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EFFECT OF LIPOSOMAL-ENCAPSULATED SUPEROXIDE DISMUTASE ON ACTIVE OXYGEN-RELATED HUMAN DISORDERS. A PRELIMINARY STUDY†

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Liposomal-encapsulated superoxide dismutase was clinically applied to patients showing an increase in neutrophil active oxygen generation, and those with diseases such as severe rheumatoid arthritis (RA), Crohn's disease and progressive systemic sclerosis (PSS) in which presence of a plasmatic clastogenic factor has been demonstrated. Liposomal SOD injection (2.5 mg twice a week) resulted in marked remission in 12 out of 16 patients with active Behçet's disease. The drug was impressively effective on patients with intestinal Behçet. Remission rates in the other diseases was 7 out of 8 mucocutaneous lymphnode syndrome (MCLS, Kawasaki disease) 3 out of 5 dermatitis herpetiformis, IgA linear bullous dermatosis or severe cement dermatitis, 4 out of 9 active and severe RA, 3 out of 3 PSS, 4 out of 4 Crohn's disease, 3 out of 4 colitis ulcerosa, and 2 out of 2 unresponsive (hemolytic) anemia. To be emphasized was that three severe active RA patients and two terminal-stage PSS patients with dyspnea due to lung fibrosis showed dramatic improvement after administration of liposomal SOD. In addition, in 13 out of 15 malignant neo plastic patients including cancer, malignant lymphoma and leucemia who were receiving radiotherapy (total dose, more than 4000 rads) and chemotherapy including anthracycline analogs (total over 450 mg/m^2) and bleomycin, the drug also prevented the appearance of myocardiac injury and fibrosis, sometimes seen as a consequence of chemotherapy. Liposomal SOD, which shows no toxicity, has various advantages compared to free SOD preparations, and is highly and broadly applicable to various clinical disorders.

Key words: Superoxide dismutase; liposomes; neutrophils; oxygen radicals; Behçet's disease; Kawasaki; Crohn; cancer therapy.

INTRODUCTION

The physical and biological characteristics, including pharmacokinetic and organ location of liposomal-encapsulated bovine copper superoxide dismutase (SOD) has

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been reported earlier[1,2,3,4]. It has also been reported that a clastogenic factor is present in the plasma of patients with autoimmune diseases. Both the activation of a non-active composant to give this factor and the chromosome breaking activity of the activated factor are inhibited by SOD[5,6]. The beneficial effect of liposomal SOD in patients with Crohn's disease and postradiotherapeutic necrosis[1,7,8,9] may be considered to be due to the inhibition of activation and of the activity of this clastogenic factor. Further, chemotherapy with drugs such as adriamycin and daunomycin[10,11,12] is known to induce myocardiac injury, while both bleomycin and radiotherapy can give rise to fibrosis due to an increased production of superoxide and other radicals[13,14].

Niwa and collaborators recently reported increased neutrophil active oxygen generation in patients with Behçet's disease[15], mucocutaneous lymphnode syndrome[16], rheumatoid arthritis (synovial neutrophils)[17] dermatitis herpetiformis including IgA linear bullous dermatosis[18] and severe and recurrent cement dermatitis[19], leading to the tissue injury of these diseases. In this study, we have applied liposomal SOD clinically for patients showing an increased neutrophil active oxygen generation and in those with clastogenic factors present in the serum. In addition, two cases of unresponsive anemia with a marked decrease in SOD activity in red cells were treated. The data obtained were compared with those using empty liposomes as placebo. Successful and sometimes impressive clinical improvement and results were obtained. Since other common therapies in the diseases tested in this study show various degree of side effects, clinical use of liposomal SOD is not only promising but can be recommended because of its efficiency and lack of toxicity.

SUBJECTS AND METHODS

A total of 63 patients were placed in the following categories and evaluated for the efficacity of liposomal SOD: 16 cases of active and complete type Behçet's disease (9 males aged 8 to 48 years and 7 females aged 28 to 45 years) including 7 patients receiving no other therapy and 7 intestinal cases; 8 cases of mucocutaneous lymph node syndrome (MCLS, Kawasaki disease, 6 males aged 5 to 8 years and 2 females 3 and 5 years) within 4 days after onset receiving no other treatment; 5 cases of dermatitis herpetiformis, IgA linear bullous dermatosis or severe and recurrent cement dermatitis (3 females aged 37 to 48 years, and 2 males aged 30 and 45 years); 9 cases of rheumatoid arthritis (RA, 2 males aged 26 and 31 years and 7 females aged 23 to 45 years) including 3 progressively advanced patients who were very resistant to all therapies concomitantly administered with low dose steroids; 3 cases of progressive systemic sclerosis (PSS, 2 males aged 40 and 47 years and a female aged 46 years) of which the two male patients were severely progressive, resistant to all therapy concomitant with low dose steroids and showing dyspnea due to lung fibrosis visible in chest X-radiography, the female patient being at a very initial stage and receiving no treatment; 4 cases of Crohn's disease (males aged 18 to 32 years); 4 cases of colitis ulcerosa (3 males aged 24 to 48 years and a female aged 32 years); 2 cases of unresponsive hemolytic anemia (females aged 20 and 23 years) resistant to all current therapies, 15 patients with cancer, malignant lymphoma and leucemia (10 males aged 8 to 56 years and 5 females aged 43 to 57 years) under treatment with adriamycin, daunomycin, bleomycin and radiation. The total dose of adriamycin administered was 90 to 270 mg over 2 to 3 months, that of daunomycin 150 to 450 mg over 2 to 3

months, bleomycin 100 to 225 mg over 2 to 3 months and that of radiation 3000 to 4000 rad within 3 weeks.

Although the present study is not a double blind trial but rather an open historical comparison, evaluation of the effectiveness of liposomal SOD was based on the following clinical conditions in each disease. First, in all the diseases tested the main evaluation bases of treatment[20] were

1) the local and general (systemic) activity of the disease (recurrence, persistence),
2) the local and general severity of the disease (number of inflammatory lesions, localization, degree of inflammation),
3) the local and general disability of the patients (degree of disability of organ functions or restriction of movement (ROM), daily and social activity).

These parameters were evaluated both subjectively and objectively. Secondly, the following laboratory examinations were also applied to the assessment of efficiency of the drug in the various diseases; peripheral blood leukocyte counts, C reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were used in Behçet's disease, MCLS, RA, Crohn's disease and colitis ulcerosa, and X-ray examination of the colon in intestinal Behçet, Crohn's disease and colitis ulcerosa, that of the joints in RA, that of the oesophagus and lung in PSS, and echocardiography in MCLS. Further, in unresponsive anemia, peripheral red cell counts and haemoglobin levels were used. Regarding the evaluation of myocardiac injury and fibrosis in malignant neoplastic patients receiving anthracycline analogues and radiotherapy, USCG (ultra sound echocardiogram), chest-X-ray pictures and skin lesions were examined. For evaluation of dermatitis herpetiformis and IgA linear bullous dermatosis, examination by direct immunofluorescence microscopy was the main laboratory assessment.

Except for 7 cases of Behçcet's disease and a PSS patient receiving no other therapy and 3 cases of dermatitis herpetiformis or IgA linear bullous dermatosis in which low dose steroids were continued, all the patients were given liposomal SOD in addition to previously administered drugs. The therapeutic efficacity was judged by comparison with that of the former treatment in absence of liposomal SOD.

Twelve different centers participated in this trial using the same protocol and prescribing the following dose regimen. Each patient received an injection of 2.5 mg liposomal SOD twice a week given deep subcutaneously or intravenously, over a period of 8 weeks, except in infant patients (MCLS) where 1.0–2.0 mg of liposomal SOD was injected every day or every other day for more than 4 days according to the age and severity of the disease.

For placebo tests, empty liposomes were administered to a total of 14 patients who consisted of sex-, age- and category-matched Behçet's disease (four), MCLS (two), RA (five) colitis ulcerosa (two) and PSS (one). Informed consents were obtained from all subjects tested before the study.

RESULTS

This uncontrolled study of 63 cases suggests that liposomal SOD is active generally on the diseases tested and especially on intestinal Behçet, colitis ulcerosa, Crohn's disease, MCLS, PSS and severe RA. In addition the drug was effective in preventing

TABLE I
Intestinal Behçet's patients treated with liposomal SOD only. Uncontrolled study

Case	Sex/Yrs	Prior to therapy: Duration of disease	Characteristics	Laboratory findings	Active oxygen	During or after therapy: Duration of therapy	Relapse	Result
1	♀ 36	7 yrs	Ileocolitis, 1978, 1980 Ilectomy. And Eruption, Uveitis, Aphthous ulcer	WBC 18,000 CRP 7 mm ESR 78	(n.d.)	4 m	no	good
2	♂ 41	5 yrs	Ileitis, 1981 ilectomy. And Aphthous ulcer, Genital ulcer	WBC 15,800 CRP 6.5 mm ESR 46	increased	3 m	yes	
3	♀ 58	15 yrs	Right colitis, 1977, 1980, 1982 right colectomy. And Genital ulcer, Aphthous ulcer	WBC 14,500 CRP 3.5 mm ESR 49	normal	5 m	no	good
4	♀ 38	18 yrs	Ileocolitis. And Eruption, Genital ulcer, Aphthous ulcer	WBC 11,000 CRP 2 mm ESR 38	slightly increased	6 m	no	good
5	♂ 51	6 yrs	Ileocolitis and sigmoid involvement. And Genital ulcer, Aphthous ulcer	WBC 18,500 CRP 6 mm ESR 80	normal	7 m	no	good
6	♂ 8	3 yrs	Ileitis. And Eruption, Aphthous ulcer, Anal ulcer	WBC 19,000 CRP 4 mm ESR 42	increased	10 m	yes	
7	♂ 48	10 yrs	right colitis, 1978, 1980 Ileo-colectomy, 1982 right colectomy with ileo-transversus anastmosis. And Uveitis, Aphthous ulcer	WBC 13,400 CRP 3 mm ESR 48	slightly increased	4 m	yes	

TABLE II
MCLS (mucocutano lymphnode syndrome) patients treated with liposomal SOD. Uncontrolled study

Case	Sex/Age	Laboratory findings on the day of first injection	Date (days after onset) of						
			Start of injection	disappearance of			Complication with coronary artery dilation	Aneurysm formation	Result
				Fever	Eruption	lymphnode swelling			
1	♂ 3 m	WBC 19,000 CRP 5 mm ESR 85	2	4	5	6	+	–	good
2	♂ 4 yrs	WBC 21,000 CRP 6 mm ESR 92	4	6	8	8	–	–	good
3	♂ 7 yrs	WBC 16,000 CRP 5 mm ESR 74	4	5	6	6	–	–	good
4	♂ 5 yrs	WBC 20,000 CRP 5.5 mm ESR 88	3	7	8	9	+	–	good
5	♂ 9 m	WBC 24,000 CRP 8 mm ESR 91	4	7	9	9	+	+	
6	♂ 7 m	WBC 19,000 CRP 6 mm ESR 73	3	6	7	8	+	–	good
7	♂ 7 yrs	WBC 24,000 CRP 6 mm ESR 78	3	6	8	8	–	–	good
8	♀ 4 yrs	WBC 20,500 CRP 6.5 mm ESR 82	2	4	5	7	–	–	good

myocardiac injury and fibrosis in the neoplasma patients receiving chemotherapy and/or radiotherapy.

Behçet's Disease

Liposomal SOD was effective in 12 out of 16 patients with Behçet's disease. It was particularly active in intestinal Behçet. In 6 out of 7 intestinal Behçet's patients, the drug was dramatically active. Three intestinal Behçet's patients who had earlier received surgical operations of the colon several times have shown complete remission without further resection (Table 1). However the drug was less active in patients showing mainly eye symptoms.

Mucocutaneous Lymphnode Syndrome (MCLS)

The drug was also beneficially effective in 7 out of 8 patients with MCLS. In patients with severe MCLS an injection was performed every day. The clinical symptoms including high fever, eruption, conjunctive hyperemia and lymphnode swelling disappeared within two to four days after the beginning of the injections (Table II). Generally, in MCLS patients it takes 10 days or more for the fever to recede as observed in a placebo test group (data not shown), whereas only two to four days were necessary in patients injected with liposomal SOD. Though complications with coronary artery dilatation were observed in four cases, aneurysm formation (often observed in such cases) was found only in one patient in whom we judged the drug ineffective.

Rheumatoid Arthritis (RA)

Liposomal SOD was active in only one out of 6 RA patients showing mild symptoms such as joint pain and slight swelling, morning stiffness and positive RF test in their serum. In contrast, the drug was dramatically effective in all three patients with very severe and highly advanced symptoms, showing deformity of many joints, marked pain and swelling of joints, restriction of movement/posture and walk (ROM) including osteodestruction visible in X-ray pictures, and who had been resistant to all orthodox therapies including low dose steroids (Figure 1). As shown in Figure 1, this severe and advanced RA patient previously showed marked swelling, pain and deformity of finger, hand and knee joints and was resistant to all previous therapies including non-steroid anti-inflammatory drugs and low dose steroids. This female patient visited our hospital for the first time on a wheel-chair. Seven to eight weeks after liposomal SOD injection, she was able to walk on crutches and a few weeks later she could limp unaided.

Progressive Systemic Sclerosis (PSS)

Two terminal stage patients who showed markedly progressive and advanced symptoms such as wide-spread scleroderma of the skin and dyspnea due to lung fibrosis as shown in chest X-rays (Figure 3a) and who had been resistant to all therapies including low dose steroids and the anti-neoplastic agent leukerin, were surprisingly improved by liposomal SOD injection (Figure 2). Two to three weeks after injection of liposomal SOD, the dyspnea subsided and the fibrosis was reduced as shown by X-

radiography (Figure 2 and Figure 3a,b). The fibrotic shadow in his chest X-ray was extremely improved three months after SOD injection. In another PSS patient in a very early stage in whom only swelling, slight edema and pain were observed in her fingers and hands, treatment with liposomal SOD alone was effective in preventing further progress of the hardness of the skin and tissue.

Unresponsive Anemia

Two patients with anemia who had been strongly resistant to all previous therapies showed a marked decrease in erythrocyte counts and haemoglobin levels. In one patient the number of red blood cells was less than half and haemoglobin levels less than one third of normal values at the most severe period (Figure 4a,b). Assessment of erythrocyte SOD levels showed that this was extremely decreased to less than one fourth of normal levels. In contrast, the serum SOD level was elevated, suggesting that Cu-SOD was released into the plasma on destruction of the erythrocytes[21]. A few weeks after administration of liposomal SOD, the anemia showed improvement, accompanied by an increase and normalization of the SOD level in red blood cells. A slight increase in SOD levels in other blood cells and a decrease in serum SOD levels were observed concurrently.

Malignant Neoplastic Patients

As shown in Table III, liposomal SOD injections were performed simultaneously with radiotherapy and chemotherapy including adriamycin, daunomycin or bleomycin in 15 patients with leucemia, malignant lymphoma and cancers. Secondary effects such as myocardiac injury and fibrosis, usually observed with these chemotherapies and irradiation, were found in only 2 cases (Table III). This incidence is less than that in patients receiving anthracycline analogues[22,23] in which more than 30% of of the cases suffered from congestive heart failure, electrocardiographic changes and severe histologic toxicity when the total amount of the drugs exceeded 450–550 mg/m^2. Not only were the effects of irradiation and chemotherapy preserved but also the clinical course of the patients was improved by the combination of therapy with liposomal SOD (not shown). This could support the presumption that the death of terminal malignant neoplastic patients is not due entirely to the disease itself but to the toxicity of the therapies applied.

Other Disorders

Liposomal SOD was dramatically active in the patients with Crohn's disease and colitis ulcerosa (remarkable efficacy was observed in 3 out of 4 colitis ulcerosa and all of Crohn's disease tested). The drug was also effective in 3 out of 5 patients with dermatitis herpetiformis, IgA linear dermatosis and severe and recurrent dermatitis in whom increased neutrophil active oxygen generation has been reported[18,19].

Except for MCLS patients in whom results were rapid, the effectiveness of liposomal SOD appeared two or three weeks after the beginning of application. With respect to secondary effects, marked induration at the site of injection was observed in 4 cases and fever which seemed to be due to heterophil protein was seen within 12 hr in 3 out of 63 cases treated. Two subjects were coincident and had both. Regarding the

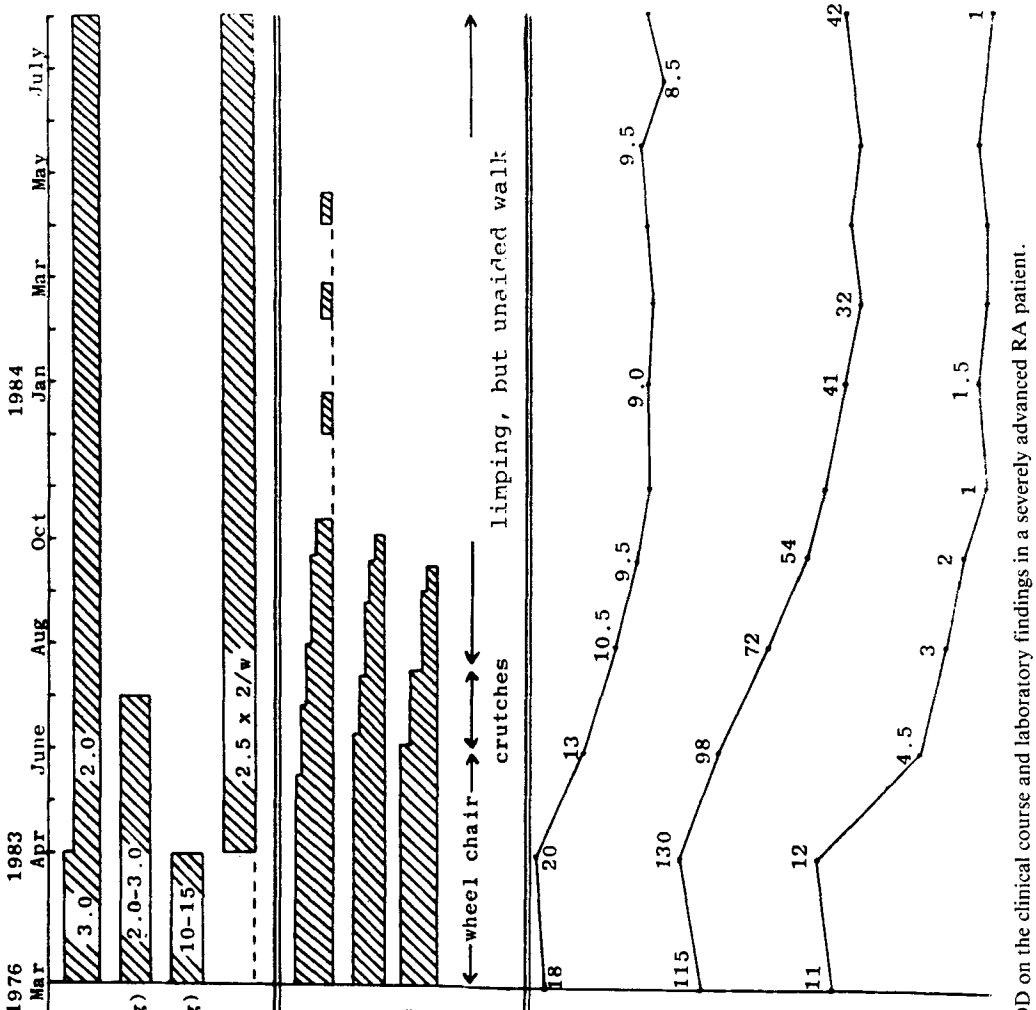

FIGURE 1 Effect of liposomal-SOD on the clinical course and laboratory findings in a severely advanced RA patient.

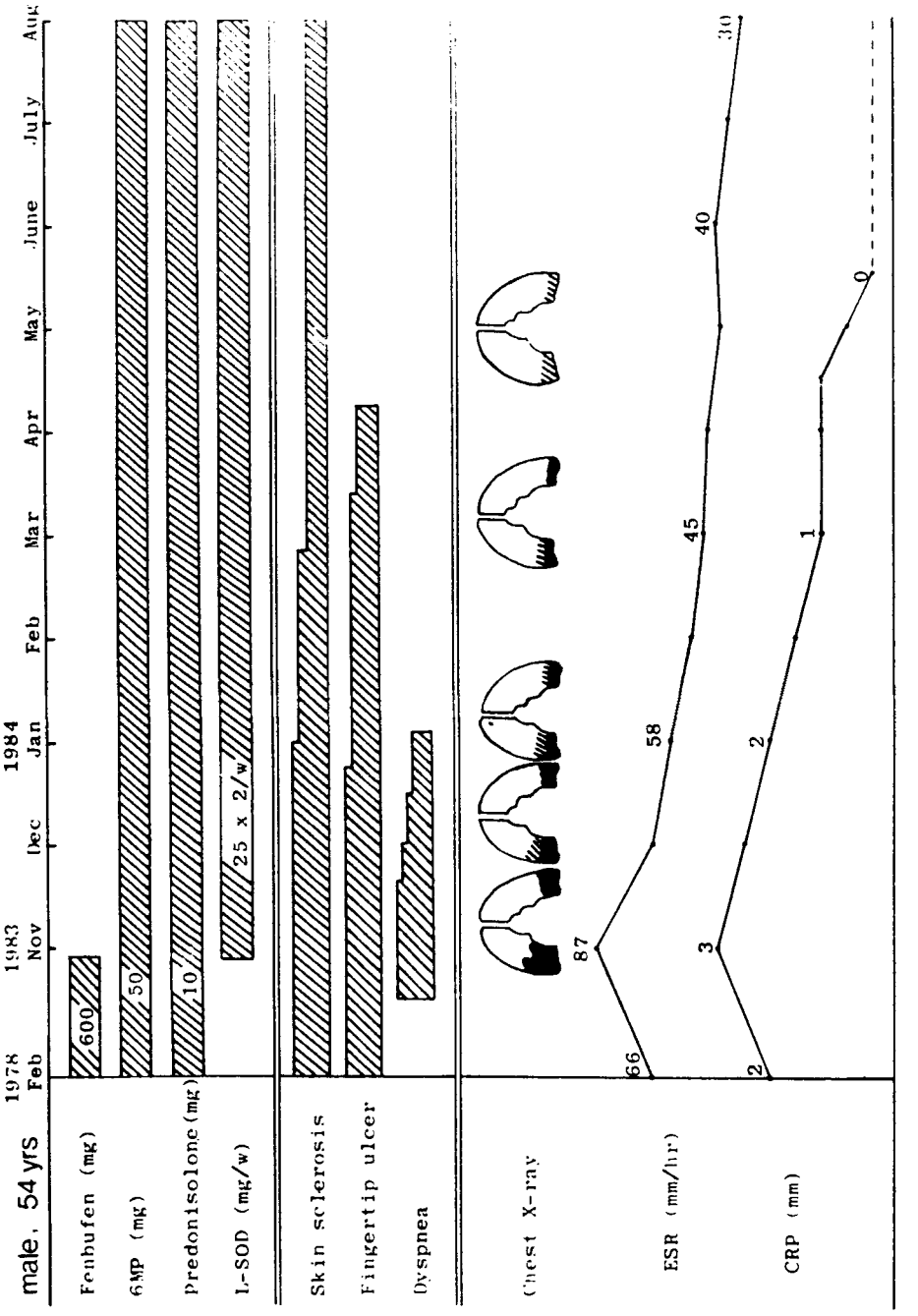

FIGURE 2 Effect of liposomal-SOD on the clinical course and laboratory findings in a severe PSS patient in terminal stage.

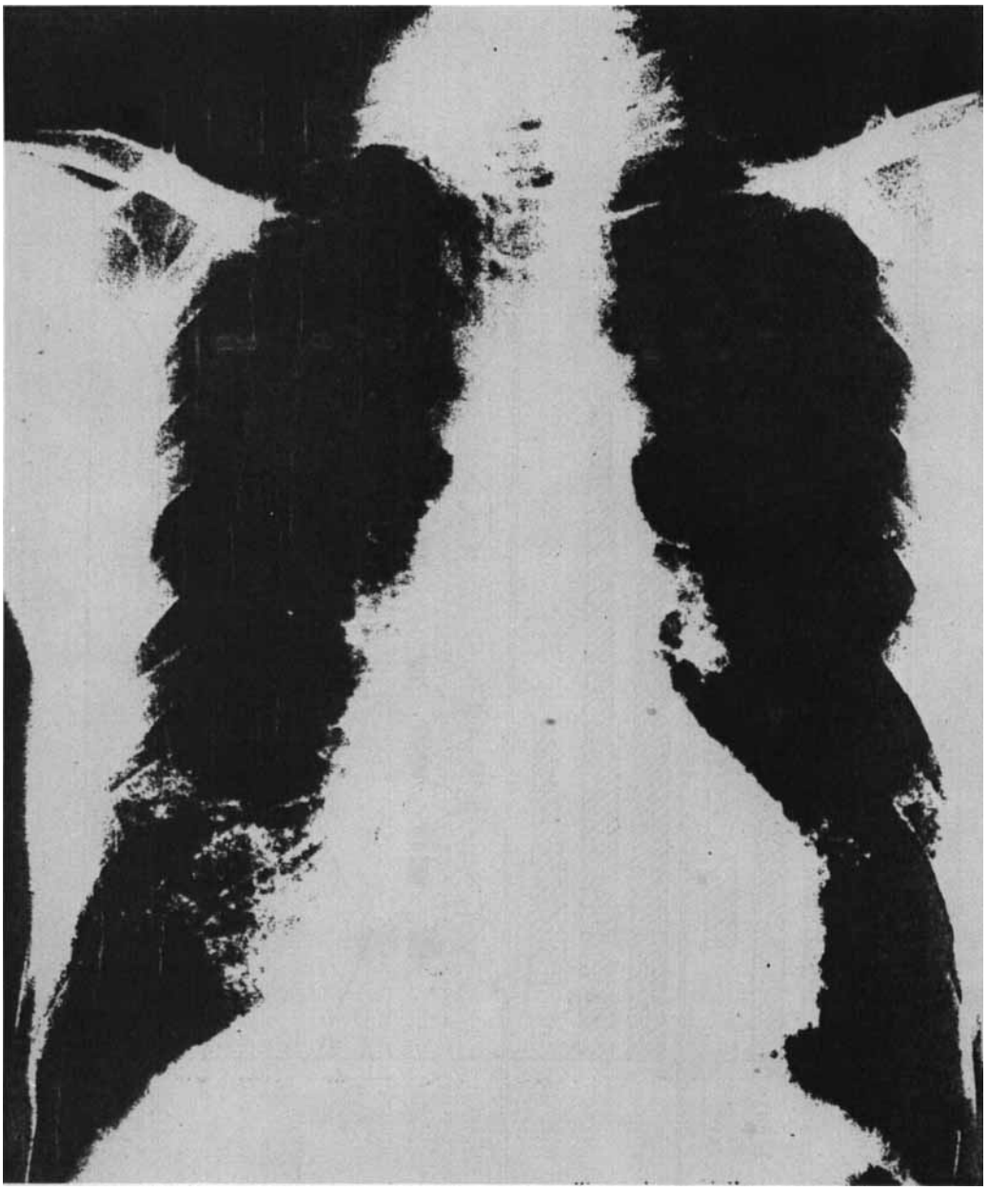

FIGURE 3a Chest X-ray picture of a PSS patient suffering from progressive lung fibrosis and dyspnea.

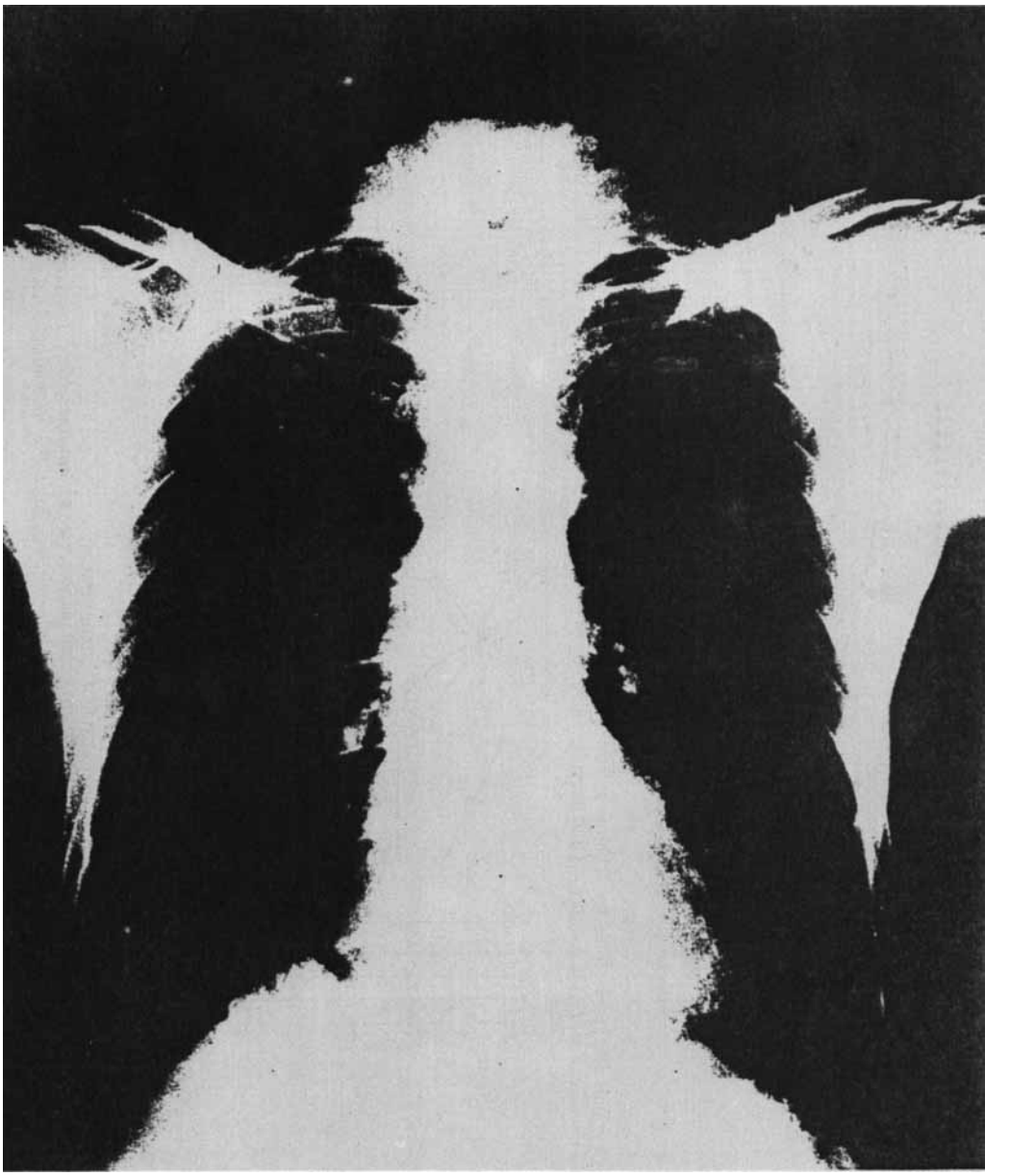

FIGURE 3b · Chest X-ray picture of this PSS patient after treatment with liposomal-SOD for 3 weeks.

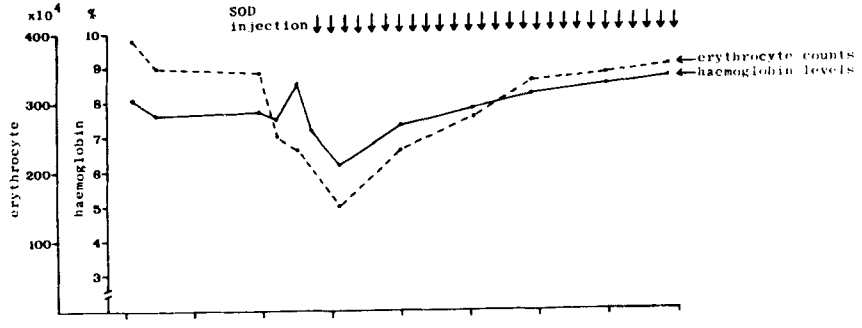

FIGURE 4a Erythrocyte counts and haemoglobin levels before and after liposomal SOD administration.
Each line (in upper figure) denotes erythrocyte counts (----) and haemoglobin levels (——), respectively.

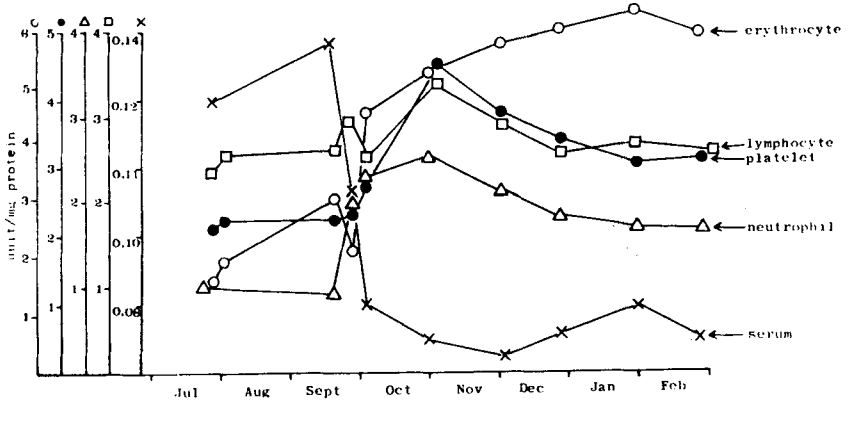

FIGURE 4b SOD levels in blood components before and after liposomal SOD administration.
Each symbol and line (in lower figure) denotes SOD levels in erythrocytes (○—○), in platelets (●—●), in neutrophils (△—△), in lymphocytes (□—□), and in serum (x—x), respectively. Normal ranges of SOD levels in erythrocytes are 6.0 ± 0.8; in platelets, 2.0 ± 0.3; in neutrophils, 0.8 ± 0.1; in lymphocytes, 2.0 ± 0,.2; and in serum, 0.08 ± 0.02 unit/mg protein.

TABLE III
Clinical course of the patierts with leucemia and cancers treated with both chemotherapy and/or irradiation, and liposomal SOD

	Diseases	Sex/Age	Chemotherapy* and radiation	Remmision	Side effect of chemotherapy	Result
Case 1	a.L.L.	♂ 8	adriamycin (460 mg)‡	yes	no	good
2	a.L.L.	♀ 7	daunomycin (500 mg)	yes	no	good
3	a.M.L.	♀ 9	daunomycin (475 mg)	yes	no	good
4	a.M.L.	♂ 8	daunomycin (500 mg)	yes	no	good
5	a.M.L.	♂ 25	adriamycin (570 mg)	yes	no	good
6	a.M.L.	♀ 38	adriamycin (460 mg)	yes	no	good
7	L.C.	♀ 51	bleomycin (450 mg) and radiation (3,000 rad)	yes	no	good
8	S.C.	♂ 62	adriamycin (480 mg)	yes	no	good
9	S.C.	♀ 56	adriamycin (630 mg) and radiation (4,000 rad)	yes	no	good
10	S.C.	♂ 49	adriamycin (640 mg)	yes	no	good
11	L.C.	♂ 68	daunomycin (650 mg)	no (early death)	myocardiac damage	
12	U.C.	♀ 54	radiation (3,000 rad)	yes	no	good
13	Hg. with§ Sq. C.C.	♂ 53	bleomycin (450 mg) and radiation (4,000 rad)	yes	myocardiac injury and lung fibrosis	good
14	Sq. C.C.	♂ 61	bleomycin (200 mg)	yes	no	good
15	Sq. C.C.	♀ 47	bleomycin (210 mg) and radiation (3,000 rad)	yes	no	good

a.L.L.: acute lymphocytic leucemia, a.M.L.: acute myelotic leucemia, L.C.: lung cancer, S.C.: stomack cancer, Hg.: Hodgikin's disease, U.C.: uterine cancer, Sq. C.C.: squamous cell carcinoma.

* Only chemotherapies correlated with an increase in active oxygen generation are described.

‡ Parenthesis denotes total amount of chemotherapies (per m^2) administered or radiation irradiated during the past two years before liposomal SOD treatment was finished.

§ This patient had complication of Hodgikin's disease with squamous cell carcinoma.

induration, improved pharmacological techniques may decrease this.

Empty liposomes of the same composition showed no effects on the symptoms of patients whatsoever.

DISCUSSION

Since free bovine Cu-SOD is rapidly concentrated in the kidney and then excreted[24] useful clinical application of the enzyme is limited and up till now perhaps the most successful use has been in intra-articular injection for rheumatoid arthritis. This rapid excretion can be diminished by coupling the protein with polyethylene glycol, albumin or other polymers[25,26,27]. However, no improvement in clinical efficacity appears to have been demonstrated with such modifications.

The advantages of liposomal SOD lie in improved pharmacokinetic properties leading to a longer life time in the organism, with a slow release of free SOD, concentration in divers organs other than the kidneys, better total distribution, greatly enhanced fixation to the outside of cell membranes compared with free SOD and greatly increased *tissue* permeability[28]. An example of *in vitro* efficiency is shown by the fact that oxidative damage of mitochondria (due to peroxide formation on UV irradiation of methyl linoleate) is not inhibited by free SOD but is prevented by the liposomal SOD used in this study due to increased membrane fixation[29]. It is to be noted that these liposomes do *not* bind to bacteria and thus do not protect against phagocytosis. *In vitro* results show that incubation of neutrophils with liposomal SOD and either *Escherichia coli* or *Staphylococcus aureus* (strain 502 A) does not significantly decrease bacterial killing[30].

Production of antibodies to bovine Cu-SOD as a result of treatment with liposomal SOD is presently under study[31]. As yet no indications of anaphylactic conditions have been observed.

The present study suggests that liposomal SOD could be more broadly applicable than the free enzyme. Increased neutrophil active oxygen generation in patients is a rather common clinical phenomenon and often such cases are difficult to treat, requiring drugs which show side effects and even toxicity. Among the cases treated with liposomal SOD, especially impressive were the remissions induced in three RA and two PSS patients with extremely advanced progressive symptoms who were entirely resistant to all other medical treatment, including low dose steroids. Since liposomal SOD was dramatically effective in RA patients with markedly severe symptoms, but was much less active in patients with mild symptoms, it may be speculated that severe RA has somewhat different etiology or pathogenesis from mild RA. This could be due to differences in levels of clastogenic factor or to active oxygen production by the neutrophils.

With respect to the two patients with unresponsive anemia, both were severe cases and were entirely resistant to various orthodox treatments for anemia. Both showed a very marked decrease in erythrocyte Cu-SOD as has been described for patients with hemolytic uremic syndrome[32,33,34] and in Machiafavi Michelli disease[35]. Treatment for three months with liposomal SOD not only greatly improved the clinical condition but appears to have resulted in permanent remission. This suggests that administration of liposomal SOD may be directly effective in the bone marrow during erythropoiesis to rectify a defect in synthesis of the enzyme in the stem cells. The results obtained in this study (Figure 4) show that as SOD levels in red cells increase, the abnormally high

plasmatic SOD is decreased in accord with the concept that such high SOD levels are due to cell lysis.

In accord with this schema it can be noted (Figure 4) that lysis, as shown by serum SOD levels, is very rapidly reduced after treatment with liposomal SOD whereas erythrocyte SOD levels, haemoglobin levels and erythrocyte count require about three months to reach normal levels, i.e. the time required to replace entirely the old erythrocytes by new red blood cells.

In Japan, free bovine Cu-SOD is mainly applied by intra-articular injection. Use of liposomal SOD by the same technique cannot be recommended since this causes increased irritation. This is probably due to activation of synovial neutrophils since *in vitro* experiments show that SOD-liposomes, albumin liposomes or empty liposomes stimulate free radical production by neutrophils[36,37] acting simply as particulate material. As previously shown[17] synovial neutrophils in cases of RA show an enhanced production of active oxygen and further stimulation by liposomes is contra-indicated.

Other roles for superoxide radicals in the initiation of inflammation may involve the activity of indoleamine dioxygenase[38,39,40] which uses O_2^- as substrate and is involved in serotonin metabolism, and the prostaglandin G_2, H_2 and arachidonic acid cascade. This may explain why liposomal SOD is also effective in patients with common inflammations which have no specific correlation with low levels of endogenous SOD or high neutrophil active oxygen production.

Clinical application of liposomal SOD can be justified in pathologies such as the following:

1) diseases in which an increased neutrophil active oxygen generation is demonstrated,
2) auto-immune diseases and post-radiotherapeutic necrosis in which high levels of clastogenic factor are present,
3) ulcerations of the skin,
4) prevention of oxidative damage in cancer patients induced by chemotherapy including adriamycin, daunomycin and bleomycin, and radiotherapy,
5) common inflammations or other conditions in which localised neutrophil concentrations due to an SOD inhibited chemotactic factor[41] give rise to high free radical production,
6) situations involving excessive platelet aggregation or perturbation of the prostaglandin system,
7) low endogenous SOD levels in specific cell populations as in certain cases of hemolytic anemia (indirectly, by action on the bone marrow) or in high energy irradiation[42] accidents,
8) ischemic and post ischemic conditions in which considerable oxidative damage is caused,
9) to reduce vascular permeability in cases of severe thermal burns,
10) treatment of post radiotherapeutic fibroses, and perhaps fibromes and fibromyomes.

In cases of radio-induced fibroses considerable success has been obtained even with fibroses existing for several years[43].

As reported elsewhere, anti-inflammatory activity of SOD is not correlated with pI, with molecular weight, with circulation lifetime or with gross pharmacokinetic properties in general[44]. In inflammation models using rats, homologous SOD is *not* anti-inflammatory and indeed heterologous SODs from different sources show widely different anti-inflammatory efficacity[45]. Perhaps a certain distance between endogenous and exogenous enzyme in terms of sequence homology is necessary. The mechanism of anti-inflammatory activity *in vivo* is complicated and cannot be explained in simplistic terms. For mouse fibroblasts in culture, it has been shown that protection by free SOD remains even when this exogenous enzyme is removed from the cell culture medium[46]. It is possible that very few molecules attached to cell membranes, even of a specific cell population, play a role much more important than removal of plasmatic superoxide radicals or increased *intracellular* protection[44]. Certainly, in clinical terms, the concept of an increase in intracellular SOD at the dose rates used (*1/1000 of the total endogenous SOD*) cannot be considered seriously. Increased attachement to membranes could account for the greater efficiency of liposomal SOD compared with the free enzyme.

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