The pharmacokinetics and effects of a long-acting preparation of superoxide dismutase (PC-SOD) in man

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Reactive oxygen species (ROS), like superoxide anion, play an important role in different disease states.
- Superoxide dismutase (SOD) acts as a free radical scavenger by catalysing the dismutation of superoxide.
- Over the last decade, therapeutic use of SOD has been explored, but the results of these experiments have indicated that this has been of limited value, probably due to unfavourable pharmaceutical characteristics of the compounds.

WHAT THIS STUDY ADDS

- Single intravenous administrations of PC-SOD (SOD covalently linked to lecithin) in doses up to 80 mg was well tolerated and biologically active for a period of 19 ± 6 h in healthy White volunteers.
- This suggests that PC-SOD has pharmaceutically appropriate characteristics and may be a possible protective agent for patients in clinical conditions characterized by acute high radical overload.

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AIM

To study the pharmacokinetics (PK), safety and tolerability of single rising doses up to 80 mg of superoxide dismutase covalently linked to lecithin (PC-SOD) in healthy White volunteers.

METHODS

This double-blind, placebo-controlled, four-period cross-over study was performed in eight healthy volunteers (four male/four female). Three doses of PC-SOD (20, 40 and 80 mg) and placebo were administered intravenously in randomized order. Serum and urinary PC-SOD concentrations were measured predose and up to 96 h after dosing. In addition to standard safety measurements, the urinary excretion of N-acetyl- β -glucosaminidase, α -glutathione S-transferase (α -GST) and π -GST was measured to evaluate renal function. The PK of PC-SOD was analysed using noncompartmental and compartmental methods.

RESULTS

All treatments were well tolerated, and no obvious relationship between adverse events and treatment was observed. No effects of PC-SOD on renal function could be detected. Dose normalized C_{max} and AUC were not different between the different dosages, indicating linearity of plasma concentrations with dose. Estimated PC-SOD clearance was 2.54 ml min^{-1} [95% confidence interval (CI) 2.07, 2.83]. The terminal half-life was estimated to be 1.54 days (95% CI 0.93, 2.15). SOD activity was elevated above baseline for 19 ± 6 h after the 80-mg dose.

CONCLUSIONS

Single intravenous administrations of PC-SOD in doses up to 80 mg were well tolerated in healthy White male and female volunteers. With the doses used, SOD activity was linearly related to the dose; after the 80-mg dose it was present for an appreciable period. These findings suggest that it is worthwhile to investigate PC-SOD in clinical conditions characterized by a high radical overload.

Introduction

Reactive oxygen species (ROS), like superoxide anion ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2), play an important role in health and disease. They have been implicated in the pathophysiology of different disease states, including anthracyclin-induced cardiotoxicity (AIC), inflammatory bowel disease, ischaemia/reperfusion injury and neurodegenerative conditions [1–8]. The hypothesis is that in these pathological conditions, relatively large amounts of ROS are produced which cause functional damage to many tissues and even apoptosis [9]. The underlying mechanism for the deleterious effect of ROS on tissues is not fully understood, but includes cell membrane damage due to lipid peroxidation, and direct damage to proteins and DNA [9].

There are different endogenous defence mechanisms against the ROS damage such as superoxide dismutase (SOD), catalase, peroxidases and vitamin A and E which all share free radical scavenger properties [10–12].

SOD acts as a free radical scavenger by catalysing the dismutation of superoxide to hydrogen peroxide and oxygen as shown below:

Three isoforms of SOD exist in humans: cytosolic Cu,Zn SOD (SOD1), mitochondrial MnSOD (SOD2) and extracellular Cu,Zn SOD (SOD3), of which the intracellular forms are the most abundant. The endothelial cell surface is protected by SOD3, but this protection seems insufficient in many clinical conditions, and it has therefore been suggested that additional protection may be of benefit [13]. Indeed, over the last decade therapeutic use of SOD has been explored, but there is consensus that up to now this has been of limited value [14]. Likely explanations for the limited success of exogenously administered SOD are that its intracellular isoforms hardly bind to the endothelium and that they are relatively short-lived [15]. In addition, particularly for SOD3, which is an attractive candidate for therapeutic use, the manufacturing process is difficult.

Therefore, there is a need for SOD preparations that are relatively easy to manufacture, show a reasonably long residence time in the body and will be taken up by organs that are relatively poorly protected against free radicals. This has resulted in the development of PC-SOD (recombinant human SOD1 covalently coupled to an average of four molecules of lecithin) and a chimeric recombinant superoxide dismutase consisting of SOD2 and SOD3 [13, 16, 17].

PC-SOD has a higher affinity to the cell membrane, enhanced distribution to various tissues and a prolonged systemic half-life compared with SOD1 alone. In addition, it has a 4.5-fold increase in oxygen-radical scavenging effects resulting in a 100-fold increase in protective effects against vascular endothelial cell injuries, compared with unmodified SOD [16, 17]. Preclinical data have shown that

PC-SOD is effective in several models, including inflammation, chemotherapy-induced cardiotoxicity, ischaemia-reperfusion injury and motor dysfunction after spinal cord injury [18, 32–38]. The preclinical data also indicated that PC-SOD is well tolerated, although multiple doses to primates were associated with the presence of lipid inclusion bodies in renal tubular cells. However, this was entirely reversible and not associated with functional impairment or necrosis of cells. Thus, PC-SOD is a potentially protective agent in pathological conditions mediated by free radical overproduction [19–22].

In a previous study in Japanese volunteers, where doses up to 20 mg were investigated, PC-SOD was well tolerated, but the duration of increased elevation of SOD activity was only 3 h, which is too short to be of likely clinical relevance. The current study was performed to assess the tolerability, pharmacokinetics (PK) and effects of single (higher) ascending doses of PC-SOD in healthy White volunteers. The study was designed so that detectable SOD activity would be present for a period of 12–24 h. Furthermore, special attention was given to the effects of the compound on renal function and tubular integrity, as this was an issue with very high doses of PC-SOD in preclinical experiments. The effects on renal function were assessed by measurement of the urinary excretion of specific markers for tubular damage (N-acetyl- β -glucosaminidase (NAG), α - and π -glutathione S-transferase (GST) and microalbumin.

Subjects and methods

The study protocol was approved by the Medical Ethical Committee of Leiden University Medical Center (LUMC) and performed according to the principles of the International Conference on Harmonization and Good Clinical Practice and the Helsinki Declaration. Written, informed consent was obtained from all subjects before study entry.

Subjects

Eight healthy subjects (four female and four male) aged between 18 and 45 years and within 20% of the normal body weight range relative to height and frame size were included in this double-blind, placebo-controlled, four-way cross-over study. Subjects were included after a full medical screening showing no clinically significant abnormalities. Subjects were excluded in case of a history of drug allergy or hypersensitivity, or drug, alcohol or nicotine abuse.

Study medication

The subjects were dosed four times using an ascending dose schedule with randomized placebo as summarized in Table 1. Dose escalation was performed when no significant clinical abnormalities were observed after the previous lower dose. The wash-out period between doses was at least 1 week.

Table 1

Administration schedule of superoxide dismutase covalently linked to lecithin (PC-SOD)

Subject code	Study day 1	Study day 2	Study day 3	Study day 4
F1/M1	20 mg PC-SOD	40 mg PC-SOD	80 mg PC-SOD	Placebo
F2/M2	20 mg PC-SOD	40 mg PC-SOD	Placebo	80 mg PC-SOD
F3/M3	20 mg PC-SOD	Placebo	40 mg PC-SOD	80 mg PC-SOD
F4/M4	Placebo	20 mg PC-SOD	40 mg PC-SOD	80 mg PC-SOD

F, Female; M, male.

The PC-SOD preparation consists of an average of four molecules lecithin derivative covalently bound to the human derived CuZn-SOD, produced by genetic recombination using *Escherichia coli* as a host cell. The lecithinized product has 3×10^3 U SOD activity per mg. For this study, a single batch of the lyophilized formulation also containing sucrose was used. Placebo consisted of sucrose. The final preparation that was administered consisted of PC-SOD or placebo diluted with distilled water and 5% mannitol.

Study days

The subjects were admitted to the research unit after an overnight fast. After preparation and baseline measurements, the study drug was administered intravenously over 60 min. During the study days, frequent measurements of vital signs, 12-lead ECG recording and evaluation of adverse events, blood sampling and fractionated urine collection took place. The subjects remained in the unit for 24 h and returned for follow-up assessments and blood sampling at 48 and 96 h after dosing. During the study days, subjects used standard meals and abstained from using xanthine-containing drinks or food.

Sampling and assays

Serum PC-SOD concentrations and SOD activity were measured in venous blood samples taken predose (twice), at 20, 40, 60, 65, 75, 90 min, and at 2, 3, 4, 8, 12, 24, 48, 96 and 168 h after start of the infusion. The last time point coincided with the first predose sample of the subsequent study day. After collection, the tubes were kept at 4°C for and subsequently centrifuged at 2000 g for 10 min at 4°C. The separated serum was stored at -20°C until analysis within 1 month after sampling.

Urine was collected during the study period over the following time spans: 0–4, 4–8, 8–12, 12–24 and 24–48 h. Immediately after voiding, urine samples were stored at 4°C and aliquots of 2 ml were taken from each collection period and stored at -20°C until analysis within 1 month after sampling. Samples to assess antibody formation were taken at completion of the last administration and at 1 and 3 weeks after the last dosing.

Blood samples for routine haematology and biochemistry were taken before and at 24 h after each infusion.

Serum and urinary PC-SOD concentrations were measured using an enzyme-linked immunosorbent assay consisting of an antibody against human Cu, Zn-SOD, and a second antibody against human Cu, Zn-SOD conjugated with horseradish peroxidase. The assay has a lower limit of quantification 626 ng ml⁻¹. Intra- and interassay variability were investigated at PC-SOD concentrations of 626, 2500 and 10 000 ng ml⁻¹ for serum and 626, 5000 and 20 000 ng ml⁻¹ for urine; each concentration in triplicate. The coefficients of variation (CV) for the intra-assay variability for the respective concentrations were 5.6, 3.2 and 1% in serum, and 7.3%, 2.3% and 2.3% in urine. The CVs for the interassay variability in serum and urine were 7.9, 2.7 and 1.3% and 4.9%, 8.2% and 1.2%, respectively. Repeated freezing and thawing had no appreciable effects (CV < 10% after three freeze–thaw cycles).

PC-SOD activity was measured using a nitrite method previously described [23].

The test is based on the principle that when hypoxanthine and xanthine-oxidase are brought together, superoxide anion is formed. When superoxide anion reacts with hydroxylamine, nitrite is formed and this can be measured by colour densitometry with the aid of a colouring reagent. SOD present in serum will inhibit the formation of nitrite by reacting with the superoxide anion. Serum SOD activity was quantified using the reduction in superoxide anion generation caused by serum added to the system. The assay had a lower limit of quantification of 3 µg ml⁻¹. Intra- and interassay variability was 3.9% and 7.5% for serum and 6.8% and 10.9% for urine, respectively. Both assays were performed at Daiichi Pure Chemicals Co. Ltd (Ibaraki, Japan).

Antibody formation against PC-SOD was measured by quantification of specific IgE, IgG and IgM titres. For anti-PC-SOD IgE antibody measurement, antihuman IgE mouse monoclonal antibody (alkaline phosphatase labelled) was used as secondary antibody. The titre was qualitatively judged using the level of the positive control (human anti-perennial rye class IgE antibody) as the reference value and was described as positive if the titre was >0.2 IU ml⁻¹. For anti-PC-SOD-IgG+IgM measurements, antihuman IgG and IgM mouse monoclonal antibody (alkaline phosphatase labelled) was used as secondary antibody. The titre was qualitatively judged in reference to the antibody level of a

pooled normal human serum sample (negative control) and indicated as positive if the value exceeded by 3.1-fold the average value of four normal human serum samples.

Urinary NAG activity was measured using a commercially available colorimetric assay (Roche Diagnostics, Basel, Switzerland; reference value 1.39–3.23 U per 24 h, detection limit 1 U l⁻¹). Urinary excretion of α -GST and π -GST was determined using validated quantitative enzyme immunoassays (Biotrin, Dublin, Ireland; limit of detection α -GST 0.09 μ g l⁻¹ and π -GST 1.72 μ g l⁻¹ and both intra- and interassay variability <6.9%). Urinary microalbumin and creatinine concentrations were measured using routine methodology at the central laboratories for clinical chemistry of LUMC.

Data analysis

Vital signs, ECG and laboratory parameters were analysed by generating average graphs of parameters over time per treatment. If these graphs suggested possible differences between treatments, areas under the effect curve over the first 12 h divided by the corresponding time span (AUC) were calculated and compared between treatments using factorial analysis of variance (factors subject and treatment).

The cumulative urinary excretion of NAG, α -GST, π -GST and creatinine over 0–4 h and over 0–48 h were calculated. For values below the detection limit, the detection limit was used. The cumulative 24-h microalbumin excretion was evaluated as the microalbumin over creatinine ratio. The values were compared between treatments using factorial analysis of variance (factors subject and treatment).

The PK of PC-SOD was assessed using a noncompartmental PK approach for $C_{\max}$, $AUC_{0-48\text{ h}}$ and $AUC_{0-7\text{ days}}$. These parameters were compared between doses after dividing the parameter by the doses using factorial analysis of variance (ANOVA; factors subject and dose) to assess dose linearity. Within-individual ratios for the different doses were compared using paired Student *t*-tests.

Compartmental PK (using a two-compartment open model) was performed on all of the profiles by analysing the data as arising from a multiple dose sequence. The analyses were performed using nonlinear mixed effect modelling, which estimates all curves for all subjects simultaneously. First-order conditional error estimation with the 'interaction' option was used and residual error was modelled as the sum of an additive and a constant coefficient of variation component.

Multiplying the urine weights with the associated concentrations and summing over 48 h calculated the cumulative excretion of PC-SOD. Average renal clearance over this period was calculated by dividing the cumulative renal excretion by the serum AUC over the same time span. Renal clearance was compared between doses using factorial analysis of variance (factors subject and treatment).

The relationship between activity and serum concentration was investigated using graphical and regression

techniques. Linear mixed effect modelling was performed to examine the relationship between PC-SOD concentration and SOD activity.

The compartmental PK analyses were performed using NONMEM version V (GloboMax LLC, Hanover, MD, USA). All statistical calculations were performed using SPSS for Windows software (SPSS, Inc., Chicago, IL, USA).

Results

General

Eight subjects (four female and four male; age range 18–27 years; mean body mass index 23.4 kg m⁻²) were included. All subjects completed the study and no important drug-related adverse events were noted. No serious adverse events occurred during the study. There was no obvious relationship between the occurrence of any adverse event and one of the treatments. The most frequently observed adverse event was an upper respiratory tract infection, which occurred on placebo (twice) as well as on active drug (twice after 20 and 40 mg and three times after 80 mg). Other common adverse events were headache and haematomas after blood sampling. One subject experienced multiple premature ventricular complexes, independent of treatment. No clinically significant changes were observed during any treatment in vital signs, ECG monitoring or routine laboratory tests. No antibodies against PC-SOD were found during two subsequent follow-up visits.

PC-SOD concentrations

On two occasions on which placebo was infused (F3 and F4; both female) concentrations of PC-SOD were found in five samples (5/543 = 0.92%). No explanation for this anomaly could be found, and these data were omitted from the analysis.

Mean plasma profiles are given in Figure 1 and the non-compartmental parameters ($C_{\max}$ and $AUC_{0-7\text{ days}}$) are summarized in Table 2. No significant changes were observed in the dose-normalized $C_{\max}$ ($P = 0.402$) and $AUC_{0-7\text{ days}}$ ($P = 0.102$) for the different doses given, indicating linear PK. The within-individual ratios (40 vs. 80 mg) were 1.98 [95% confidence interval (CI) 1.80, 2.14], 1.99 (95% CI 1.71, 2.27) and 1.88 (95% CI 1.60, 2.16) for $C_{\max}$, $AUC_{0-48\text{ h}}$ and $AUC_{0-7\text{ days}}$, respectively, which confirmed that no significant dose effect was present. The mean cumulative excretion of PC-SOD over 48 h increased with higher doses (Table 3), but renal clearance was independent of the dose ($P = 0.154$).

When the profiles were modelled using a two-compartment model and as if originating from a multiple-dose regimen, a good fit of the data was obtained (Figure 1; Table 2). When the model parameters are expressed differently, estimates for the half-lives can be calculated. This showed that the initial half-life ($t_{1/2\alpha}$) was 11.0 h (95% CI 5.0,

17.0) and terminal half-life ($t_{1/2\beta}$) was 1.54 days (95% CI 0.93, 2.15).

PC-SOD activity

After the 20-mg dose, SOD activity could not be detected for a number of individuals, which may be attributed to the

Figure 1
Mean (+SD) observed superoxide dismutase covalently linked to lecithin (PC-SOD) serum concentration–time profiles (symbols) following intravenous administration of PC-SOD. The lines indicate the predicted profiles based upon the pharmacokinetic modelling. 20 mg, (■); 40 mg, (○); 80 mg, (●)

relatively high limit of quantification. At each higher dose, a higher SOD activity was observed which was present for a longer time period (Figure 2). Mean $\pm$ SD maximum SOD activity increased from $10.4 \pm 2.8 \mu\text{g ml}^{-1}$ after 40 mg to $18.7 \pm 2.0 \mu\text{g ml}^{-1}$ after 80 mg PC-SOD dosing. Analysis after log-transformation revealed a (back-transformed) geometric mean ratio of 1.85 (95% CI 1.53, 2.24), indicating a doubling of activity with a doubling of administered dose. The mean $\pm$ SD duration of the period during which SOD activity was above the limit of quantification increased from 8 ± 3 h after the 40-mg dose to 19 ± 6 h after the 80-mg dose.

Relationship between activity and concentration in serum

Individual graphs indicated that a linear model was most suitable to describe the PC-SOD concentration–SOD activity relationship. The average estimated linear relationship between PC-SOD concentration and SOD activity had an intercept of 650 ng ml^{-1} (95% CI $-746, 2046$) and a slope of 0.913 ng (0.790, 1.036) SOD activity per ng PC-SOD.

Effects on renal function

The urinary excretion of NAG, α -GST, π -GST and microalbumin/creatinine ratio over both 4 h (not shown) and 48 h (Table 3) after each subsequent dose, did not differ between active drug and placebo.

Table 2

Mean (SD; $n=8$) pharmacokinetic parameters of superoxide dismutase covalently linked to lecithin (PC-SOD) administered as an intravenous infusion over 1 h

Parameter	Non-compartmental pharmacokinetic parameters for i.v. SOD, dose (mg)		
	20	40	80
C_{max} ($\mu\text{g ml}^{-1}$)	4.95 (0.91)	9.33 (1.12)	18.38 (2.58)
Dose-normalized C_{max} ($\text{ng ml}^{-1} \text{ mg}^{-1}$)	247 (46)	233 (28)	230 (32)
$\text{AUC}_{0-7 \text{ days}}$ ($\mu\text{g ml}^{-1} \text{ day}^{-1}$)	6.73 (1.77)	11.63 (2.21)	21.67 (4.64)
Dose-normalized $\text{AUC}_{0-7 \text{ days}}$ ($\text{ng ml}^{-1} \text{ day}^{-1} \text{ mg}^{-1}$)	336 (88)	291 (55)	271 (58)
	Compartmental pharmacokinetic parameters for i.v. PC-SOD		
	Mean	95% CI	
Clearance (l day^{-1})	3.53	2.98, 4.08	
Intercompartmental clearance (l day^{-1})	1.17	0.57, 1.77	
Central volume (l)	4.98	4.37, 5.59	
Steady-state volume (l)	7.44	6.70, 8.18	
Initial half-life* (days)	0.47	0.21, 0.72	
Terminal half-life* (days)	1.54	0.93, 2.15	
Residual error			
Constant CV (%)	42.1		
Additive SD (ng ml^{-1})	20.2		

The summary of the noncompartmental analyses is given in the upper part of table and the parameters based upon the population pharmacokinetic approach using a two-compartment pharmacokinetic model are given in the lower part. CV, Interindividual variability in population parameters. *Results from alternative parameterization.

Table 3

Summary of urinary superoxide dismutase covalently linked to lecithin (PC-SOD) excretion

PC-SOD dose	Urinary PC-SOD excretion				P-value
	Placebo	20 mg	40 mg	80 mg	
Cumulative PC-SOD excretion (% dose per 48 h)	NA	1.58 (0.56)	1.03 (0.76)	1.52 (0.54)	NA
Renal clearance PC-SOD over 48 h† (ml min ⁻¹)	NA	0.048 (0.015)	0.036 (0.026)	0.057 (0.022)	NA
Renal safety parameters	Placebo	20 mg	40 mg	80 mg	P-value
NAG (U)*	4.1 (1.8)	4.0 (1.6)	3.7 (1.4)	4.3 (1.4)	0.77
α-GST (μg)*	14.1 (6.5)	18.6 (12.6)	14.5 (8.7)	15.1 (9.1)	0.35
π-GST (μg)*	9.5 (3.2)	10.4 (3.2)	8.9 (2.6)	9.6 (3.1)	0.53
Microalbumin/creatinine ratio 24 h after dose	0.043 (0.031)	0.039 (0.023)	0.044 (0.024)	0.03 (0.017)	0.53

Urinary PC-SOD excretion in 48 h (percentage of dose, SD; n = 8) and renal clearance of PC SOD over 48 h (upper panel). Cumulative urinary excretion of N-acetyl-β-glucosaminidase (NAG), α-glutathione S-transferase (α-GST) and π-GST over 48 h and the ratio of microalbumin over creatinine 24 h after i.v. administration of PC-SOD (lower panel) †Average renal clearance was calculated using the serum AUC over 48 h: renal clearance 0–48 h = cumulative renal excretion 0–48 h/serum AUC 0–48 h. No difference in renal clearance between the different doses was observed (P = 0.154). *Normal values: NAG excretion: 2.8–6.4 U per 48 h; α-GST: <22.2 μg per 48 h; π-GST: <85.2 μg per 48 h.

Figure 2

Mean (SD) superoxide dismutase (SOD) activity profile after intravenous administration of 20, 40 and 80 mg SOD covalently linked to lecithin. 80 mg, (●); 40 mg, (○); 20 mg, (■)

Discussion

This study has shown that single intravenous (i.v.) administration of PC-SOD in doses up to 80 mg was well tolerated in healthy White volunteers. For all safety parameters that were assessed, no treatment effect was observed. Particularly, the absence of effect on renal function is important, as there were indications from preclinical data that PC-SOD could possibly affect renal function. All markers for evaluation of renal function, including protein and creatinine excretion, show no differences between the different PC-SOD doses and placebo. In our assessment urinary NAG, α- and π-GST were included, enzymes used to evaluate tubular damage. The first is derived from tubular lyso-

somes, the latter are cytosolic enzymes that are found in the proximal and distal tubular cells, respectively. All these markers are specific for tubular damage and are very sensitive in detecting renal dysfunction at a very early stage [24]. These findings suggest that single i.v. doses of PC-SOD up to 80 mg are not associated with untoward effects on renal function in humans.

Noncompartmental PK analyses indicated linearity of serum concentrations with increasing dose. The compartmental PK analysis of the PC-SOD profiles was complicated by the occurrence of detectable PC-SOD concentrations in five samples of two subjects (<1% of the total amount of samples) during placebo treatment. Sampling and environmental factors were investigated for these samples, but no explanation was found for the aberrant results. It may be that an interfering endogenous compound was present in these subjects. The data of these samples were omitted and this resulted in an adequate description of the concentration profiles. It was shown that the compound has a relatively small central volume of distribution (5 l) and a low clearance (2.5 ml min⁻¹). As the renal clearance was only approximately 0.05 ml min⁻¹, it is concluded that the clearance is predominantly extrarenal. This is in keeping with data in nonhuman primates using ³H-labelled PC-SOD, showing that only 10% of PC-SOD is excreted unchanged in the urine. Although the exact clearance mechanism of PC-SOD remains to be elucidated, it is likely that the compound is cleared through multiple mechanisms, in which utilization in various biochemical processes, hepatic clearance and inactivation by esterases may play a role.

Previous trials with SOD preparations have failed to show beneficial effects in humans [25]. A cause of this failure could be the short half-life of these compounds. With the doses used in this study, it was shown that SOD activity was linearly related to the dose, and that it was present for an appreciable period. After the 80-mg dose,

SOD activity was elevated above baseline for at least 24 h. This indicates that PC-SOD could be beneficial in pathological conditions characterized by acute ROS overload, such as ischaemia/reperfusion injury, neurological ischaemic disease and AIC [19, 26–28]. Another reason why earlier trials with SOD preparations in humans showed no beneficial effects may be explained by the finding that in these trials the target such as the cytosol and the mitochondria was not reached. This seems necessary, as the intracellular isoforms of SOD, especially, play an important role in protection against myocardial damage after ischaemia/reperfusion [29–31]. Due to its increased affinity for the cell membrane it is possible that with PC-SOD this problem can be overcome [16, 17]. Indeed, several *in vitro* and *in vivo* studies have shown beneficial effects of PC-SOD in various disease models [18, 32–38].

The present study has some shortcomings. First, only serum PC-SOD activity was measured and no information is provided on the presence of PC-SOD intracellularly or in the cell membrane. A small volume of distribution of PC-SOD in humans was found. This suggests that the drug does not have high intracellular penetration and hence its likely therapeutic benefit will be assessable only after demonstration of intracellular activity. However, it may also be that the beneficial effects of PC-SOD are not dependent on the intracellular activity, as the volume of distribution (range 0.05–0.10 l kg⁻¹) in animal species in which the compound was tested for efficacy is comparable to that in humans (0.07 l kg⁻¹). Second, it seems paradoxical that SOD converts O₂⁻ in H₂O₂, which is also a ROS, and is therefore potentially harmful. However, although the exact mechanism is not elucidated, it is apparent that this does not translate into 'clinical damage'. Indeed, many laboratory models have shown that administration of exogenous SOD provides protection against damage induced by free radicals [20, 26–28, 32–38]. Moreover, in protection against free radical-induced damage during the reperfusion phase of ischaemia-reperfusion injury, there are strong indications that SOD is of prime importance [39].

In summary, this study has shown that PC-SOD in doses up to 80 mg was well tolerated in healthy White volunteers. For the 80-mg dose, serum SOD activity was elevated above baseline for at least 19 ± 6 h. These findings suggest that it is worthwhile to investigate PC-SOD further as a protective agent in patients with clinical conditions associated with a high radical overload.

Competing interests:

This study was financially supported by Seikagaku Corporation, Tokyo, Japan and the data will be used for further development of the compound by LTT-Bio-Pharma, Tokyo, Japan. J.S. and Y.M. are employees of LTT-Bio-Pharma.

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