Universiteit Leiden
The Netherlands

Anthracycline-induced cardiotoxicity, a pathophysiology based approach for early detection and protective strategies

Broeyer, F.J.F.

CHAPTER 6

The pharmacokinetics of PC-SOD, a lecithinized recombinant superoxide dismutase, after single- and multiple-dose administration to healthy Japanese and Caucasian volunteers

Based on: J Clin Pharmacol. 2008 Feb;48(2):184-92

Suzuki J, Broeyer FJ, Cohen AF, Takebe M, Burggraaf J, Mizushima Y.

ABSTRACT

To study the pharmacokinetics (PK) of single rising intravenous doses (40-160mg) and repeated doses (80mg for 7 days) of lecithinized superoxide dismutase (PC-SOD) in Japanese volunteers and to compare the PK of PC-SOD between Caucasians and Japanese.

The Japanese study consisted of two parts: a single dose, open-label, dose-escalation and a multiple dose, single-blind, placebo-controlled part. The PK of PC-SOD was determined using non-compartmental and compartmental methods. PK-data from a study with PC-SOD in Caucasians was reanalyzed using the same methodology.

The mean (SD) terminal half-life of PC-SOD in Japanese subjects was 25 (4) hours for the 40mg and 80mg and 31 (15) hours for the 160mg dose. There was non-linearity between dose-normalized C_{max} and clearance (p-values 0.002 and 0.022). After multiple dosing, steady state was reached after 5 days. The observed accumulation ratio was 2.6 (0.5).

The PK of the single 80 mg dose was similar for Japanese and Caucasians.

The PK of PC-SOD was shown to be non-linear with dose which may be attributable to a saturable clearing mechanism. The relative long half-life of PC-SOD (>24 hrs) suggests that it is worthwhile to study the compound as protective agent in clinical conditions with free radical overload.

INTRODUCTION

Overproduction of free radicals, such as the superoxide anion, is associated with the pathology of different diseases.(1-3) Superoxide dismutase (SOD), which catalyses the dismutation of superoxide to hydrogen peroxide and oxygen, is important in the defense against free radical overload.(4) It thus seems logical to develop SOD as a potential treatment modality. However, attempts to achieve this have failed mainly because exogenous SOD has a low affinity for the cell-membrane and has unfavorable pharmacokinetics (e.g. a very short half-life).(5) These characteristics limit the clinical use of SOD, as especially the intra-cellular isoforms of SOD play a role in protection against free-radical induced damage and exogenous SOD needs to be active for a certain period of time to exert its potential protective effect.(5-7)

Therefore a recombinant Cu,Zn SOD, covalently bound to on average 4 molecules of lecithin (PC-SOD), have been developed. In pre-clinical experiments PC-SOD has a 4.5 times greater oxygen-radical scavenging effect, which leads to a 100-fold increase in protective effect against O_2^- induced vascular endothelial cell damage compared with unmodified SOD.(8) In addition, a stronger binding to human vascular endothelial cells was demonstrated.(9) Furthermore, studies in rats showed that PC-SOD had a prolonged residence time, compared to unmodified SOD and was effective in various animal models.(2;3;10-19) These characteristics make PC-SOD a potentially protective agent in various pathological conditions that involve free radical overproduction.

Previous phase I trials in Caucasians demonstrated that PC-SOD was well tolerated in doses up to 80mg, but the pharmacokinetics in other ethnic groups has not been reported yet. This may be of particular importance for the clearance of PC-SOD as apparently most differences caused by ethnic factors occur during drug metabolism.(20)

Therefore, a pharmacokinetic study with single iv doses (up to 160mg) and repeated iv doses (80mg/day for 7days) of PC-SOD in healthy Japanese volunteers was performed. As a previously performed PK study in Caucasians used the same methodology, the PK of the single iv 80 mg dose were compared.

SUBJECTS AND METHODS

Subjects

For the study performed in Japan, eligible for study participation were male Japanese volunteers, within 20% of the normal body weight range relative to height and frame size. All subjects were screened prior to study participation and considered healthy based on history, physical examination and laboratory assessment. This study protocol was approved by IRB of The Kitasato Institute, Research Center for Clinical Pharmacology (formerly known as The Kitasato Institute Bio-Iatric Center). The study in Caucasian subjects was performed as previously described.(21) This protocol was approved by the Medical Ethics Committee of Leiden University Medical Center. From both Japanese and Caucausian subject's written informed consent was obtained before screening.

Study design

The study in Japanese subjects was done in three cohorts of six male volunteers who received escalating single doses of PC-SOD (40, 80 and 160 mg) in an open-label fashion and a single cohort of eight male volunteers who received seven daily doses of PC-SOD (80 mg) in a placebo-controlled design (6 active treatment, 2 placebo). Dose escalation occurred when no clinically significant safety issues were observed in the previous dose-level. The multiple dose part of the study started after completion of the highest dose of the single dose study.

The study in Caucasian subjects consisted of eight healthy subjects (4 female and 4 male) who received single doses of PC-SOD (20, 40 and 80 mg) in a double blind, placebo-controlled, 4-way cross-over study.

Trial medication

Recombinant human SOD (rhSOD) was produced in *Escherichia coli*, the exact procedure is described elsewhere.(8) One of the cysteine residues of rhSOD was converted to S-(2-hydroxyethyl-thio-) cysteine and phosphatidylcholine derivatives were then covalently bound to this modified rhSOD to produce PC-SOD. The specific activity of PC-SOD was about 3,000 U/mg of protein when assayed with the cytochrome C method using a xanthine-xanthine oxidase-cytochrome C system. Vials for injection containing 30 mg of PC-SOD were produced by a freeze-drying process with purified sucrose as an additive. The test drug was dissolved in xylitol 5% (Japan) or mannitol 5% (Netherlands). Placebo consisted of either xylitol or mannitol.

Study days (Japanese)

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. For the participants of the multiple-dose cohort the study drug was administered 7 times with an interval of 24 hours in between. 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 48 hrs (multiple dose: 72 hrs) and returned for follow-up assessments and blood sampling one and two weeks after (last) dosing. During the study days subjects had standard meals and abstained from using xanthine-containing drinks or food.

Sampling (Japanese)

PC-SOD serum concentrations were assessed before administration and at 30, 60, 90 minutes and 2, 3, 5, 9, 13, 25, and 48 hours after dosing (single dose). For the multiple dose part serum PC-SOD concentrations were assessed 60 minutes prior to each administration and at 30, 60, 90 minutes and 2, 3, 5, 9 and 13 hours on day 1 and 4. In addition PC-SOD concentrations were determined 23, 48, 72 and 168 hours after the last administration.

Cumulative urinary PC-SOD concentrations were measured at -12-0, 0-6, 6-12, 12-24, and 24-48 hours (and 48-72 hours for

the multiple dose cohort) after the start of administration, for the single dose cohorts and the first day and the last day of the multiple dose cohort. In addition during day 2 to 6 cumulative urinary PC-SOD concentration was measured for each 24 hour period.

For all cohorts safety laboratory assessments were done before each administration, at 24 hours and 1 week after PC-SOD administration. For the multiple dose cohort additional safety assessments were done at 48 and 72 hours after the last dose.

The study outline for the Caucasian subjects was comparable to those of the Japanese volunteers.(21)

Serum and urinary PC-SOD concentrations were measured using an enzyme linked immunosorbent assay (ELISA), 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 0.626 µg/mL. The intra-assay variability and inter-assay was investigated at PC-SOD concentrations of 0.626, 2.50 and 10.0 µg/ml for serum and 0.626, 5.0 and 20.0 µg/ml for urine (each concentration in triplicate). The coefficients of variation for the intra-assay variability for the respective concentrations were 5.6, 3.2 and 1.0% in serum, and 7.3%, 2.3% and 2.3% in urine. The coefficients of variation for the inter-assay 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 3 freeze/thaw cycles).

Non-compartmental pharmacokinetic analyses

The data were analysed using non-compartmental analysis with estimation of the elimination half-life (ln2/Đz) using log-linear regression of the terminal part of the curve, where the number of included points was determined by the software program WinNonlin V5.0 (Pharsight Corp, Mountain View, CA). Extrapolation of the $AUC_{0-\infty}$ was done using the calculated AUC_{0-last} to which $C_{last/\lambda z}$ was added. The pharmacokinetic parameters of PC-SOD after single doses (for both the Japanese and Caucasian subjects, only 80mg data) were analysed for C_{max}, (maximum observed plasma drug concentration), AUC_{0-24} (area under the plasma drug concentration curve (AUC) from time 0 to 24 hours), AUC_{0-last} (AUC from time 0 to last point measured), $AUC_{0-\infty}$ (AUC from time 0 to infinity), clearance (Cl), volume of distribution (Vd) and terminal elimination half-life (t1/2). The degree of accumulation of PC-SOD expected during the multiple-dose regimen was predicted based on the single-dose data. The predicted accumulation ratio (Rpred) was defined as the $AUC_{0-\infty}$ of the 80mg single-dose cohort divided by AUC_{0-24} of the 80mg single-dose cohort.
After multiple-dose administration, the following parameters were determined from the PC-SOD concentration versus time data: C_{max}, $AUC_{0-\infty}$, AUC_{0-24} and t1/2 after the first administration and C_{max}, AUC_{int} (AUC over the 24 dosing interval during steady state) and t1/2 after the last administration. The observed accumulation ratio (Robs) was defined as AUC_{int} (AUC over the 24 hour dosing interval) on day 7 of the multiple-dose cohort divided by AUC_{0-24} on day 1. The accumulation of PC-SOD in serum at steady-state (Rss, steady-state accumulation ratio) was defined as the AUC_{int} on day 7 of the multiple-dose cohort divided by $AUC_{0-\infty}$ on day 1.

Compartmental pharmacokinetic analyses

Compartmental analysis was performed using the software program WinNonlin V5.0 (Pharsight Corp, Mountain View, CA). A 2-compartment model with macro-constants was used. Observations were iteratively reweighted using the square of the predicted concentration corresponding to a constant coefficient of variation residual error model. Using this model C_{max}, Cl, initial half-life ($t_{½,\ initial}$), terminal half-life ($t_{½,\ initial}$) and Vd were determined for both single- and multiple dose data .

Statistical analysis

Pharmacokinetic parameters were summarized using mean, standard deviation (SD), median, minimum and maximum. Tolerability and safety variables were summarized using descriptive statistics (n, mean, SD, median, minimum and maximum for continuous variables).

Dose-normalized C_{max} and total clearance were used to assess dose-linearity using single factor factorial analysis of variance on log-transformed data (ANOVA; factor dose) to assess dose-linearity. Mean differences and 90%-CI intervals in C_{max} (µg/mL), $t_{½}$ (hr), clearance (mL/hr), volume of distribution and $AUC_{0-\infty}$ (µgαhr/mL) between Japanese and Caucasian were determined using two-sample student t-tests on log-transformed data assuming unequal variances.

RESULTS

General

Twenty-six male Japanese volunteers (age: 20-32, mean BMI: 21.4 kg/m²) were included. In the Caucasian study eight subjects (4 female/4 male, age: 18-27, mean BMI: 23.4 kg/m²) participated.

All Japanese subjects completed the study. No adverse events were observed in 40 and 80mg single dose groups. The most common adverse event was mild diarrhea (twice in the 160mg-group, once in the 80mg multiple dose group, in one subject receiving placebo). These events were considered possibly related to the study drug. Other adverse events were headache, muscle pain, fatigue, pain in the right hip and influenza. These events occurred once and were considered not to be related to the study medication. In one subject in the multiple doses group antibodies against PC-SOD were detected at the first follow up. Follow up at 6 months showed that these antibodies were no longer present.

Safety analysis in Caucasians did not indicate any safety issues, results of the safety analyses are reported elsewhere.(21)

Non-compartmental pharmacokinetic analyses

The mean serum concentrations of PC-SOD versus time curves for the single-dose and the multiple-dose regimens are shown in figure 1 and 2 respectively. A summary of the pharmacokinetic parameters is given in table 1 (single dose) and table 2 (multiple-dose).

Following single-dose intravenous PC-SOD administration in Japanese serum PC-SOD concentrations were elevated above baseline for 24 hours in all doses used. Mean (SD) terminal half-life (t1/2) of PC-SOD was 24.7 (4.3), 24.9 (3.5), 31.3 (14.6) hours for the 3 ascending doses respectively. After 80mg single dose in Caucasian a terminal half-life of 26.1 (11.2) hours was found.

Dose-normalized C_{max} and clearance (Japanese) were 259.4 (31.4), 254.9 (24.7) and 358.0 (74.1) ng/ml/mg and 167.4 (27.4), 143.9 (18.9) and 119.4 (30.0) ml/hr for 40, 80 and 160mg PC-SOD respectively. These data indicated that the pharmacokinetics of PC-SOD is dose-dependent. (p-values 0.002 and 0.022).

Urinary PC-SOD concentrations were below the limit of quantification for the 40mg, and 80mg, but after 160mg PC-SOD the cumulative urinary excretion 0-48hr was 2.28 (1.34) mg, which is 1.4 (0.8)% of the administered dose.

After multiple-dose administration of PC-SOD 80mg C_{max}, day7 was 38.1 (2.1) µg/mL. The AUC_{int} was 649.7 (98.3) hr*µg /mL. Based on the seven trough serum PC-SOD concentrations, steady state was reached after 5 days. The Robs 2.6 (0.4) was greater than the value calculated from the single dose data (Rpred: 2.0 (0.2), p=0.02).Urinary PC-SOD concentrations were below limit of quantification during the multiple-dose regimen.

Compartmental pharmacokinetic analyses

When data were modeled using a 2-compartmental model a good fit was obtained. In two subjects (in the 80 and 160 mg single dose cohort) no adequate estimation of half-life could be calculated. The results after compartmental analyses were comparable to those obtained with non-compartmental analyses (table 3).

Comparison Caucasians-Japanese

The non-compartmental pharmacokinetics of the 80mg single dose administrations were compared between Japanese and Caucasians using C_{max}, clearance, volume of distribution, half-life and $AUC_{0-\infty}$.(table 1)

DISCUSSION

In this study we evaluated the pharmacokinetic profile of PC-SOD following single doses of 40, 80 and 160mg and multiple doses (80mg/day for 7 days) in Japanese volunteers. Additionally, pharmacokinetics of 80mg single dose PC-SOD in Japanese and Caucasian subjects was compared.

The mean plasma concentration versus time curve for the 48 hours following a single dose of PC-SOD was characterized by bi-exponential decline from peak plasma concentration. Half-lives were more than 24 hours for all investigated doses, which is substantially longer than previous reports in trials with unlecithinized SOD.(22;23) The excretion of PC-SOD is predominantly extra-renal, as urinary excretion was less than 2% in the 160mg cohort. This is in line with findings from a previous study in healthy Caucasians, but in contradiction with results in earlier trials with unlecithinized recombinant SOD, where urinary excretions up to 57% were reported. These data suggest that the diminished urinary excretion, and possibly the prolonged half-life, can be attributed to the addition of lecithin to SOD.(22;23) In contradiction with earlier studies in Japanese and Caucasians dose-dependency of the pharmacokinetic parameters was shown, likely because in this study higher doses were studied. As also, C_{max} showed dose-dependency, this strongly suggests a saturable clearance for PC-SOD.

After multiple dosing steady state was reached after 5 days. Pharmacokinetics after the multiple dose regimen showed a similar pattern of distribution and elimination as observed during the single dose cohorts. But some differences were observed. First, terminal half-life was longer than during single dose regimen (56.8 vs 24.9 hours), second a slightly higher accumulation ratio than predicted on the single dose data (R_{pred} 2.0 vs R_{obs} 2.6) was found.

For the higher than expected accumulation and longer half-life of PC-SOD after multiple dosing, some possible explanations can be given. First, during the multiple-dose regimen the final part of $AUC_{0-\infty}$ is better characterized due to longer sampling (48 hours vs. 168 hours in the single- and multiple dose cohort respectively). It is therefore highly likely that the calculated $AUC_{0-\infty}$ during single-dose and following the first dose in the multiple dose regimen is underestimated because of incomplete characterization of the terminal elimination phase. Second, it may be that at higher exposures as in the multiple-dose part makes the observed non-linearity in the single-dose cohorts clearer. When the pharmacokinetic profiles were modeled using a 2-compartment model the estimated pharmacokinetic parameters were comparable to those determined with non-compartmental methods, indicating that we adequately described the pharmacokinetic properties of PC-SOD. Nevertheless, the finding that steady state is reached after approximately 5 days, which is more compatible with a half-life of 24 hrs, may suggest that there is a 'deep' compartment containing very little amounts of drug.(24) Thus for practical reasons it seems that the relevant elimination half-life of PC-SOD is in the order of 24 hours.

Based on our data there are no indications that after 80mg single dose of PC-SOD there are differences of clinical significance between Japanese and Caucasian subjects.

Generally, PC-SOD was well tolerated in doses up to 160 mg. The observation that one of the Japanese subjects developed antibodies against PC-SOD after multiple doses of PC-SOD requires further investigation and the development of antibodies should be monitored in future trials.

In conclusion, this study demonstrates that PC-SOD concentrations were elevated above baseline for at least 24 hours after single doses of PC-SOD greater or equal of 40mg. Dose non-linearity was demonstrated after single doses, indicating saturable clearance. During the multiple-dose regimen steady state was reached after 5 days. Accumulation was slightly higher than expected. It was shown that PK after a single iv dose of 80 mg PC-SOD is similar for healthy Japanese and Caucasian subjects. The pharmacokinetics of PC-SOD make it is worthwhile to further investigate PC-SOD in patients with diseases characterized by high free radical overload.

ACKNOWLEDGEMENT

The authors wish to thank mr Wolf Ondracek MA, who skilfully translated the Japanese documents and was indispensable for the communication between the investigators.

1 Jourd'heuil D, Morise Z, Conner EM, Grisham MB. Oxidants, transcription factors, and intestinal inflammation. J Clin Gastroenterol 1997;25 Suppl 1:S61-S72.

2 Minotti G, Menna P, Salvatorelli E, Cairo G, Gianni L. Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. Pharmacol Rev 2004 Jun;56(2):185-229.

3 Dhalla NS, Elmoselhi AB, Hata T, Makino N. Status of myocardial antioxidants in ischemia-reperfusion injury. Cardiovasc Res 2000 Aug 18;47(3):446-56.

4 Halliwell B, Gutteridge JM. Oxadative stress:adaptation, damage, repair and death. In: Halliwell B, Gutteridge JM, editors. Free Radicals in Biology and Medicine. 3rd ed. Oxford: Oxford University Press; 1999. p. 246-350.

5 McCord JM, Edeas MA. SOD, oxidative stress and human pathologies: a brief history and a future vision. Biomed Pharmacother 2005 May;59(4):139-42.

6 Tanaka M, Mokhtari GK, Terry RD, Balsam LB, Lee KH, Kofidis T, et al. Overexpression of human copper/zinc superoxide dismutase (SOD1) suppresses ischemia-reperfusion injury and subsequent development of graft coronary artery disease in murine cardiac grafts. Circulation 2004 Sep 14;110(11 Suppl 1):II200-II206.

7 Yoshida T, Maulik N, Engelman RM, Ho YS, Das DK. Targeted disruption of the mouse Sod I gene makes the hearts vulnerable to ischemic reperfusion injury. Circ Res 2000 Feb 18;86(3):264-9.

8 Igarashi R, Hoshino J, Takenaga M, Kawai S, Morizawa Y, Yasuda A, et al. Lecithinization of superoxide dismutase potentiates its protective effect against Forssman antiserum-induced elevation in guinea pig airway resistance. J Pharmacol Exp Ther 1992 Sep;262(3):1214-9.

9 Igarashi R, Hoshino J, Ochiai A, Morizawa Y, Mizushima Y. Lecithinized superoxide dismutase enhances its pharmacologic potency by increasing its cell membrane affinity. J Pharmacol Exp Ther 1994 Dec;271(3):1672-7.

10 Logue SE, Gustafsson AB, Samali A, Gottlieb RA. Ischemia/reperfusion injury at the intersection with cell death. J Mol Cell Cardiol 2005 Jan;38(1):21-33.

11 Chan PH. Role of Oxidants in Ischemic Brain Damage. Stroke 1996 Jun 1;27(6):1124-9.

12 Yunoki M, Kawauchi M, Ukita N, Sugiura T, Ohmoto T. Effects of lecithinized superoxide dismutase on neuronal cell loss in CA3 hippocampus after traumatic brain injury in rats. Surg Neurol 2003 Mar;59(3):156-60.

13 Nakagawa K, Koo DD, Davies DR, Gray DW, McLaren AJ, Welsh KI, et al. Lecithinized superoxide dismutase reduces cold ischemia-induced chronic allograft dysfunction. Kidney Int 2002 Mar;61(3):1160-9.

14 Nakajima H, Hangaishi M, Ishizaka N, Taguchi J, Igarashi R, Mizushima Y, et al. Lecithinized copper, zinc-superoxide dismutase ameliorates ischemia-induced myocardial damage. Life Sci 2001 Jul 13;69(8):935-44.

15 Hangaishi M, Nakajima H, Taguchi J, Igarashi R, Hoshino J, Kurokawa K, et al. Lecithinized Cu, Zn-superoxide dismutase limits the infarct size following ischemia-reperfusion injury in rat hearts in vivo. Biochem Biophys Res Commun 2001 Aug 3;285(5):1220-5.

16 Chikawa T, Ikata T, Katoh S, Hamada Y, Kogure K, Fukuzawa K. Preventive effects of lecithinized superoxide dismutase and methylprednisolone on spinal cord injury in rats: transcriptional regulation of inflammatory and neurotrophic genes. J Neurotrauma 2001 Jan;18(1):93-103.

17 Nakajima H, Ishizaka N, Hangaishi M, Taguchi J, Itoh J, Igarashi R, et al. Lecithinized copper, zinc-superoxide dismutase ameliorates prolonged hypoxia-induced injury of cardiomyocytes. Free Radic Biol Med 2000 Jul 1;29(1):34-41.

18 Shimmura S, Igarashi R, Yaguchi H, Ohashi Y, Shimazaki J, Tsubota K. Lecithin-bound superoxide dismutase in the treatment of noninfectious corneal ulcers. Am J Ophthalmol 2003 May;135(5):613-9.

19 Tsubokawa T, Jadhav V, Solaroglu I, Shiokawa Y, Konishi Y, Zhang JH. Lecithinized superoxide dismutase improves outcomes and attenuates focal cerebral ischemic injury via antiapoptotic mechanisms in rats. Stroke 2007 Mar;38(3):1057-62.

20 Kim K, Johnson JA, Derendorf H. Differences in drug pharmacokinetics between East Asians and Caucasians and the role of genetic polymorphisms. J Clin Pharmacol 2004 Oct;44(10):1083-105.

21 Broeyer FJF, van Aken BE, Suzuki J, Kemme MJB, Schoemaker RC, Cohen AF, et al. The pharmacokinetics and effects of a long-acting preparation of superoxide dismutase (PC-SOD) in man. British Journal of Clinical Pharmacology. In press 2007.

22 Uematsu T, Nagashima S, Umemura K, Kanamaru M, Nakashima M. Pharmacokinetics and safety of intravenous recombinant human superoxide dismutase (NK341) in healthy subjects. Int J Clin Pharmacol Ther 1994 Dec;32(12):638-41.

23 Tsao C, Greene P, Odlind B, Brater DC. Pharmacokinetics of recombinant human superoxide dismutase in healthy volunteers. Clin Pharmacol Ther 1991 Dec;50(6):713-20.

24 Darwish M, Kirby M, Robertson P, Jr., Hellriegel E, Jiang JG. Single-Dose and Steady-State Pharmacokinetics of Fentanyl Buccal Tablet in Healthy Volunteers. The Journal of Clinical Pharmacology 2007 Jan 1;47(1):56-63.

Table 1 Non-compartmental pharmacokinetic parameters in Japanese and Caucasian volunteers.

		Japanese			Caucasians	Caucasians vs. Japanese
DoseParameter		40 mg (N=6)	80 (N=6)	160 (N=6)	80 mg (N=8)	Mean of difference†
C_{max} (µg/mL)	Mean (SD)	10.4 (1.3)	20.4 (2.0)	57.3 (11.9)	18.4 (2.6)	0.86 (0.73-1.02)
	Median (min-max)	10.0 (9.3-12.6)	21.1 (17.7-22.9)	54.9 (44.0-79.4)	18.2 (14.3-23.0)	
t ½ (hr)	Mean (SD)	24.7 (4.3)	24.9 (3.5)	31.3 (14.6)	26.1 (11.2)	1.02 (0.77-1.34)
	Median (min-max)	25.3 (18.9-29.4)	25.5 (21.1-30.5)	25.3 (22.2-60.1)	23.0 (14.7-48.1)	
Clearance (mL/hr)	Mean (SD)	167.4 (27.4)	143.9 (18.9)	119.4 (30.0)	167.9 (35.3)	0.86 (0.73-1.02)
	Median (min-max)	169.2 (134.7-208.7)	153.9 (116.9-160.5)	121.4 (66.4-157.4)	173.4 (123.3-219.0)	
Vd (L)	Mean (SD)	5.62 (1.31)	4.88 (0.55)	4.58 (0.58)	5.81 (2.38)	1.02 (0.77-1.34)
	Median (min-max)	5.7 (3.9-7.0)	4.7 (4.3-5.7)	4.7 (3.8-5.4)	5.03 (3.73-10.74)	
$AUC_{0-\infty}$ (µgαhr/mL)	Mean (SD)	244.4 (40.0)	564.6 (81.2)	1440.0 (493.7)	496.3 (108.3)	1.16 (0.98-1.37)
	Median (min-max)	236.4 (191.6-297.0)	519.7 (498.4-684.3)	1318.5 (1016.7-2411.2)	461.4 (365.2-648.8)	
Percent extrapolation	Mean (SD)	25.1(6.4)	24.9(3.5)	30.7(12.2)	24.9(11.6)	NA
	Median (min-max)	27.1 (15.7-31.4)	25.5 (21.1-30.5)	25.5 (22.2-54.4)	21.9 (8.1-44.6)	

C_{max}, maximum observed serum drug concentration; $t_{1/2}$, half-life; V_d, volume of distribution; $AUC_{0-\infty}$, AUC from time 0 to infinity; † 90%-confidence intervals between brackets.

Table 2 Comparison of the pharmacokinetic parameters of intravenous single-dose and multiple-dose PC-SOD administrations.

Parameter		Single dose PC-SOD 80mg	Multiple dose PC-SOD 80 mg dose (after first dose)	Multiple dose PC-SOD 80 mg dose (after last dose)
Cmax (µg/mL)		20.4 (2.0)	20.4 (1.4)	38.1 (2.1)
		21.1 (17.7-22.9)	21.0 (18.0-21.8)	39.2 (35.2-39.9)
AUC0-24 (hrαµg/mL)	Mean (SD)	281.5 (30.6)	253.9 (53.9)	NA
	Median (min-max)	283.6 (241.3-321.9)	255.8 (170.7-321.5)	NA
AUCint (hrαµg/mL)	Mean (SD)	NA	NA	649.7 (98.3)
	Median (min-max)	NA	NA	673.8 (514.4-742.2)
$AUC_{0-\infty}$ (hrαµg/mL)	Mean (SD)	564.6 (81.2)	411.8 (151.3)	NA
	Median (min-max)	236.4 (191.6-297.0)	397.3 (215.0-620.3)	NA
Percent extrapolation	Mean (SD)	26.1 (3.9)	37.0(12.6)	20.0 (4.4)
	Median (min-max)	26.6 (21.2-31.8)	36.8 (22.0-51.3)	19.9 (14.5-25.5)
t ½ (hours)	Mean (SD)	24.9 (3.5)	16.5 (5.1)	56.8 (20.8)
	Median (min-max)	25.5 (21.1-30.5)	16.2 (10.6-22.1)	58.3 (34.0-87.1)
Predicted accumulation ratio, Rpred	Mean (SD)	2.0 (0.2)	NA	NA
	Median (min-max)	2.0 (1.8-2.2)	NA	NA
Observed accumulation ratio, Robs	Mean (SD)	NA	NA	2.6 (0.4)
	Median (min-max)	NA	NA	2.5 (2.0-3.2)
Steady-state accumulation ratio, Rss	Mean (SD)	NA	NA	1.7 (0.6)
	Median (min-max)	NA	NA	1.7(1.0-2.4)

C_{max}, maximum observed serum drug concentration; $t_{0-\infty}$, half-life; AUC_{0-24}, area under the plasma drug concentration versus time curve (AUC) from time 0 to 24 hours; AUC_{int}, AUC over 1 dosing interval during steady state; $AUC_{0-\infty}$, AUC from time 0 to infinity.
R_{pred}, predicted accumulation ratio, defined as $AUC_{0-\infty}$ divided by AUC_{0-24}; R_{obs}, observed accumulation ratio, defined at AUC_{int} on day 7 divided by AUC_{0-24} on day 1; R_{ss}, steady-state accumulation ratio, defined as AUC_{int} on day 7 of the multiple-dose cohort divided by $AUC_{0-\infty}$ on day 1.

Table 3 Compartmental analyses of pharmacokinetic parameters in Japanese volunteers.

Parameter		Single dose PC-SOD 40 mg intravenous		Single dose PC-SOD 80 mg intravenous		Single dose PC-SOD 160 mg intravenous		Multiple dose PC-SOD 80 mg dose	
		Estimate	StandardError	Estimate	StandardError	Estimate	StandardError	Estimate	StandardError
C_{max} (µg/mL)	Mean (SD)	11.3 (2.4)	0.8 (0.4)	20.1 (2.7)	0.9 (0.5)	51.4 (5.8)	3.1 (1.6)	19.9 (1.1)	1.2 (0.3)
	Median (min-max)	11.0 (8.9-15.2)	0.7 (0.5-1.5)	20.2 (16.8-24.1)	0.8 (0.3-1.4)	49.8 (45.3-61.7)	2.7 (1.8-6.1)	20.3 (18.1-20.9)	1.2 (0.9-1.6)
	%-CV	7.2 (2.5)		4.2 (2.0)		5.9 (2.3)		5.9 (1.3)	
Vd (L)	Mean (SD)	6.0 (1.5)	0.6 (0.3)	5.4 (1.0)	1.3 (1.6)	6.5 (2.2)	3.4 (3.0)	7.5 (1.4)	0.5 (0.3)
	Median (min-max)	6.1 (4.0-8.1)	0.6 (0.3-1.0)	5.4 (4.2-6.5)	0.7 (0.3-4.1)	6.4 (4.2-10.1)	2.8 (0.8-8.1)	7.1 (5.7-9.4)	0.4 (0.3-0.9)
	%-CV	9.5 (2.3)		21.8 (23.7)		46.4 (27.6)		6.4 (2.1)	
Clearance (mL/hr)	Mean (SD)	163.0(27.1)	15.5(7.4)	134.6(20.0)	20.5(18.7)	93.4(30.4)	45.3(29.0)	110.7(22.3)	4.1(2.3)
	Median (min-max)	162.5 (132.0-208.8)	13.3 (8.6-25.6)	127.8 (109.7-158.6)	14.5 (7.7-53.4)	95.0 (47.2-126.8)	46.4 (14.4-84.7)	106.0 (84.7-149.5)	3.3 (2.5-8.4)
	%-CV	9.5 (4.3)		15.8 (15.0)		58.2 (42.3)		3.6 (1.2)	
t ½, initial (hours)	Mean (SD)	1.4 (0.8)	0.8 (0.6)	3.9 (3.0)	2.6 (1.8)	4.5 (1.1)	2.2 (0.7)	5.5 (3.2)	1.7 (0.8)
	Median (min-max)	1.7 (0.4-2.3)	0.7 (0.2-1.9)	3.2 (0.7-8.0)	2.5(0.6-5.3)	4.3 (3.3-5.9)	2.0 (1.5-3.3)	4.9 (2.3-5.5)	1.7 (0.7-1.7)
	%-CV	56.0 (29.8)		72.0 (14.7)		50.9 (19.8)		32.5 (8.8)	
t ½, terminal (hours)	Mean (SD)	27.2 (6.0)	5.0 (2.6)	31.0 (9.4)	16.8 (22.5)	63.7 (35.7)	81.7 (71.9)	54.7 (10.2)	6.0 (2.6)
	Median (min-max)	28.4 (20.2-35.2)	4.7 (2.3-8.6)	27.7 (22.1-43.5)	6.6 (2.4-55.9)	54.8 (29.1-103.8)	75.0 (9.7-166.6)	56.5 (38.4-54.7)	6.5 (2.4-6.0)
	%-CV	17.3 (5.8)		43.6 (49.1)		104.6 (60.8)		10.6 (3.7)	

C_{max}, estimated maximum drug concentration; $t_{½\ initial}$,initial half-life; $t_{½\ initial}$, terminal half-life; V_d, volume of distribution; %-cv, mean coefficient of variation in percentage with standard deviation between brackets.

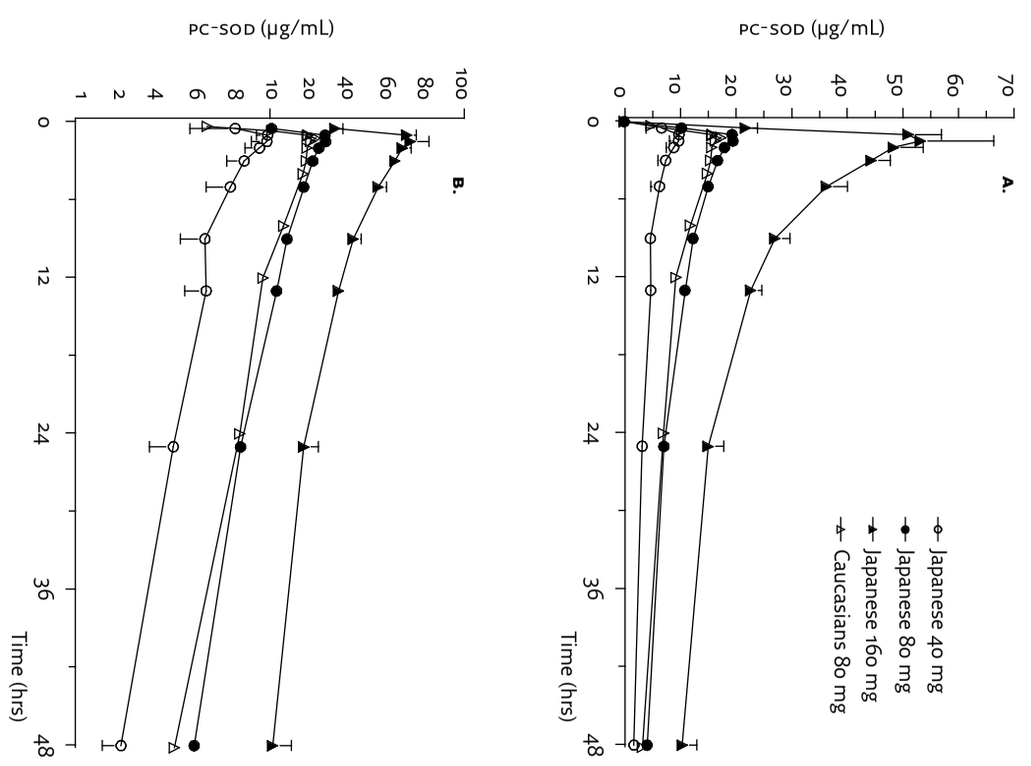

Figure 1A & B Serum PC-SOD concentration (mean ± SD) after single intravenous doses in Japanese (N=6, open circles 40 mg, closed circles 80 mg, closed triangles 160 mg) Caucasian (N=8, open triangles 80 mg) volunteers.

Figure 2 Serum PC-SOD concentration (mean) after repeated administration of 80 mg/day intravenously for seven days in Japanese volunteers.