Rheumatoid arthritis (RA) is a chronic and systemic disorder characterized by synovial inflammation together with subsequent destruction and deformity of synovial joints including bone and cartilage, while RA has a prevalence of approximately 1% in the world population with a three times more incidence rate in women than in men. Although we have previously demonstrated the possible involvement of glutamatergic signaling machineries in the pathogenesis of RA (1), the underlying pathological mechanisms for this disabling disease are still not well understood to date (2).

Naturally occurring polyamines such as spermidine (SPD) and spermine (SPM) are known to be indispensable regulators of diverse cellular processes such as gene transcription and translation, in addition to modulating cellular growth, differentiation, and apoptosis (3). Several independent lines of evidence indicate the possible relationship between particular polyamines and pathophysiology in some disorders such as Alzheimer’s disease (4) and skeletal muscle hypertrophy (5). Recently, we have demonstrated that daily oral supplementation of both SPD and SPM prevents bone loss in ovariectomized mice in vivo as well as osteoclastic maturation induced by the mater regulator, receptor activator of nuclear factor-κB ligand (RANKL) in vitro (6). In the present study, therefore, we focused on pharmacological properties of those naturally occurring polyamines given to rats with type-II collagen–induced arthritis (CIA) as a model of RA in vivo.

The protocol employed here meets the guidelines of The Japanese Pharmacological Society and was approved by the Committee for Ethical Use of Experimental Animals at Kanazawa University (permit number: AP-101806). The experimental CIA model was generated by intradermally injecting 500 μL emulsion containing 500 μg of type-II collagen (BD Biosciences, Franklin Lakes, NJ, USA) and complete Freund’s adjuvant (Difco Laboratories, Detroit, MI, USA) into the root of the tail and the back of 8-week-old male Lewis rats under anesthesia (1). After 7 and 14 days, a second and a third immunization booster (100 μg of type-II collagen, each immunization) was administered under similar experimental protocols. Animals were daily given oral supplementation of either SPD or SPM (Sigma Chemicals, St. Louis, MO, USA) freshly dissolved in drinking water at a concentration of 3 mM for 28 consecutive days after the first immunization ad libitum. No significant changes were seen in the amount of daily intake of drinking water (approximately 35 mL per day), as well as spontaneous behaviors, in rats given each polyamine (data not shown). Four weeks after the first immunization, the knee joints

Short Communication

Amelioration by the Natural Polyamine Spermine of Cartilage and Bone Destruction in Rats With Collagen-Induced Arthritis

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Abstract. We investigated pharmacological properties of naturally occurring polyamines on cartilage and bone destruction seen in joints of rats with collagen-induced arthritis (CIA). Daily supplementation of spermine (SPM), but not spermidine, significantly inhibited increases in the hind paw volume and arthritis score in CIA rats, in addition to the increased mRNA expression of receptor activator of nuclear factor-κB ligand in both cartilage and synovial tissues. Histological analysis clearly revealed a drastic prevention by SPM of the cartilage and bone destruction in synovial joints of CIA rats. Particular natural polyamines would be beneficial for the prophylaxis of synovial joint destruction in rheumatoid arthritis.

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and the interphalangeal articulations of rat feet were excised for subsequent biochemical and histological analyses. The arthritis score counting was based on a scale of 0 to 4 using the criteria in a blinded manner (7).

Results are all expressed as the mean ± S.E.M. and the statistical significance was determined by the two-tailed and unpaired Student’s t-test or one-way analysis of variance ANOVA with the Bonferroni/Dunnett post hoc test.

Apparent swelling was seen in hind paws of CIA model rats 14 days after the first immunization, with a significant increase in the hind paw volume (Fig. 1A). A marked prevention was seen in the increased hind paw volume in CIA rats given SPM, but not SPD, when determined at 14 days after the first immunization. However, neither SPD nor SPM significantly prevented the sustained increase in hind paw volume in CIA rats when determined 21 to 28 days after the first immunization. No arthritis score was obtained in naïve animals irrespective of the administration of SPD or SPM, while the score was drastically increased from 14 to 28 days after the first immunization in CIA rats (Fig. 1B). In animals with daily oral administration of SPM, but not SPD, a significant prevention was found in the increased arthritis score in CIA rats when determined at 14 and 21 days. However, both SPD and SPM failed to significantly alleviate the increased score at 28 days in CIA rats.

mRNA was extracted from cartilage and synovial tissues of knee joints. A significant increase was seen in mRNA expression of tumor necrosis factor-α (TNF-α) and RANKL in both cartilage (Fig. 2A) and synovial tissues (Fig. 2B) isolated from CIA rats. Although both SPD and SPM failed to significantly prevent the increased TNF-α mRNA expression in both cartilage and synovial tissues, SPM was invariably effective in significantly inhibiting the increase in RANKL mRNA expression in both cartilage and synovial tissues of CIA rats. However, SPD prevented the increased RANKL mRNA expression in synovial tissues, without significantly affecting that in cartilage, of CIA rats.

Histological analysis was performed on sections dissected from the interphalangeal articulations of rat feet. Von Kossa staining clearly revealed quite severe bone and cartilage destruction in the metacarpophalangeal articulations isolated between metacarpal bone and first phalanx of the middle finger of CIA rat feet. The daily

![Fig. 1.](image-url) Oral supplementation of SPM inhibits CIA-induced increases in hind paw volume and arthritis score. Eight-week-old male Lewis rats were subjected to CIA, followed by daily oral supplementation of SPD or SPM at 3 mM and subsequent determination of hind paw volume (A) and arthritis score (B) once a week for 28 consecutive days. **P < 0.01, significantly different from each control value obtained in naïve rats. #P < 0.05, significantly different from the value obtained in CIA rats. The arthritis score was based on a scale of 0 to 4 using the following criteria: 0, normal; 1, mild, but definite redness and swelling of the ankle or wrist, or apparent redness and swelling limited to individual digits, regardless of the number of affected digits; 2, moderate redness and swelling of ankle and wrist; 3, severe redness and swelling of the entire paw, including digits; 4, maximally inflamed limb with involvement of multiple joints. U.D., under detection.
administration of SPM led to a drastic amelioration of the bone and cartilage destruction in the metacarpophalangeal articulations of the middle finger, with SPD being ineffective (Fig. 3A). Repetition of these histological evaluations with different animals for quantitative scoring clearly revealed the high effectiveness of SPM, but not SPD, in alleviating the bone and cartilage destruction in CIA rats (Fig. 3B).

The essential importance of the present findings is that daily oral supplementation of SPM significantly prevented the bone and cartilage destruction in CIA rats in vivo. Moreover, SPM markedly prevented the increased RANKL mRNA expression in both cartilage and synovial tissues of CIA rats. Although SPM is shown to inhibit the experimental inflammation in association with modulation of the production of pro-inflammatory cytokines, such as TNF-α, interleukin-1 (8), and lymphocyte function-associated antigen type 1 (9), this is the first direct demonstration of the amelioration by SPM of bone and cartilage destruction through a mechanism relevant to the suppression of promoted expression of RANKL responsible for osteoclast differentiation and activation in cartilage and synovial tissues of knee joints in CIA rats. Both SPD and SPM markedly prevent the RANKL-induced osteoclastic differentiation in conjunction with the inhibition of phosphorylation and transcriptional activity of the transcription factor nuclear factor-κB in vitro (6). Taken together, SPM could ameliorate the bone and cartilage destruction of CIA rat joints in a manner related to RANKL expression in cartilage and synovial tissues, in addition to a direct action on osteoclasts. Rather large in vivo experimental variations would at least in part account for the apparent differential pharmacological profiles between SPD and SPM in inhibiting increased mRNA expression of TNF-α and RANKL in joint cartilage and synovial tissues of CIA rats. Our previous findings that SPM is more effective than SPD in inhibiting osteoclastic differentiation mediated by RANKL (6), argue in favor of an idea that overall amelioration would involve the repression of osteoclastogenesis mediated by RANKL, in addition to downregulation of RANKL expression in cartilage and synovial tissues, in RA.
The reason why SPD failed to significantly ameliorate the increased volume and arthritis score in CIA rats in spite of the significant inhibition of RANKL mRNA expression in synovial tissues is not clarified so far. One possible speculation is that inflammatory processes would be responsible for both swelling and arthritis in hind paws along with bone and cartilage destruction mediated by overactivation of osteoclastogenesis in knee joints during CIA immunization. The fact that both SPD and SPM failed to significantly ameliorate upregulation of the inflammatory cytokine TNF-\(\alpha\) in both cartilage and synovial tissues in CIA rats gives support to this speculation.

Soybean and various types of soy products have long been consumed as daily foods in Japan, while soybeans have the highest amount of SPD (1430 nmol/g) and SPM (340 nmol/g) amongst different natural foods (10). A long-term intake of polyamine-rich foods is shown to gradually increase blood polyamine levels in humans and animals (11). By taking into consideration the average daily intake (35 mL per day) of drinking water containing 3 mM SPM, rats are supposed to be daily given 100 \(\mu\)mol of SPM for 28 consecutive days under our experimental protocols. Accordingly, the dose of SPM used here seems not to be excessive from a viewpoint of the pharmacological relevance to daily consumption of soybeans. The anti-TNF antibody administration reduces the inflammation in CIA animals (12), whereas cathepsin K inhibitor ameliorates the inflammation and bone erosion in experimental arthritis (13). On the basis of the present findings along with the protection by soy protein against RA (14), we propose that the appropriate dietary consumption of soybean and soy products would be beneficial for the maintenance of synovial joint health toward the prophylaxis of RA through a mechanism relevant to the amelioration by particular polyamines of

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**Fig. 3.** Oral supplementation of SPM inhibits CIA-induced bone and cartilage destruction. Eight-week-old male Lewis rats were subjected to CIA, followed by daily oral supplementation of SPD or SPM at 3 mM for 28 consecutive days. Rats were decapitated for histological analysis of the interphalangeal articulations of their feet by Von Kossa staining. Typical micrographic pictures are shown in the left panel (A), while quantitative destruction scoring data are shown in the right panel (B). *P < 0.05, significantly different from the value obtained in CIA rats. The destruction score of synovial joints was based on a scale of 0 to 4 using the following criteria: 0, no erosion of cartilage or bone; 1, unequivocal erosion less than 10% of cartilage or bone cross-sections; 2, erosion of less than 50%; 3, erosion of 50% – 90%; and 4, erosion of more than 90% of cartilage and bone cross-sections. U.D., under detection.
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excessively activated osteoclastogenesis.

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References


