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FREE RADICALS AND SOD ACTIVITY OF JAW CYST. DIRECT MEASUREMENT AND SPIN TRAPPING STUDIES BY ESR

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Free radicals produced in the fluid of jaw cysts were directly measured at room temperature using ESR. With these samples, SOD activity of the cyst fluid was measured by the ESR spin trapping method with DMPO as a trapping agent. Freeze-dried samples of cyst fluid showed a broad ESR signal at $g = 2.005$. Relative signal intensity of samples from jaw cysts with inflammation was higher than jaw cysts without inflammation. SOD activity of cyst fluid with high viscosity showed higher values than that of cyst fluid with low viscosity. We suggest that free radicals produced in jaw cyst damage tissues while higher SOD activity of cyst fluid play a role in a self-defense mechanism against free radicals.

KEY WORDS: Jaw cyst, free radicals, SOD activity, DMPO, ESR spin trapping.

INTRODUCTION

Cysts of the jaws slowly expand within the maxilla or mandible. It has been previously described that the existence of a positive intracystic fluid pressure is the only explanation for cyst enlargement.[1] Recently, Suzuki[2] has identified marked changes in leukocyte number and lipidperoxide values in cyst fluid which is infected with bacteria. Therefore, we hypothesized that active oxygens and free radicals generated in jaw cyst damaged cyst wall tissues with bacterial infection. However, SOD as an elimination factor of active oxygen, is present and plays some role in various diseases and inflammation.[3] The present paper will report the free radicals and SOD activity of cyst fluid measured by ESR.

MATERIALS AND METHODS

Preparation of Samples

Samples for ESR measurement consisted of 18 fluids collected from jaw cysts (4 radicular, 1 residual, 3 dentigerous, 1 globulomaxillary, 2 incisive canal, 2 post-operative) of 13 patients. Fluid samples were immediately centrifuged at room temperature and a part of supernatant was frozen and stored at $-80\,^{\circ}C$ for free radical measurement. The remaining supernatant was sampled for SOD activity using the ESR spin trapping method.

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Free Radicals in Cyst Fluid by Direct ESR Measurement

To measure free radicals, freeze-dried samples were prepared and transferred into cylindrical quartz sample tubes with approximately 10–20 mg of supernatant and placed in the cavity of the ESR. A JEOL JES-FR80 was used as the ESR spectrometer. The measurement conditions were Microwave power 1 mW, center field 336 ± 5 mT, modulation width 0.63 mT, amplitude 2.5 × 100, time constant 0.3 sec, sweep time 2 min. The peak height ESR signal was calculated as the comparative value (H_S/H_M) to the peak height of standard sample (Mn^{2+}), which was expressed as relative ESR signal intensity (RSI) (Figure 1). The value was divided RSI × 10 by sample weight (mg) and expressed as units signal intensity (USI).

SOD Activity of Cyst Fluid by ESR Spin Trapping

The SOD activity was measured by ESR spin trapping method utilizing 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) as a spin trapping agent. The measurement is based on the theory of detecting superoxide anion (O_2^-) as DMPO spin adducts. O_2^- radicals were generated by the reaction of Hypoxanthine (HX) and Xanthine oxidase (XOD). Reagents were purchased from: (1) HX (Sigma), (2) Diethylene-triamine-pentacetic acid (DETAPAC; Wako), (3) Superoxide dismutase (SOD, from bovine erythrocyte; Sigma), (4) DMPO (labotec k.k.), (5) XOD (from butter milk; Boehringer Mannheim). The reagents were resolved in phosphate buffer (pH 7.8) and dispensed into test-tubes in the above order. Instantly after adding XOD, a reaction mixture was moved to a quartz ESR flat cell (maximum capacity 160 μl). Exactly 1 minute after XOD addition, ESR measurement was started at room temperature using JEOL JES-FR80. The signal intensity at the beginning of DMPO-OOH spin adducts is calculated by comparing the (I_S/I_M) value to the signal intensity of standard sample (Mn^{2+}) (Figure 2). A standard curve was made from the comparative value (I_S/I_M) decrease in spin adducts of DMPO-OOH when SOD solution of 0–50 units/ml existed in this reaction mixture. SOD activity of each sample was determined by both this standard curve and the comparative value (I_S/I_M), when adding cyst fluid instead of SOD. Measurement conditions were the following: Microwave power 8 mW, center

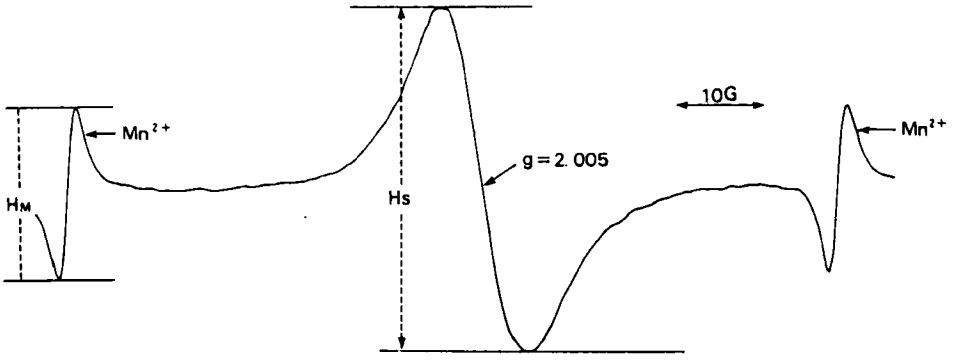

FIGURE 1 ESR spectrum of a freeze-dried sample. A value (H_S/H_M) is expressed as RSI. The value divided RSI × 10 by sample weight (mg) was calculated as Units Signal Intensity (USI). USI = RSI × 10/weight.

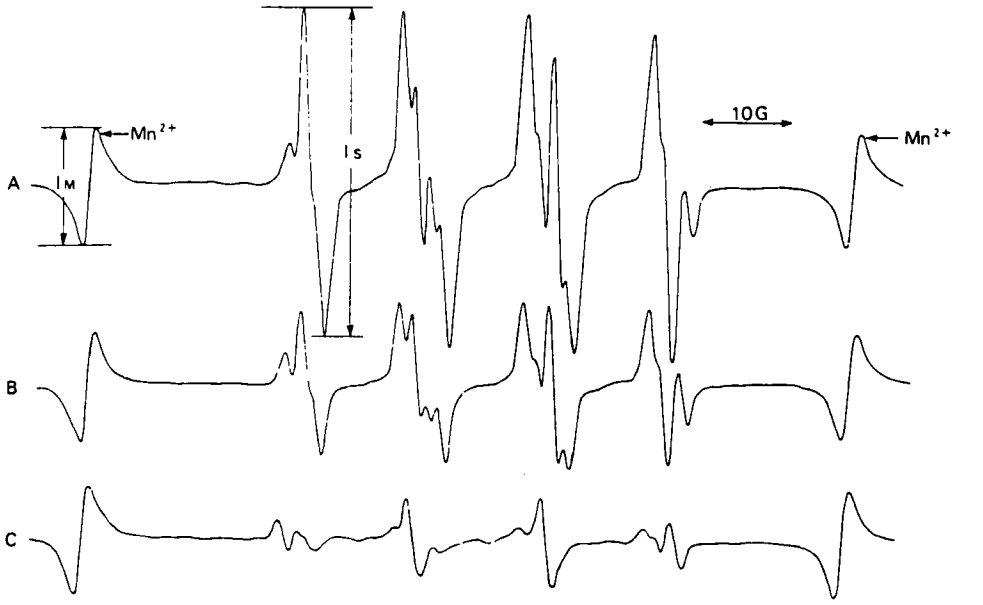

FIGURE 2 ESR spectra of DMPO-OOH spin adducts. DMPO spin adducts shows the superoxide anion (O_2^-) generated by the reaction of Hypoxanthine (HX) and Xanthine oxidase (XOD). A standard reaction mixture contained 0.5 mM HX, 0.96 mM DETAPAC, 7.5 mM DMPO, 0.1 U/ml XOD and various concentrations of standard SOD or sample (50 μl) in phosphate buffer (pH 7.8). Spectrum A is without SOD. B is 6.25 units/ml of SOD. C is 50 units/ml of SOD.

field 335.3 ± 5 mT, modulation width 0.1 mT, amplitude 2.5 × 100, time constant 0.3 sec, sweep time 2 min.

Relationship between the Results of ESR Measurement and Clinical View of Jaw Cyst

We examined the relationship between the results of the ESR measurement and the viscosity of cyst fluid, and the inflammatory view on clinic. In addition, we analyzed statistically the correlation between free radicals and SOD activity in the same samples.

RESULTS

Free Radicals of Cyst Fluid (Table 1)

In measuring the freeze-dried sample with ESR, a broad signal was detected at

TABLE 1
Free Radicals of Cyst Fluid

Clinical view of cyst fluid	ESR signal intensity (USI)
With inflammation (n = 5)	1.27 ± 0.56*
Without inflammation (n = 13)	0.34 ± 0.19

Values show mean ± SD. *Significantly different from the cyst fluid without inflammation ($p < 0.05$).

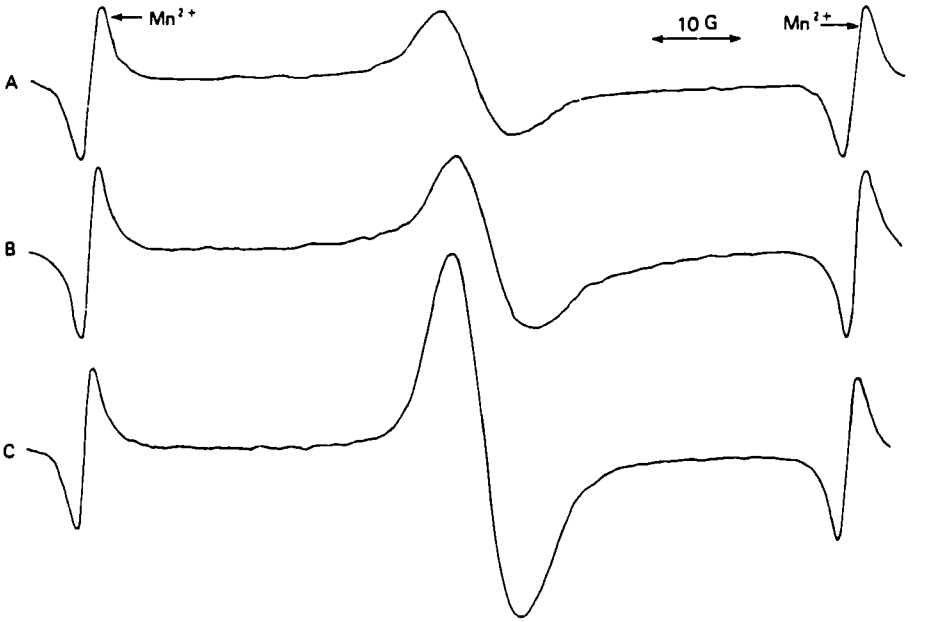

FIGURE 3 ESR spectra of freeze-dried cyst fluids with inflammation. Spectrum A: USI = 0.864, B: USI = 0.857, C: USI = 2.194.

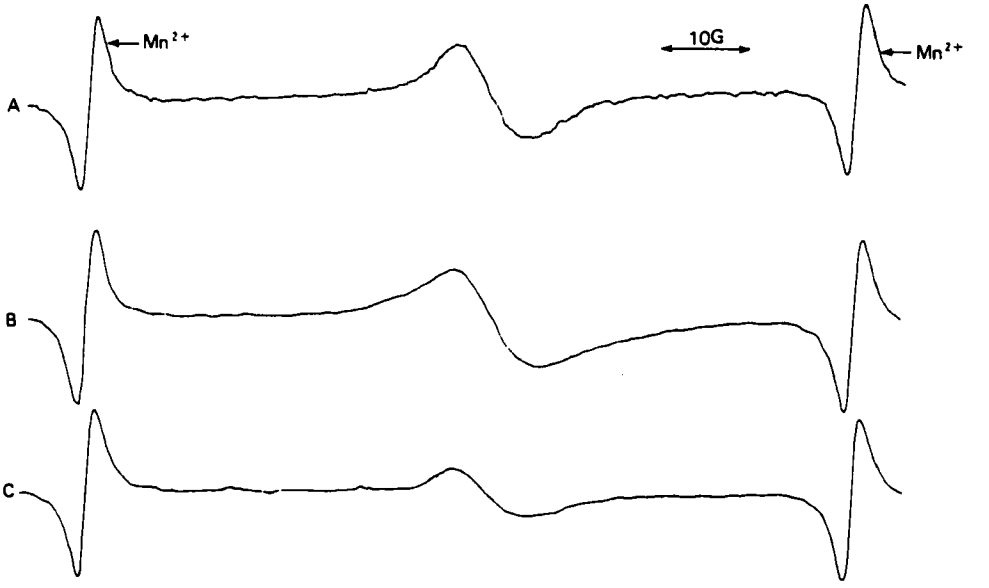

FIGURE 4 ESR spectra of freeze-dried cyst fluids without inflammation. Spectrum A: USI = 0.772, B: USI = 0.242, C: USI = 0.212.

TABLE 2
SOD Activity of Cyst Fluid

Clinical view of cyst fluid	SOD Activity (Units/ml)
With inflammation (n = 5)	24.38 ± 17.6
High viscosity without inflammation (n = 4)	26.12 ± 2.51*
Low viscosity without inflammation (n = 9)	12.77 ± 8.71

Values show mean ± SD. *Significantly different from the low viscosity cyst fluid without inflammation ($p < 0.05$).

$g = 2.005$. USI showed high values, when cyst fluid was accompanied with some inflammatory symptoms (Figure 3). In contrast, the cyst fluid without inflammation showed significantly lower USI values ($p < 0.05$) (Figure 4).

SOD Activity of Cyst Fluid (Table 2)

A case of jaw cyst accompanied with bacterial infection showed markedly high SOD activity. Moreover, all of the high viscosity fluid without inflammation showed high SOD activity. In contrast, low viscosity fluid showed significantly lower SOD activity ($p < 0.05$) (Figure 5).

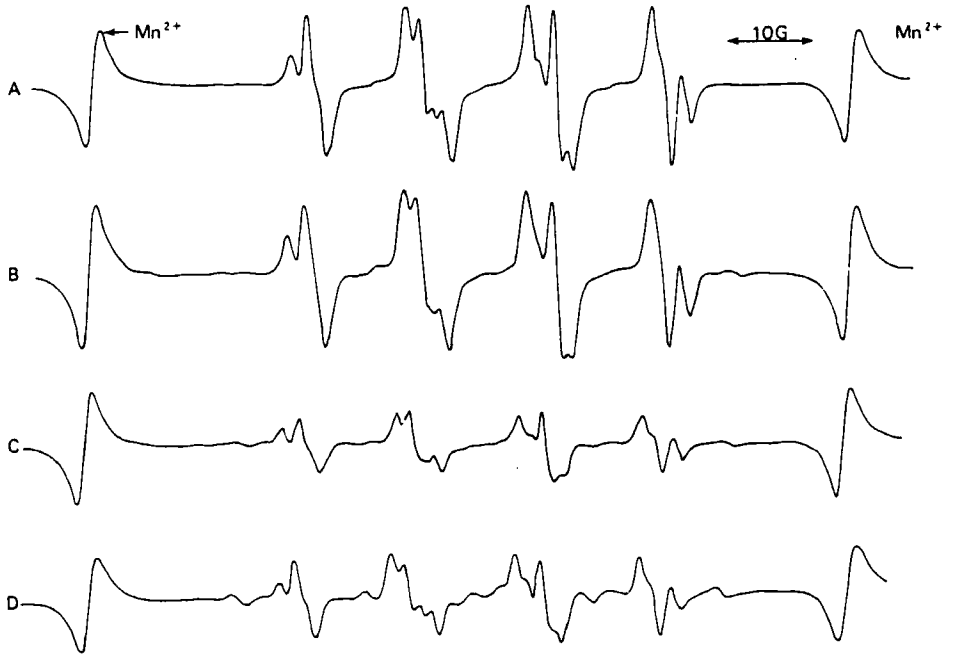

FIGURE 5 ESR spectra of DMPO-OOH spin adducts in each sample. SOD activity and clinical view of cyst fluid are as follows. Spectrum A = 6.71: low viscosity fluid without inflammation. B = 6.22: low viscosity fluid without inflammation. C = 25.79: high viscosity fluid without inflammation. D = 54.24: low viscosity fluid with severe bacterial infection (spectra D is the sample diluted with 5 volumes of PBS).

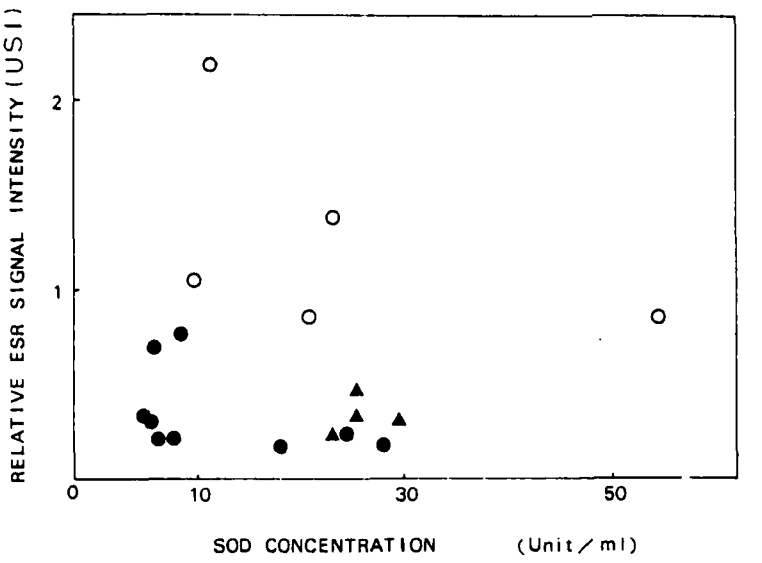

FIGURE 6 Correlation between the free radicals (USI) and SOD activity in same samples. ○: the cyst fluid with inflammation. ▲: the high viscosity cyst fluid without inflammation. ●: the low viscosity cyst fluid without inflammation.

Correlation between Free Radicals and SOD Activity (Figure 6)

The cyst fluid accompanied with inflammation showed relatively high values of combined free radicals (USI) and SOD activity. Among the cyst fluids without inflammation, high values of SOD activity correlated with low values of USI. However, there is statistically no proportional correlation between USI and SOD activity.

DISCUSSION

In recent years, it has been reported that free radicals generated in cyst fluid reflected the clinical condition of jaw cyst.[4] In our study, ESR signal intensity (USI) of freeze-dried cyst fluids showed high values when jaw cysts occurred in conjunction with inflammation, and decreased with less inflammatory symptoms. Thus, USI might correspond with the degree of tissue damage of cyst wall. But it is unknown what type of free radical was detected at $g = 2.005$ from the freeze-dried samples. Skaug[5] reported that β-lipoprotein was abundant in cyst fluid. We may hypothesize that the carbon-centred protein radical was generated in the process of the preoxidation of lipids united with proteins.[6] When jaw cyst is accompanied with bacterial infection, leukocytes produce active oxygens[7] to generate free radicals in lipid-peroxidation by radical chain reaction. These processes create an obstacle at cyst wall tissues.

However, SOD activity of the cyst fluid was in proportion with the liquid viscosity of fluids. It is reported that the viscous element in cyst fluid is hyaluronic acid,[7] and that hyaluronic acid controls free radicals.[8] Results of this research certified that viscous elements of cyst fluid play an important role in the self-defense mechanism

against free radicals and that high values of SOD activity of cyst fluids without inflammation correlated with low values of free radicals. But, we could not make clear the relationship between free radicals and SOD activity in jaw cysts, so the accumulation of data on more cases is needed hereafter.

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