

Superoxide Dismutase Activity in Arthropathy: Its Role and Measurement in the Joints

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ABSTRACT

The electron spin resonance (ESR) method was used to measure the superoxide dismutase (SOD) activity in synovial fluids from the knee joints of 73 patients with rheumatoid arthritis (RA), and the results were compared with those of 50 patients with osteoarthritis (OA) and posttraumatic arthritis (PA). The SOD activity in RA and OA knee joint fluids was higher than in the control patients with PA. Patients with moderate RA (grade III or IV according to Larsen's classification of rheumatoid knee radiographs) showed higher SOD activities in joint fluids than patients with early (grade I or II) and terminal (grade V) stages of RA. Our results suggest that the SOD activity in joint fluids is a valid index of articular destruction and repair.

Key words: *Electron spin resonance (ESR), Superoxide dismutase (SOD), Rheumatoid arthritis(RA), Osteoarthritis(OA)*

The inflammatory synovium in rheumatoid arthritis (RA) infiltrated with chronic inflammation releases reactive oxygen species (ROS), which appear to be involved in joint destruction. Although various aspects of the role of ROS have been investigated in patients with RA and osteoarthritis (OA) by several methods, the results obtained have not always been consistent.

In general, ROS consists of four kinds of oxygen; superoxide radicals (O_2^-), hydroxyl radicals ($\cdot OH$), singlet oxygen (1O_2), and hydrogen peroxide (H_2O_2). O_2^- especially has been thoroughly investigated. It has also been suggested that ROS might be responsible for joint destruction in RA. In particular, attention has been devoted to the relation between O_2^- and superoxide dismutase (SOD), which dismutates O_2^- .

The content of ROS and SOD activity is measured by various methods. Although their relation to various pathological conditions has been reported, the results are not always consistent and their roles have not yet been completely defined. Patients with RA show significantly higher

SOD activity in the synovial fluid than those with OA¹¹⁾. However there are contradictory results indicating that SOD activity in the synovial fluid is higher in OA patients than in RA patients²⁾. The Cu,Zn-SOD levels in RA knee joint fluid are higher when compared with those in patients with posttraumatic arthritis, but extracellular SOD activity is lower in RA patients¹⁶⁾.

The conventional procedure to measure SOD activity is commonly employed as follows. When O_2^- is reacted with SOD, another reduced substance is also indirectly measured by colorimetry^{2,10,16)}. Recently, the development of a trapping agent has made it possible to detect the radical reaction of ROS as a relative stable spin adduct by the electron spin resonance (ESR) spectrometer¹²⁾. ESR measurement of SOD activity determines the amount of O_2^- as a substrate more precisely and selectively. The determination of SOD activity by the ESR method can be more reliable⁵⁻⁸⁾. In the present study, SOD activity in RA knee joint fluid and synovial tissues was measured according to this ESR technique. The clini-

cal implications concerning SOD and the relation between SOD activity and articular destruction were investigated.

MATERIALS AND METHODS

The synovial fluids were collected from 76 rheumatoid knees in 73 RA patients (9 knees of 9 males, and 67 knees of 64 females) after informed consent. RA was diagnosed according to the criteria of the American Rheumatism Association (ARA, 1987)¹⁾ and classified according to the Steinbrocker's classification¹⁸⁾. The mean age of the patients was 63.1 years (range; 33–84 years). Clinical evaluation was carried out using the criteria for the therapeutic response of RA knees scored by the Japanese Orthopaedic Association (JOA). For radiological observation, the antero-posterior view of the knee joint was evaluated according to Larsen's classification¹⁵⁾.

The osteoarthritic joint fluids were obtained from 50 knees of 47 OA patients with a mean age of 69 years (range; 38–96 years), and from 11 knees of 11 posttraumatic arthritis (PA) patients as a control. For the synovial tissue controls, 5 knees of 4 OA cases and 2 knees of 2 knee amputees were used. Serum samples from 12 patients with RA, 4 with OA, and 24 normal were measured.

After aspiration from the knee joints, the synovial fluids were centrifuged at 3,000 rpm for 10 min., and the supernatants were stored in a freezer at -80°C until used. The serum samples were similarly stored in a freezer. The synovial fluid and serum were returned gradually to room temperature and assayed as follows. SOD activity was determined according to the ESR method⁸⁾. In brief, 50 μl of 2 mM hypoxanthine (HX) (Sigma Co.), 35 μl of 11 mM diethylenetriaminepentaacetic acid (Sigma Co.), 50 μl of the sample, and 15 μl of 5,5-dimethyl-1-pyrroline-1-oxide (DMPO) (Daiichi Pure Chemicals Co., Ltd.) as a trapping agent were mixed into a test tube by an automatic mixer, and 50 μl of xanthine oxidase (XOD) (Boehringer Mannheim Co.) was added. After stirring, the reaction mixture was transferred into a special flat cell (169 μl , JEOL Ltd.), and analyzed for DMPO- O_2^- spin adducts by an ESR spectrometer (JES-FE1XG, Nihon Electron Co.). The calibration curve was constructed by the use of 0.78–25 units/ml of standard SOD. Magnetic oxide was checked as an internal standard. Spin numbers were calculated as a ratio of the signal height intensity of 2,2,6,6-tetramethyl-4-hydropiperidine with a known spin quantity.

RESULTS

The SOD activity in the synovial fluid was 6.93 ± 4.24 U/ml in the RA patients (76 knees in 73 patients) and 7.03 ± 3.77 U/ml in the OA pa-

tients (50 knees in 47 patients). There was no significant difference between the RA and OA groups, but there was a significant difference between these groups and the PA group ($p < 0.01$) (Fig. 1). The values of the SOD activity for the RA and OA groups were higher than that for the PA group, in which the SOD activity of the synovial fluid was 4.20 ± 1.99 U/ml. The SOD activity of the synovial fluid of the RA or OA patients was also higher than that in the serum from the RA patients (4.69 ± 1.81), OA patients (3.56 ± 1.43), and normal serum control (4.43 ± 1.79) (Fig. 1).

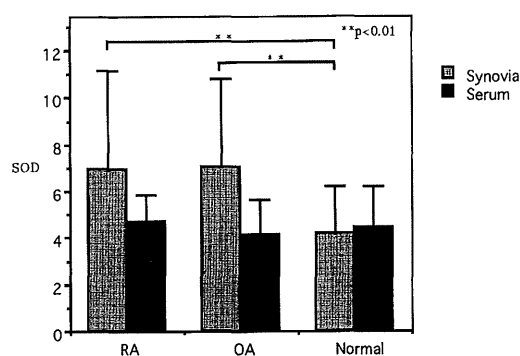
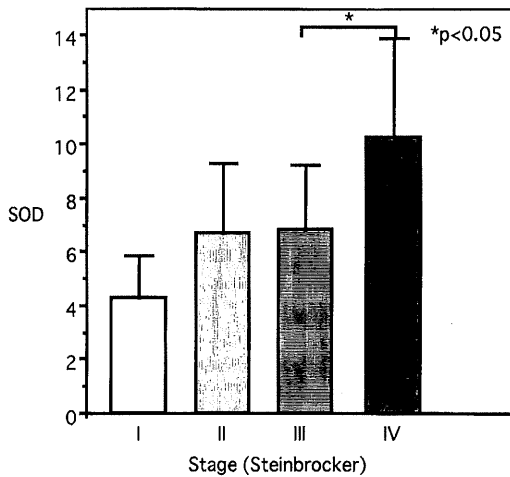


Fig. 1. SOD activity in RA synovial fluid and synovial tissues obtained from RA patients during surgery.

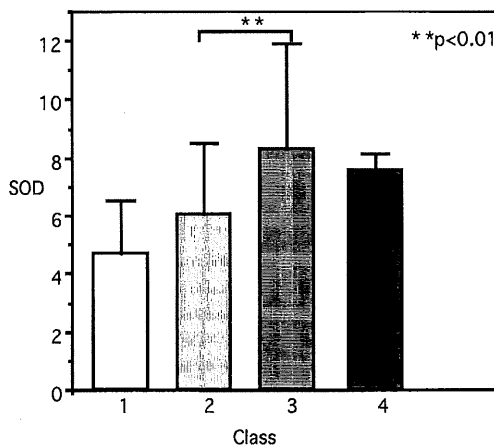
Using Steinbrocker's classification, the SOD activity in the synovial fluid of stage III and IV RA patients was 6.11 ± 3.34 U/ml (stage III) and 10.73 ± 5.10 (stage IV) U/ml, respectively, indicating a relatively t-statistical difference ($p < 0.05$). Moreover, considerable differences were also observed between Class 2 and Class 3 in the SOD activity of the RA synovial fluid (6.06 ± 2.11 U/ml (Class 2) and 8.86 ± 4.52 U/ml (Class 3)) (Fig. 2). With Larsen's classification for knee X-ray radiographic changes, the SOD level in the synovial fluid was seen to be highest in grade IV RA patients, while the patients with early RA (grade I or II) and terminal RA (grade V) tended to have lower levels of SOD activity (Fig. 3). When the relation between SOD activity and the JOA clinical score was examined, the highest SOD activity was seen in the score range of 40 to 59 (Fig. 4). As with RA, SOD activity was highest in the middle stages of OA and tended to be lower in the patients with early and terminal OA stages.

The synovial fluid SOD activity in RA patients with a past medical history of steroid injection was 6.92 ± 3.90 . No correlation between synovial fluid SOD activity and a past medical history of

steroid injection or calcification in OA could be detected by the ESR method.



(A)



(B)

Fig. 2. The relationship between Steinbrocker's classification stage (A), class (B) and SOD activity in RA synovial fluids

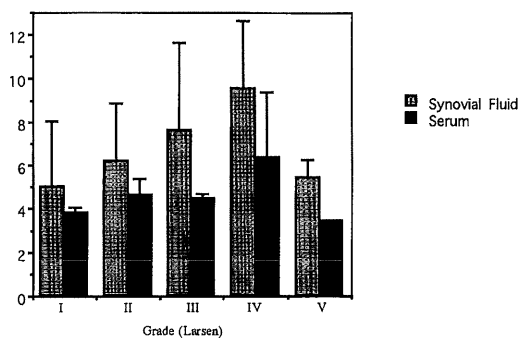


Fig. 3. The relationship between the Larsen's grades and SOD activity in the synovial fluid or serum from patients with RA.

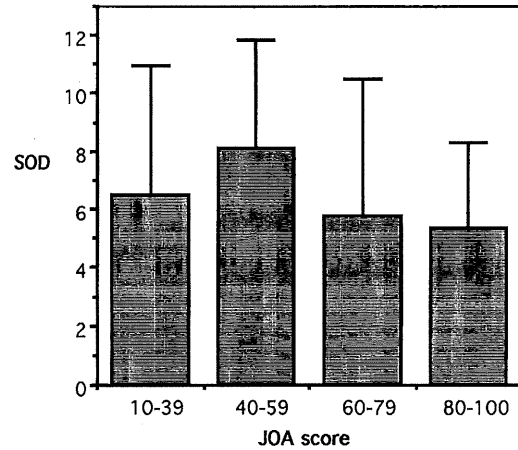


Fig. 4. The evaluation of RA knee function according to the score of JOA and SOD activity of RA knees, and the SOD activity of RA synovial fluid.

DISCUSSION

It has been reported that various factors are responsible for osteocartilaginous destruction and inflammation in the joints^{19,20}. Direct cartilaginous destruction can be caused by hyperplasia of the pannus, or collagenase and lysosome produced by the synovial membrane as synovitis progresses²¹. Leukocytes and synovial lining cells capture immune complexes in the synovial fluid and are stimulated by interleukin-1 or other cytokines. Reactive oxygen species (ROS) are released during the phagocytosis of foreign bodies. When phagocytosis is excessively stimulated, the production and secretion of ROS increases synchronously, resulting in a variety of deleterious effects on the local tissues¹³. Then leukocytes and synovial lining cells release ROS, prostaglandin E₂, proteases, arachidonic acid metabolites, causing inflammation and the destruction of joint tissues.

ROS bring about the degradation of hyaluronic acid in the joint tissue, resulting in the reduced viscosity of synovial fluid. The superoxide radical (O₂⁻) has a harmful effect on tissues when overproduced *in vivo*. The superoxide radical, which is decomposed into water and H₂O₂ by SOD, is eliminated rapidly *in vivo*. This protection is accelerated 10⁴ times in the presence of SOD. Thereafter, the H₂O₂, generated from the superoxide radical by SOD, is quickly changed to oxygen and water without toxicity by catalase or glutathione peroxidase around the tissue. SOD was discovered by McCord and Fridorich⁴, who found Cu-Zn-SOD, Fe-SOD, and Mn-SOD present in mankind and Cu, Zn-SOD (Mr 32,200) and Mn-SOD (Mr 40,000) present in cytoplasm and in mitochondria, respectively.

SOD activity in RA joint fluid has been measured, and the roles of ROS and SOD in RA arthritis has been suggested^{11,16}. However, there

are no definitive views about the role of SOD in the synovial fluid. Most of the previous measurements of SOD activity involved the detection of other reaction substances, such as the cytochrome C method, the nitro blue tetrazolium (NBT) method, the epinephrine method, the pyrogallol method and the ascorbic acid method. The compounds in all these methods were reduced by O_2^- and were detected by the colorimetry method^{2,11,16}. The enzyme immunoassay (EIA) and radioimmunoassay (RIA) have been adapted to determine the amount of SOD.

The radical reactions involving ROS occur so rapidly that it was difficult to detect them directly and selectively. However, better radical trapping agents have enabled the detection of radical reactions with the use of ESR, which converts ROS into stable spin adducts¹². This ESR method for measuring SOD activity makes it possible to determine the amount of O_2^- more accurately and selectively⁵⁻⁸, and to determine SOD activity more reliably.

Using conventional indirect methods, it was reported that RA patients showed a four-fold increase in the SOD activity of synovial fluid when compared with OA patients¹¹, while others, using the same methods, reported that SOD activity was lower in the RA synovial fluid². In the present ESR study, the joint fluids in RA patients in Steinbrocker's stage IV showed significantly higher SOD activity levels than lower stages according to the joint destruction seen on X-ray. The joint fluid in RA patients of class 2 or 3 on activities of daily living tests similarly showed significantly increased SOD activity. The joint fluids in RA patients with grade IV in Larsen's classification showed the highest SOD activity, while those with early (grade I or II) and terminal RA (grade V) showed the lower SOD activity. This tendency in RA was similar to that in OA. The patients with moderate OA showed a higher SOD activity compared with the early and terminal OA patients. SOD activity in the joints may play a role in the prevention of articular destruction from ROS, and in promoting the repair process. Therefore, the SOD activity of RA synovial fluid determined by ESR can be assessed as an index of articular destruction and repair.

The leukocytes in the synovial fluid contained high levels of SOD activity. We suggest that, along with the increase of leukocytes in inflammatory lesions, the activity of inflammatory cells is enhanced, accelerating the production of ROS and SOD activity in synovial fluid, and that the SOD activity of synovial fluid reflects the amount of SOD in the joint tissue. When the articular destruction is progressing from moderate to more severe stages, SOD activity increases in the synovial fluids. At the severe stage, the SOD activity could be rapidly consumed due to the massive

damage in the joints, thus accelerating destruction. SOD activity may have an important role in the prevention of destruction and in repair in the joints in RA and OA.

SOD may prevent the overproduction of ROS and joint destruction as an antioxidant. A recent attempt to inject SOD into joints was intended to prevent the spread of inflammation¹⁴. The overdestruction of joint tissue may reduce SOD activity. In the present study, patients with moderate RA (grade III or IV on Larsen's classification¹⁵) showed a higher SOD activity than patients with early (grade I or II) and terminal RA (grade V). Our results suggest that the SOD activity examined by ESR in RA knee synovial fluid is a useful index of articular destruction and repair and that SOD prevents osteocartilaginous destruction in the joints by ROS.

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