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STAPHYLOCOCCAL ARTHRITIS – EFFECTS OF SUPEROXIDE DISMUTASE ON INFECTED KNEE JOINTS OF RABBITS

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The effect of SOD on staphylococcal arthritis has not been successfully evaluated to date. We developed an animal model to investigate the correlation. Using 16 rabbits divided into four groups, we injected two groups with staphylococcus aureus and the other two with NaCl. One group in each was also injected with SOD.

The presence of SOD activity in untreated and infected knee joints of rabbits over a period of 72 hours showed no significant difference.

TBA-reactive substances (TBARS) measured in joint fluid and plasma did differ in each of the groups, with the highest values found in animals with septic arthritis treated with SOD. This finding corresponded especially with the histological investigation. Joints of infected animals intra-articularly injected with SOD also showed histologically significantly more inflammation, a higher amount of bacteria in the joint cavity, and more distinct joint damage than joints injected only with bacteria.

The mechanisms responsible for this SOD effect remain to be determined.

KEY WORDS: Bacterial arthritis, superoxide dismutase, TBA-reactive substances, joint damage.

INTRODUCTION

Bacterial joint infection remains an unsolved problem despite aggressive surgical intervention and appropriate antibiotic treatment. The infection is devastatingly destructive to all articular structures such as cartilage, synovial membrane, and bone. This is especially true of septic arthritis caused by staphylococcus aureus.[1,2]

The role of oxygen radicals in causing inflammatory disease has received much attention in recent years.[3-8] As early as 1924, Phemister concluded that the deterioration of cartilage from bacterial joint infection was caused by polymorphonuclear leukocytes rather than by bacteria.[9] To our knowledge nothing has been published to date indicating a correlation between reactive oxygen species (ROS) and joint damage by septic arthritis. Superoxide dismutase (SOD) is assumed to protect cells against the attack of ROS produced by inflammatory cells.[5,6,10-13] Extracellular fluids appear to be less protected against oxygen damage.[6,10]

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We have developed an animal model to investigate the effect of SOD on staphylococcal arthritis in rabbits. Using a standardized procedure, our model permits repeated aspiration of joints, taking small amounts of fluid from them.[14]

Our study also estimated the presence of activity of intra-articularly injected SOD and measured the TBA-reactive substances (TBARS) in joint fluid and plasma of infected and control animals.

MATERIALS AND METHODS

Experimental protocol

For this experiment sixteen rabbits were used, four groups of four animals. They were anaesthetised using a Neurolept-Ketamin analgesia. The right knee joint of each rabbit was injected with 2 ml NaCl 0.9% (group I), or 1 ml NaCl 0.9% and 1 ml SOD, 120 U (group II), or 1 ml staph. aureus solution and 1 ml NaCl 0.9% (group III), or 1 ml staphylococcus aureus solution and 1 ml SOD, 120 U (group IV). The joint fluid was then aspirated 12, 24, 36, 48, 60, 72 hours after this first inoculation, 1 ml NaCl was subsequently injected into each joint and 0.2–0.3 ml fluid was immediately aspirated after distribution of it by manipulating the joint. Finally, 2 ml NaCl 0.9% (groups I and III) and 1.5 ml NaCl 0.9% and 0.5 ml SOD, 60 U (group II and IV) were injected into the joint. Blood samples were taken using the ear veins for venous access.

After 72 hours the animals were sacrificed and the joints were freed of soft tissues and prepared for histological examination in a routine manner.

Infecting inoculum

The infecting inoculum was a coagulase-positive staphylococcus aureus of the type ATCC 6538. Colony counts were performed on overnight cultures in casein-peptone solution agar (CLS). The bacterial concentration in this culture was found to be 1.4–2.7 × 10 9 bacteria/ml.

Three samples of staph. aureus of the type ATCC 6538 (1.12 × 10 7 organisms/ml) in CLS with SOD (4 mg/ml) were incubated and were compared with three other samples of the same type of organisms (1.24 × 10 7 organisms/ml) cultivated in a similar agar with an addition of 1 ml of NaCl and incubated for the same length of time at 37 degrees. Colony counts were performed before and after incubation.

Biochemical methods

The method according to Fridovich was used to estimate the presence of SOD activity in joints.[15] This method is an indirect test for the activity of SOD with xanthine-xanthine oxidase serving as a source of superoxide anions. It is based on the competitive reaction between SOD and oxidized cytochrome C for the superoxide radical. To measure the amount of reduced cytochrome C photometrically it is necessary to dilute the sample. 1 Unit of SOD is defined as the amount of activity which inhibits 50% of the reduction of oxidized cytochrome C under the described conditions.[15]

The method of Yaqi *et al.* was used to estimate TBA-reactive substances (TBARS) in plasma and joint fluid.[16] In this test, thiobarbituric acid (TBA) reacts with TBARS. The reaction products are estimated fluorometrically based on a predetermined

standard. The aspirated joint fluid was treated in the same way as recommended for the plasma.[16]

All samples were centrifuged before assay of SOD and TBARS to avoid contributions from the phagocytes present.

Statistics

The values of TBARS measured in joint fluid and plasma differed widely from a normal distribution. Therefore, they are expressed by extreme and median values as shown in Tables II and III. To test the single effects of treatment and of time

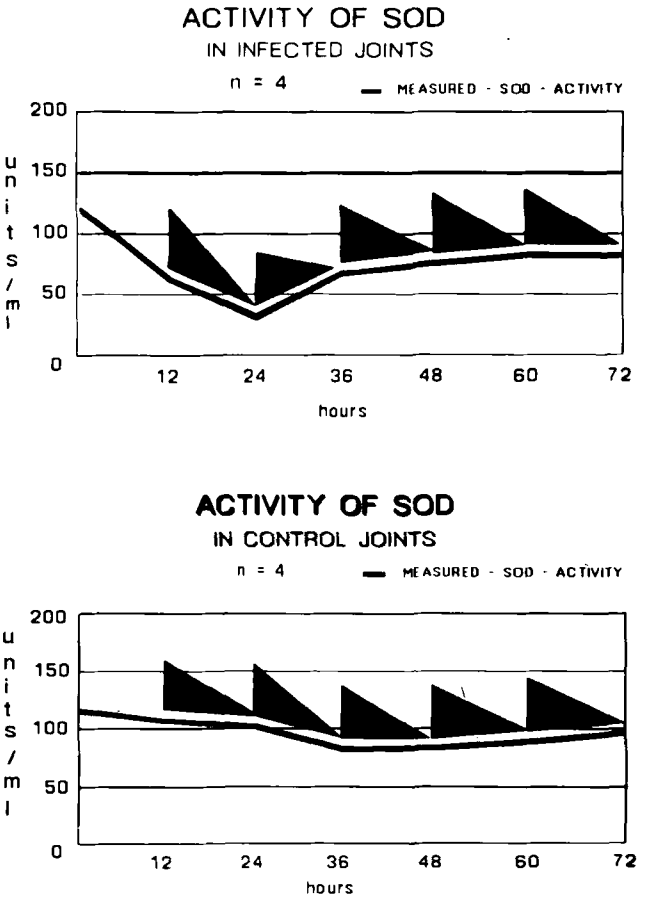

FIGURE 1a) and 1b) Activity of SOD in infected and control joints. 120 units SOD were injected at time 0. After 12 hours, 1 ml NaCl was injected and small volumes of joint fluid (0.2–0.3 ml) aspirated to measure intra-articular SOD activity. Thereafter, 60 units were again injected. The same procedure was repeated at intervals of 12 hours over a period of 72 hours. The expected values were calculated with the assumption that the amount of fluid added to the joint prior to the aspiration was negligible. The triangles point out the injected SOD activity added to the measured SOD activity as shown by the baseline and the decrease of activity in 12 hours.

independence the Kruskal-Wallis-H-test was used. In spite of the divergences from normal distribution, the simultaneous influence of group effects (treatment) and of time effects was tested with a two-way variance analysis and was also examined using a nonparametric median-polishing procedure.[17] This procedure was used to check the possible influence of extreme values on the results.

RESULTS

The presence of injected SOD activity in untreated and in infected joints showed only slight differences as shown in Figures 1a and 1b. Under both conditions at least 50% of the injected SOD activity was found in the joint fluid 12 hours after injection (Table I). No effect of SOD *in vitro* on its efficiacy for growth and formation of colonies of staphylococcus aureus was observed.

TBARS estimated in the joint fluid displayed extreme variability. However, TBARS levels in animals of group IV were found to be higher than those in animals of other groups (Table II).

TBARS levels in plasma were different in each of the groups. In general, they were lower in noninfected animals (I and II). The lowest values were found in animals

TABLE I
SOD units/ml Synovial fluid

SOD treatment	Group II	Group IV
h	$\bar{x} \pm$ SE	$\bar{x} \pm$ SE
12	110 ± 14.7	62 ± 27.6
24	106 ± 8.2	30 ± 13.6
36	82 ± 17.1	67 ± 26.7
48	86 ± 21.5	75 ± 23.2
60	92 ± 39.0	75 ± 16.5
72	100 ± 32.3	82 ± 21.7

TABLE II
TBARS nmoles/ml joint fluid (n = 12)

		12–36 H	48–72 H
I	min.	0.000	0.000
	med.	0.000	4.330
	max.	0.120	28.300
II	min.	0.750	0.100
	med.	4.550	3.300
	max.	25.250	9.360
III	min.	0.790	0.000
	med.	4.760	6.780
	max.	23.670	37.210
IV	min.	1.380	3.260
	med.	6.990	13.120
	max.	32.270	41.640

TABLE III
TBARS nmoles/ml plasma (n = 8)

		0–6 H	12–24 H	36–48 H	60–72 H
	min.	0.000	0.000	1.290	1.830
I	med.	0.000	3.250	1.500	8.540
	max.	2.250	8.220	11.450	12.100
	min.	0.000	1.740	2.500	0.000
II	med.	0.000	2.000	3.120	0.000
	max.	3.270	4.200	7.680	6.700
	min.	0.000	2.170	4.550	1.420
III	med.	0.000	3.900	10.480	15.660
	max.	5.150	8.640	17.820	21.170
	min.	0.420	0.000	4.770	7.140
IV	med.	1.520	3.900	10.270	15.330
	max.	5.950	7.030	21.600	36.710

treated with SOD only (II; Table III). On the other hand, the TBARS values in plasma were found to be highest in infected animals treated with SOD (IV; Table III).

Histological investigation of the joints revealed increased quantities of bacteria, more pronounced inflammation, and more extensive joint damage in animals of group IV compared with those of group III (Figures 2 and 3).

Statistics

TBARS levels in plasma differed not only between groups but also between time intervals. Above all, the time differences are evident between groups III and IV ($p < 0.01$). The most distinct differences between the groups were found at 48 hours. Differences are even more pronounced when two time intervals are pooled together (Table III).

TBARS measured from joint fluid showed differences only between groups but not between time intervals. Therefore, the values of several time intervals were pooled together. By adding together the intervals of 12–36 and 48–72 the differences in the groups are even more distinct. The time differences are only small ($p < 10\%$). There is a significant difference between group IV and the other groups at 48–72 hours ($p < 0.05$) and between group I and the other groups at 12–36 hours ($p < 0.01$; Table II).

DISCUSSION

SOD remains the only documented example of a clinically used "antioxidant enzyme".[6] SOD therapy is based on the belief that free radicals are pathophysiologically important.[6] Although SOD is physiologically present in high concentration in cells, it is generally absent or present only in very low concentration in the extracellular space apparently providing little protection against damage caused by $O_2^{\cdot -}$.[6,10] When SOD is injected it can therefore be assumed that it is distributed in the

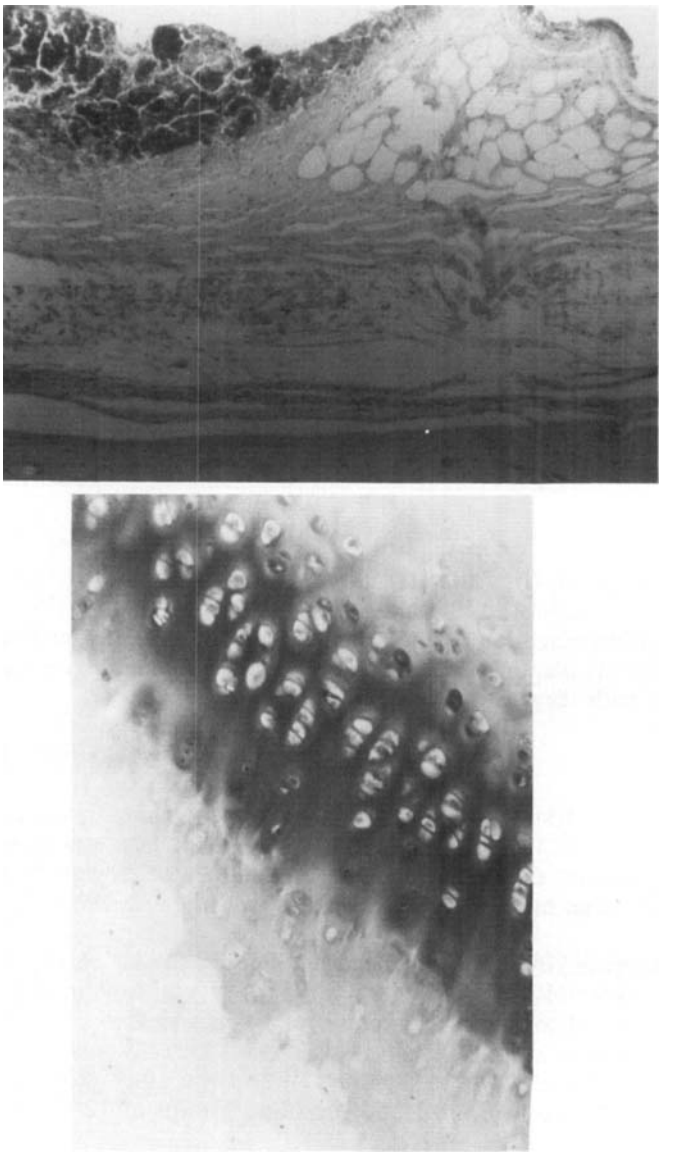

FIGURE 2 Group III (infected). a) Synovial membrane: Loss of synovial cells – clusters of organisms on the surface with severe infiltration of leukocytes and abscess formation mainly in superficial layers. Haematoxylin & Eosin × 100; b) Cartilage: Mild degenerative changes and loss of metachromasia within the superficial third of cartilage. Toluidin blue × 100.

extracellular space and does not enter cells. As a consequence, injected SOD should only have beneficial effects in situations where an overproduction of superoxide ($O_2^{\cdot -}$) in the extracellular space induces pathological phenomena.[6] The conditions mentioned above are likely to occur in septic arthritis where there is a

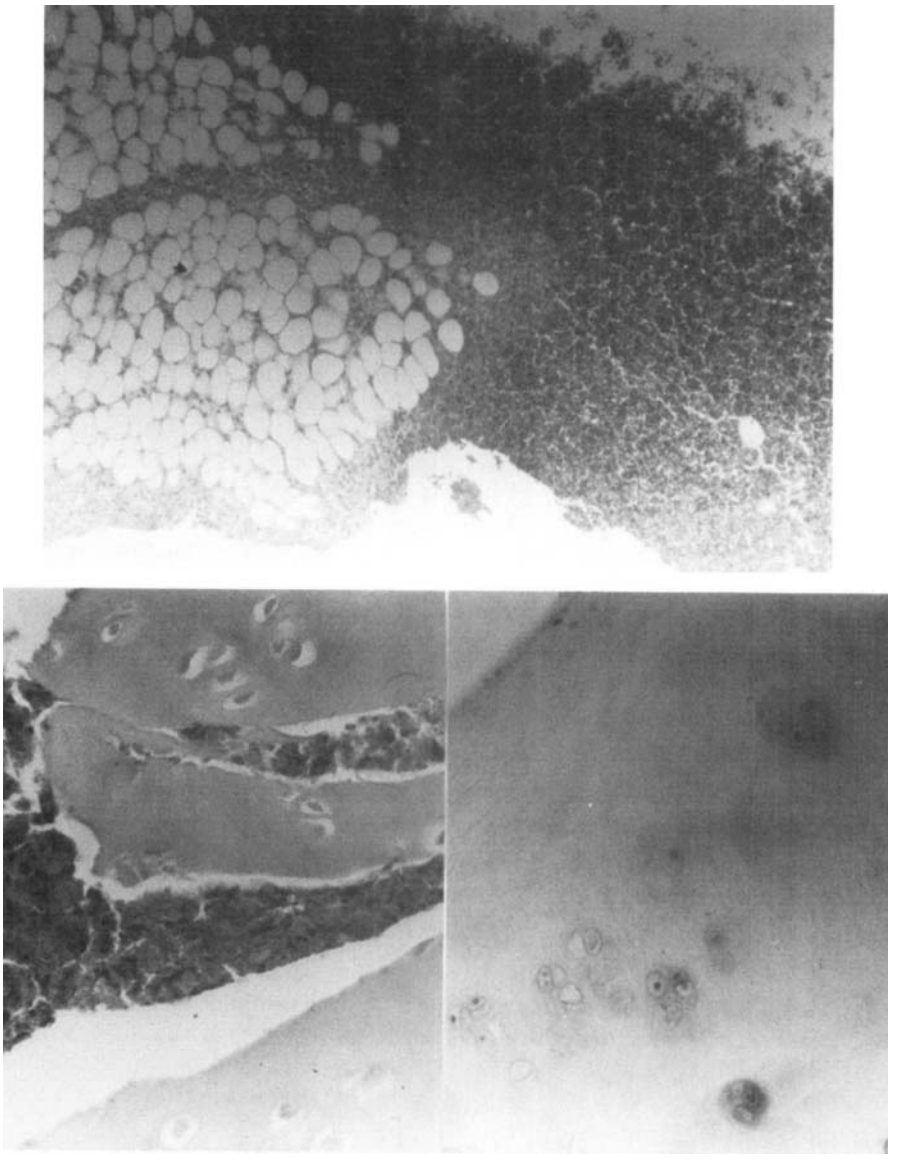

FIGURE 3 Group IV (infected and SOD). a) Synovial membrane: Extensive inflammation with numerous phlemonic and necrotic areas affecting all synovial and subsynovial layers. Haematoxylin & Eosin × 100; b) Cartilage: Multiple superficial erosions with masses of organisms within the cartilage. Distinct degenerative changes and loss of metachromasia affecting more than two-thirds of the cartilage. Haematoxylin & Eosin × 100.

1) high flux of superoxide radicals ($O_2^{\cdot -}$) in the joint cavity;

2) massive destruction of joint tissues due to reactive oxygen species;

3) low extracellular SOD concentration.

We used a model for bacterial joint inflammation to investigate the potential beneficial effects of SOD.

It can be assumed from our results that joint infection does not substantially influence intra-articular persistence of injected SOD activity (Figures 1a and 1b).

Control animals in groups I and II showed low TBARS concentrations in joint fluid and plasma in contrast to infected animals of group III and IV treated with bacteria. However, the TBARS levels did not demonstrate the effect of SOD we had expected to obtain. The lowest values were found to be in controls treated with SOD (II), the highest in infected animals treated with SOD (IV). The reasons why this group showed these high values in joint fluid and in plasma are not yet clear.

The high TBARS concentration in animals of group IV corresponded particularly with our histological investigation. The highest degree of joint destruction with enormous quantities of staphylococcus in joint of animals was found in group IV (Figure 3). Because of this histological finding, we investigated the effects *in vitro* of SOD on staphylococcus aureus cultures. This study clearly showed that SOD has no effect on the growth and colonies forming efficiency of staphylococcus aureus.

The reasons for this rather unexpected histologic finding showing increased bacterial growth and increased damage of joints inoculated with SOD may be that:

1) SOD prevents the killing of bacteria in septic arthritis by removing the superoxide radical ($O_2^{\cdot -}$),[18]

2) extensive joint destruction is caused by the action of bacteria or by ROS produced by inflammatory cells;[3,4,8,18]

3) there is increased hydrogen peroxide production from SOD activity;[19]

4) SOD induces increased oxygen tension, facilitating bacterial growth *in vivo*;

5) catalytic iron produced by the destruction of bacteria or by damaged tissues and microbleeding may impair the phagocytic activity of PML;[20]

6) mobilised iron may have become a strong pro-oxidant under our experimental conditions by injecting SOD.[4,7,8]

At the present time it is not possible to single out which mechanisms are responsible for this apparent, negative SOD effect. Nevertheless, in view of the intra-articular application of SOD,[16–18] it is possible that this finding is of clinical importance.

Acknowledgements

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