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

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CHAPTER 5

Disturbed iron metabolism due to anthracycline-based chemotherapy in early stage, surgically cured female breast cancer patients

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ABSTRACT

AIM To study the pharmacokinetics, safety and tolerability of single rising doses up to 80mg of PC-SOD in healthy Caucasian volunteers.

METHODS This double blind, placebo controlled, 4-period cross over study was performed in eight healthy volunteers (4 male/4 female). Three doses of PC-SOD (20, 40 and 80 mg) and placebo were administered iv in randomised order. Serum and urinary PC-SOD concentrations were measured pre-dose and up to 96 hours after dosing. In addition to standard safety measurements, the urinary excretion of NAG, α -GST, p-GST was measured to evaluate renal function. The PK of PC-SOD was analysed using non-compartmental 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 normalised 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 (95%-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 hours after the 80mg dose.

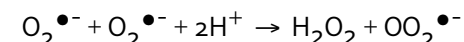
DISCUSSION Single iv administrations of PC-SOD in doses up to 80 mg were well tolerated in healthy Caucasian male and female volunteers. With the doses used, SOD-activity was linearly related to the dose, after the 80mg dose it was present for an appreciable period. These findings suggest that it is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

INTRODUCTION

Reactive oxygen species (ROS), like super oxide anion ($O_2^{\bullet-}$) 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 anthracycline-induced cardiotoxicity (AIC), inflammatory bowel disease, ischemia/reperfusion injury and neurodegenerative conditions.(1-8) The hypothesis is that in these pathologic 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 totally unravelled, 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 iso-forms 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 more abundant. The endothelial cell surface is protected by SOD3, but this protection seems insufficient in many clinical conditions and therefore it has 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 the intracellular iso-forms of SOD hardly bind to the endothelium and that they are relatively short-lived. In addition, particularly for SOD3, which is an attractive candidate for therapeutic use, the manufacturing process is difficult.(15)

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 4 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, an enhanced distribution to various tissues and a prolonged systemic half-life compared to 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 to unmodified sOD. (16) Pre-clinical data showed that PC-sOD is effective in several models including inflammation, chemotherapy-induced cardiotoxicity, ischemia-reperfusion injury, and motor dysfunction after spinal cord injury. (18) The pre-clinical data also indicated that PC-sOD is well tolerated, although multiple doses to monkeys 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 20mg were investigated, PC-sOD was well tolerated, but the duration of increased elevation of sOD activity was only 3 hrs which is too short to be of likely clinical relevance. The current study was performed to assess the tolerability, pharmacokinetics and effects of single (higher) ascending doses of PC-sOD in healthy Caucasian volunteers. The study was designed such that detectable sOD activity would be present for a period of 12-24 hrs. 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 pre-clinical 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 γ -glutathione S-transferase (GST)) and microalbumin.

SUBJECTS AND METHODS

The study protocol was approved by the Medical Ethical Committee of the Leiden University Medical Center and performed according to the principles of the International Conference on Harmonisation and Good Clinical Practise and the Helsinki Declaration. Written informed consent was obtained for all subjects before study entry.

Subjects

Eight healthy subjects (4 female and 4 male) aged between 18-45 years and within 20% of the normal body weight range relative to height and frame size were included in this double blind, placebo-controlled, 4-way cross over study. Subjects were included after a full medical screening showed 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 4 times using an ascending dose schedule with randomised placebo as summarised in table 1. Dose escalation was performed when no significant clinical abnormalities were observed after the previous lower dose. The washout period between doses was at least 1 week.

The PC-sOD preparation consists of an average of 4 molecules lecithin derivative covalently bound to the human derived CuZn-sOD, produced by genetic recombination using *E.coli* as a host cell. The lecithinised product has 3x10³ U sOD-activity per mg. For this study, a single batch of the lyophilised 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 hrs and returned for follow-up assessments and blood sampling at 48 and 96 hours after dosing. During the study day's 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 that were taken pre-dose (twice), at 20, 40, 60, 65, 75, 90 min, and at 2, 3, 4, 8, 12, 24, 48, 96 and 168 hrs after start of the infusion. The last time point coincided with the first pre-dose sample of the subsequent study day. After collection, the tubes were kept at 4°C for and subsequently centrifuged at 2000g for 10 minutes 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 hr, 4-8 hr, 8-12 hr, 12-24 hr, and 24-48 hr. Urine samples were, immediately after voiding, stored at 4°C and from each collection period, aliquots of 2 ml were taken 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 hrs after each infusion.

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 626 ng/mL. The intra-assay variability and inter-assay was investigated at

PC-SOD concentrations of 626, 2500 and 10000 ng/ml for serum and 626, 5000 and 20000 ng/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% 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).

PC-SOD activity was measured using a nitrite method previously described.(23)

The test is based upon 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. 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, anti-human 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 higher than 0.2 IU/ml. For anti-PC-SOD-IgG+IgM measurements anti-human 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 the average value of 4 normal human serum samples 3.1-fold.

Urinary NAG activity was measured using a commercially available colorimetric assay (Roche Diagnostics, Switzerland,

reference value: 1.39 - 3.23 U/24hr, detection limit 1 U/L). Urinary excretion of alpha-glutathione S-transferase (α -GST) and pi-glutathione S-transferase (π -GST) was determined using validated quantitative enzyme immunoassays (Biotrin, Dublin, Ireland; limit of detection: α -GST 0.09 μ g/L and π -GST 1.72 μ g/L and both intra- and inter assay variability below 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 hours 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 NAC, α -GST, π -GST and creatinine over 0-4h and over 0-48h were calculated. For values below the detection limit, the detection limit was used. The cumulative 24 hours 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 pharmacokinetics of PC-SOD was assessed using a non-compartmental PK approach for $C_{\max}$, AUC_{0-48hr} and $AUC_{0-7days}$. 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 pharmacokinetics (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 non-linear 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 hours 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 pharmacokinetic analyses were performed using NONMEM version V (GloboMax LLC, Hanover, MD). All statistical calculations were performed using SPSS for Windows software (SPSS, Inc., Chicago, IL).

RESULTS

General

Eight subjects (4 female and 4 male; age range: 18-27 years; mean BMI: 23.4 kg/m²) 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 haematoma's after blood sampling. One subject experienced multiple premature ventricular complexes, independent of treatment. No clinical significant changes were observed during any treatment in vital signs, ECG-monitoring, and the routine laboratory tests. No antibodies against PC-SOD were found during 2 subsequent follow up visits.

PC-SOD concentrations

For two occasions at which placebo was infused (F3 and F4; both female) concentrations of PC-SOD were found in 5 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 summarised in table 2. No significant changes were observed in the dose normalised $C_{\max}$ ($p=0.402$) and $AUC_{0-7\text{days}}$ ($p=0.102$) for the different doses given, indicating linear pharmacokinetics. The within individual ratio's (40 vs 80mg) were 1.98 (95%-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} 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 hours increased with higher doses (table 3), but renal clearance was independent of the dose ($p=0.154$).

When the profiles were modelled using a 2-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 hours (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 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 ± 2.8 $\mu\text{g}/\text{ml}$ after 40 mg to 18.7 ± 2.0 $\mu\text{g}/\text{ml}$ 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 hours after the 40mg dose to 19 ± 6 hours after the 80mg 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 (95% CI: -746 - 2046) and a slope of 0.913 (0.790 - 1.036) ng 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 (not shown) and 48 hours (table 3) after each subsequent dose, did not differ between active drug and placebo.

DISCUSSION

This study showed that single iv administration of PC-SOD in doses up to 80 mg was well tolerated in healthy Caucasian 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 pre-clinical data that PC-SOD could possibly affect renal function. All markers for evaluation of renal function, including protein and creatinine excretion, did not show 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 lysosomes, 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 in a very early stage.(24)

These findings suggest that single iv doses of PC-SOD up to 80 mg is not associated with untoward effects on renal function in humans.

Non-compartmental pharmacokinetic analyses indicated linearity of serum concentrations with increasing dose. The compartmental pharmacokinetic analysis of the PC-SOD profiles was complicated by the occurrence of detectable PC-SOD concentrations in 5 samples of 2 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). As the renal clearance was only approximately 0.05 ml/min, it is concluded that the clearance is predominantly extra-renal. This is in keeping with data in non-human 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 among which utilization in various biochemical processes, hepatic clearance and inactivation by esterase's may play a role.

Previous trials with SOD-preparations 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, the SOD-activity was elevated above baseline for at least 24 hrs. This indicates that PC-SOD could be beneficial in pathological conditions characterised with an acute ROS-overload, like ischemia/reperfusion injury, neurological ischemic disease and AIC.(19;26-28) Another reason why earlier trials with SOD-preparations in humans did not show 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 especially the intra-cellular isoforms of SOD play

an important role in the protection against myocardial damage after ischemia/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) Indeed, several in vitro and in vivo studies showed beneficial effects of PC-SOD in various disease models.(19;26-28;32-38)

The study reported here has some shortcomings. First, only serum PC-SOD activity was measured and no information is provided on the presence of PC-SOD intracellularly or at the cell membrane. In this study we found a small volume of distribution of PC-SOD in humans. This suggests that the drug does not have high intracellular penetration and hence its likely therapeutic benefit will only be assessable 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) in animal species in which the compound was tested for efficacy is comparable to the volume of distribution in humans (0.07 L/kg). Second, it seems paradoxical that SOD converts $O_2^{\bullet-}$ in H_2O_2 which is also a ROS, and 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 show that administration of exogenous SOD provides protection against damage induced by free radicals.(19;26-28;32-38) Moreover, in the protection against free radical induced damage during the reperfusion phase of ischemia-reperfusion injury, there are strong indications that SOD is of prime importance.(39)

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

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Table 1 Administration schedule of 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

Table 2 Mean (SD; n=8) Pharmacokinetic parameters of PC-SOD administered as iv-infusion over 1 hour. The summary of the non-compartmental analyses is given in the upper part of table and the parameters based upon population pharmacokinetic approach using a 2-compartment pharmacokinetic model are given in the lower part of the table.

Non-compartmental pharmacokinetic parameters for iv sod			
	DOSE (mg)		
Parameter	20	40	80
C _{max} (µg/ml)	4.95 (0.91)	9.33 (1.12)	18.38 (2.58)
Dose-normalised C _{max} (ng/ml/mg)	247 (46)	233 (28)	230 (32)
AUC _{0-7days} (µg/ml•day)	6.73 (1.77)	11.63 (2.21)	21.67 (4.64)
Dose-normalised AUC _{0-7days} (ng/ml•day/mg)	336 (88)	291 (55)	271 (58)
Compartmental pharmacokinetic parameters for iv PC-SOD			
	MEAN	95%-confidence interval	
Clearance(L/day)	3.53	2.98 – 4.08	
Intercompartmental clearance(L/day)	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)	20.2		

cv: inter-individual variability in population parameters; *results from alternative parameterisation.

Table 3 Summary of urinary PC-SOD excretion. Urinary PC-SOD excretion in 48 hours (percentage of dose, SD; n=8) and renal clearance of PC SOD over 48 hours. (upper panel) Cumulative urinary excretion of NAG, a-GST and p-GST over 48 hours and the ratio of microalbumin over creatinine 24 hr after iv administration of PC-SOD. (lower panel)

Urinary PC-SOD excretion					
PC-SOD dose	Placebo	20 mg	40 mg	80 mg	P-value
Cumulative PC-SOD excretion (% dose per 48 hours)	NA	1.58 (0.56)	1.03 (0.76)	1.52 (0.54)	NA
Renal clearance PC-SOD over 48 hours† (ml/min)	NA	0.048 (0.015)	0.036 (0.026)	0.057 (0.022)	NA
Renal safety parameters					
PC-SOD dose	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
a-GST (µg)*	14.1 (6.5)	18.6 (12.6)	14.5 (8.7)	15.1 (9.1)	0.35
p-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

† Average renal clearance was calculated using the serum AUC over 48 hours: renal clearance_{0-48h} = cumulative renal excretion_{0-48h}/serum AUC_{0-48h} No difference in renal clearance between the different doses was observed (p=0.154)

* normal values: NAG-excretion: 2.8 – 6.4U per 48 hours;a-GST: < 22.2 µg per 48 hours; p-GST: < 85.2 µg per 48 hours

Figure 1 Mean (+SD) observed pc-sod serum concentration-time profiles (symbols) following iv administration of pc-sod. The lines indicate the predicted profiles based upon the pharmacokinetic modelling.

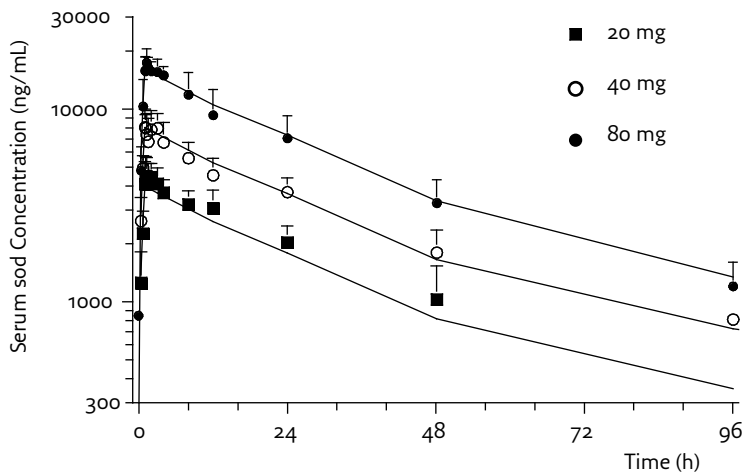


Figure 2 Mean (SD) sod activity profile after intravenous administration of 20, 40 and 80 mg pc-sod.

