Oxidative Stress is more Associated with the Pathogenesis of Rheumatoid Arthritis than Osteoarthritis and Pseudogout.

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Introduction
Increased reactive oxygen (ROS) and nitrogen species (RNS) production has been suggested in the pathogenesis of rheumatoid arthritis (RA) and osteoarthritis (OA) (1). ROS have been implicated as mediators of tissue damage in patients with RA (2). In addition, in OA pathogenesis, chondrocyte senescence plays an important role and cause cartilage degeneration (3). However, because of the highly reactive nature of these oxygen reactive species and their short half-lives, it has been difficult to investigate oxidative damage in vivo. Recently d-reactive oxygen metabolites (d-ROM) test has been widely used to measure reactive oxygen metabolites (ROMs) very easily.

In previous meeting, we presented that oxidative stress is highly upregulated in sera of RA patients and that biologics decreased it (4).

This time oxidative stress in synovial fluids in RA patients was compared with other arthritis such as OA and pseudogout. In addition, nitrotyrosine, a reaction product of ROS and nitric oxide (NO), has been used as evidence of oxidative damage in several aging tissues, OA cartilage, and RA inflamed joints (1). It is reported that 3-nitrotyrosine level in plasma was much higher in models of RA such as adjuvant- and collagen- induced arthritis than in a model of OA such as sodium mononoacetate-induced arthritis (5). So, we also asked if the expression of nitrotyrosine is higher in synovium of RA than OA.

Materials and Methods
Synovial fluid (SF) samples were obtained from patients with RA (15 cases), psoriatic arthritis (PsA) (2 cases), OA (28 cases), and pseudogout (4 cases) with hyaluronic acid in knee joints. Of 15 RA patients, 5 cases were treated with biologics (etanercpt;3, tociluzumab;1, abatacept;1). All PsA patients were also treated with biologics (infliximab;1, adalimumab;1). The samples were collected from our facility and our affiliated hospitals. All the RA, OA, PsA, and pseudogout patients fulfilled each diagnostic criterion. The protocol for obtaining synovial fluids was approved by each institutional ethics review board, and joint aspiration and the following injection of hyaluronic acid were performed only when medically necessary. Each SF sample was subjected to d-ROM test by utilizing Free Radical Elective Evaluator (F.R.E.E.) (Diacon, Italy). d-ROM values in each group were analyzed statistically (Mann-Whitney U-test or Student t-test) and simple regression analysis was performed between d-ROM value and age or CRP. Each group was further subdivided by using Kellgren-Lawrence classification for OA patients and Larsen grade classification for RA patients. Then the associations between d-ROM value and their radiological disease stages were analyzed. Significant difference was determined less than p<0.05.

To investigate the contribution of RNS in pathogenesis of arthritis, synovial tissue samples were obtained when they were harvested on total knee replacement surgery. Informed consent had been performed before surgery. These specimens were fixed with 10% formalin, and then paraffin section was prepared. Immunohistochemical staining of anti-nitrotyrosine antibody (Millipore, Temecula, CA, USA) was performed as manufacture’s protocol.

Results
The mean d-ROM value was 153 U.CARR (26 to 369 U.CARR in range). There was no significant difference between d-ROM value and age and CRP. No significant difference in d-ROM value was detected between male (130 U.CARR) and female (158 U.CARR) (Student t-test, p=0.25). The value in RA patients (209 U.CARR) was significantly higher than those in OA (124 U.CARR) (Mann-Whitney U-test, p<0.01) and pseudogout patients (121 U.CARR) (Mann-Whitney U-test, p<0.05) (Figure 1). On the other hand, there was no significant difference between OA and pseudogout (Student t-test, p=0.88). The average d-ROM value in PsA patients was 134 U.CARR. In RA patients treated with biologics, d-ROM value (110 U.CARR) was significantly lower than RA patients treated without biologics (264 U.CARR) (Mann-Whitney U-test, p<0.001).

In OA patients, d-ROM values of K-L classification were 125 (K-L I), 119 (K-L II), 134 (K-L III), and 123 (K-L IV) U.CARR, respectively. In RA patients, d-ROM values of Larsen grade classification were 222 (Larsen II), 216 (Larsen III), and 201 (Larsen IV), respectively. There was no significant difference between K-L classification, either between Larsen grade.

Immunohistochemical analysis of nitrotyrosine demonstrated that abundant nitrotyrosine-positive cells were observed in RA synovium, but only a few nitrotyrosine-positive cells were observed in OA synovium (Figure 2).

Discussion
Our data suggest that oxidative stress is measurable in synovial fluids. Neither correlation between d-ROM value and sex nor age suggests that d-ROM in synovial fluid reflects the degree of oxidative stress in each hyalarthrosis independently of sex and age. However, d-ROM was neither significantly associated with CRP level nor their radiological disease stages.

Significant increased level of d-ROM value in RA patients compared with those in OA and pseudogout and high expression of nitrotyrosine in one RA patient indicate that oxidative stress is more associated with the pathogenesis of RA than OA and pseudogout. In RA patients, the finding that biologics decreased d-ROM value compared with RA patients treated without biologics was in agreement with our previous data in sera (4). Further examinations are required to clarify the detailed mechanism.

Significance
Significant increased level of d-ROM value in RA patients compared with those in OA and pseudogout and high expression of nitrotyrosine in one RA patient indicate that oxidative stress is more associated with the pathogenesis of RA than OA and pseudogout.

Figure 1 d-ROM value in each arthritis group.

Figure 2 Immunohistochemistry of nitrotyrosine in RA (A) and OA synovium (B). Original magnification; x 200.

References