tibiae were prepared for the histologic analyses.

2.5. Determination of bone metabolic markers

Serum osteocalcin (OC) levels were determined using an enzyme immunoassay kit specific for rat OC (rat OC enzyme-linked immunosorbent assay; DRG Diagnostics, DRG Instruments, Marburg, Germany). Serum osteoclast-derived tartrate-resistant acid phosphatase (TRAP) levels were determined using a solid-phase immunofixed-enzyme activity assay (RatTRAP Assay, Code TR 102; Suomen Bioanalytiikka Oy, SBA Sciences, Oulu, Finland).

2.6. Histologic evaluation

The prepared right tibiae were fixed in 40% ethanol. Dehydration was done stepwise up to an ethanol concentration of 100%, and clearing was processed in xylene. The samples were infiltrated and embedded in methylmethacrylate softened with Plastoid N (Röhm Pharma, Weiterstadt, Germany). After slow hardening, blocks were cut in thin (5 μg) serial sections in a standard sagittal plane with a Microm HM355 heavy duty microtome (Microm Laborgeräte, Walldorf, Germany) and mounted on gelatin-coated glass slides. The sections were deplasted, and 4 sections of each rat tibia were stained using a modified Masson-Goldner trichrome method [15]. Trabecular bone volume, subcortical osteoid thickness, number of osteoclasts on bone surface, trabecular number, trabecular separation, and eroded surface/bone surface in the distal tibia were measured at 10× objective magnification using a computer image analyser (MCID, Model M1; Imazing Research, Brock University, Ontario, Canada).

2.7. Statistical analyses

Statistical significances of the differences between the groups were calculated using the 1-way analysis of variance, with further comparison made using Tukey honestly

Table 1
Average body weight of the rats and joint dimension in the hind limbs of the rats at the beginning and at the end of the experiment (mean ± SD)

	CI rats (n = 20)	CI-X rats (n = 20)	Control rats (n = 20)
Body weight at the beginning of the experiment (g)	116 ± 8	115 ± 7	117 ± 8
Body weight at the end of the experiment (g)	131 ± 15	132 ± 7	139 ± 7
Joint dimension in the hind limbs at the beginning of the experiment (mm)	6.5 ± 0.2	6.6 ± 0.2	6.6 ± 0.2
Joint dimension in the hind limbs at the end of the experiment (mm)	8.9 ± 0.6[b]	8.8 ± 0.5[b]	6.9 ± 0.2[a]

Statistical significance of the differences between the groups was calculated using the 1-way analysis of variance; further comparison was made by Tukey HSD test. Groups with different superscript letters differ significantly ($P < .01$).

significantly different (HSD) test. The statistical program used was SPSS 12.0.1 for Windows (SPSS, Chicago, IL).

3. Results

The average body weight at the end of the experiment was somewhat lower in the CI and CI-X groups as compared with the controls. However, the difference between the groups did not reach statistical significance (Table 1). All the collagen-injected rats had arthritic symptoms, swelling, and redness of the hind limbs at the end of the experiment. Joint dimension in the hind limbs of the rats was significantly greater in the CI and CI-X groups as compared with the controls (Table 1).

Serum OC was significantly higher in the CI-X group than in the CI group and in the control group (Table 2). Serum TRAP was significantly higher in both arthritic groups compared with the controls (Table 2). Trabecular bone volume was significantly lower in the CI group compared with the CI-X group and the control group (Table 3). Osteoid thickness was greatest in the control group, but it was significantly greater also in the CI-X group than in the CI group (Table 3). The number of osteoclasts on the bone surface was lowest in the control group, but it was significantly lower also in the CI-X group than in the CI group (Table 3). Trabecular number was lower and trabecular separation was greater in the CI group as compared with the other groups (Table 3). Eroded surface/bone surface was lowest in the control group, but also lower in the CI-X group than in the CI group (Table 3).

Table 2
Bone metabolic markers at the end of the experiment (mean ± SD)

	CI rats (n = 20)	CI-X rats (n = 20)	Control rats (n = 20)
Serum OC (μg/L)	17.8 ± 7.4[a]	33.3 ± 9.6[b]	17.4 ± 3.5[a]
Serum osteoclast-derived TRAP (U/L)	19.8 ± 6.5[b]	18.7 ± 6.0[b]	13.1 ± 2.2[a]

Statistical significance of the differences between the groups was calculated using the 1-way analysis of variance; further comparison was made by Tukey HSD test. Groups with different superscript letters differ significantly ($P < .01$).

Table 3
Bone histologic markers at the end of the experiment (mean ± SD)

	CI rats (n = 20)	CI-X rats (n = 20)	Control rats (n = 20)
Trabecular bone volume (%)	18.9 ± 5.7[a]	27.0 ± 4.8[b]	27.1 ± 5.1[b]
Osteoid thickness (μm)	4.20 ± 2.59[a]	9.75 ± 3.93[b]	17.98 ± 3.19[c]
No. of osteoclasts on bone surface (1/mm)	2.51 ± 0.69[c]	1.79 ± 0.46[b]	0.93 ± 0.44[a]
Trabecular number (1/mm)	1.58 ± 0.29[a]	2.17 ± 0.25[b]	2.21 ± 0.33[b]
Trabecular separation (μm)	359 ± 39[b]	323 ± 37[a]	319 ± 30[a]
Eroded surface/bone surface (%)	11.1 ± 1.7[c]	9.4 ± 1.4[b]	7.7 ± 1.1[a]

Statistical significance of the differences between the groups was calculated using the 1-way analysis of variance; further comparison was made by Tukey HSD test. Groups with different superscript letters differ significantly ($P < .01$).

4. Discussion

Dark agouti has been detected to be a suitable rat strain in modeling rheumatoid arthritis [10]. The high susceptibility of the DA strain to develop experimental arthritis was also seen in the present study, in which arthritis was induced by a recently marketed type II homologous rat collagen. All the collagen-injected rats had arthritic symptoms at the end of the experiment. The use of a homologous rat collagen instead of the widely used heterologous collagen was preferred because arthritis in rats induced with native rat type II collagen has been detected to have a better resemblance to rheumatoid arthritis [16].

The weight of the rats at the end of the experiment was slightly smaller in the arthritic groups, which is well in accordance with previous reports [17]. There are conflicting data in earlier studies concerning the background of the osteoporotic changes during arthritis. Increased bone resorption [2,3], decreased bone formation [4], or both [5] have been detected. The results of the present study suggest that, at the time of the appearance of arthritic symptoms, bone resorption activity is high, as measured by serum TRAP, whereas bone formation seems not to be severely affected, as seen in the serum OC levels. This is in accordance with the study of Österman et al [8] and confirms the results of the human studies made by Shimizu et al [2] and Gough et al [3].

Dietary xylitol supplementation induced a significant protective effect against the imbalance of bone metabolism in the rats caused by collagen type II–induced arthritis. This was seen in greater values of trabecular bone volume, trabecular number, and osteoid thickness, as well as in lower values of number of osteoclasts on bone surface, trabecular separation, and eroded surface/bone surface in the xylitol-fed

arthritic rats compared with the arthritic rats not fed xylitol. These observations may partly be explained by the increased bone formation activity induced by xylitol, as suggested by the significantly higher serum OC levels in the xylitol-fed rats. Although the serum TRAP values were not significantly lower in the CI-X rats as compared with the CI rats, the lower number of osteoclasts on bone surface and the lower eroded surface/bone surface ratio in the xylitol-fed rats may also suggest a diminished bone resorption activity.

The xylitol-induced protection against osteoporotic changes is well in accordance with our previous studies with ovariectomized rats [13,14]. Langman et al [18] have shown that arthritic rats have ineffective intestinal calcium absorption that is associated with vitamin D deficiency and reduced OC levels. Interestingly, xylitol is known to increase calcium absorption independently of vitamin D action [19]. Xylitol may thus be able to maintain sufficient intestinal calcium absorption despite the apparent vitamin D resistance in arthritic rats.

In conclusion, the results of the present study suggest that, at the time of the appearance of arthritic symptoms, bone resorption activity is high, but bone formation is not severely affected. Furthermore, dietary xylitol seems to protect against the imbalance of bone metabolism during the early phase of collagen type II–induced arthritis in DA rats.

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