Pharmacokinetics and Preventive Effects of Targeted Catalase Derivatives on Hydrogen Peroxide-Induced Injury in Perfused Rat Liver

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Purpose. To investigate the pharmacokinetics and preventive effects of liver-targeted catalase (CAT) derivatives on hepatic injury caused by reactive oxygen species.

Methods. The hepatic uptake of ^{111}In-CAT, galactosylated (Gal-), mannosylated (Man-) and succinylated (Suc-) CAT was investigated in isolated perfused rat livers in a single-pass constant infusion mode. Then, pharmacokinetic parameters were obtained by fitting equations derived from a one-organ pharmacokinetic model to the outflow profile. Their effects in preventing hydrogen peroxide-induced injury were determined by lactate dehydrogenase (LDH) release from the perfused liver.

Results. The extraction of CAT derivatives by the liver was dose-dependent, and increased by the chemical modifications described. After being bound to the cell surface, chemically modified CAT derivatives were internalized by the liver faster than CAT. Pre-perfusion of a CAT derivative significantly reduced LDH release by hydrogen peroxide at least for 30 min, and Man-CAT and Suc-CAT effectively inhibited this release.

Conclusions. Internalized CAT derivatives are also effective in degrading hydrogen peroxide and targeted delivery of CAT to liver nonparenchymal cells by mannosylation or succinylation is a useful method for the prevention of hepatic injury caused by reactive oxygen species.

KEY WORDS: catalase; pharmacokinetics; perfused rat liver; chemical modification; reactive oxygen species.

INTRODUCTION

There is a great deal of evidence to suggest that reactive oxygen species (ROS) are implicated in the pathogenesis of many diseases, including atherosclerosis, cancer and Alzheimer's disease (1–3). Therefore, antioxidant enzymes such as catalase (CAT) and superoxide dismutase (SOD) have been considered as therapeutic agents for ROS-mediated injuries and diseases. In a series of studies, we have demonstrated that bovine liver CAT rapidly accumulates in hepatocytes (or liver parenchymal cells; PC) after intravenous injection (4). We developed several derivatives of CAT and SOD by chemically modifying them in order to control their pharmacokinetic behavior (5,6). It has been shown that targeted delivery of SOD and CAT to liver nonparenchymal cells (NPC) through receptor-mediated endocytosis is a promising approach to prevent hepatic ischemia/reperfusion injury (6–8). However, the major fraction of these enzyme derivatives taken up by cells via receptor-mediated endocytosis is normally transferred to and degraded in lysosomes. The binding and internalization characteristics of these chemically modified enzymes, as far as liver cells are concerned, should depend on the physicochemical properties of the ligand and the receptor involved in the uptake. To obtain the maximal therapeutic effects following delivery of these enzymes, the spatial and temporal relationships between the enzyme delivery and ROS production need to be quantitatively examined.

In this study, the hepatic disposition characteristics of ^{111}In-CAT derivatives were investigated in isolated perfused rat livers. The venous outflow patterns of these derivatives were analyzed based on a one-organ pharmacokinetic model including the binding and internalization processes to obtain the binding constant, maximum binding site and internalization rate of each derivative. Then, the inhibitory effects of pre-treatment with CAT derivatives on hydrogen peroxide (H_2O_2)-induced hepatic injury were evaluated by measuring lactate dehydrogenase (LDH) release to the outflow medium of the perfused liver. Based on the results obtained, targeted delivery of CAT to liver NPC was revealed to be an effective approach to inhibit hepatic injury caused by H_2O_2.

MATERIALS AND METHODS

Animals

Male Wistar rats (180–220 g) were purchased from the Shizuoka Agricultural Cooperative Association for Laboratory Animals (Shizuoka, Japan). All animal experiments were carried out in accordance with the Principles of Laboratory Animal Care as adopted and promulgated by the US National Institutes of Health and with the Guideline for Animal Experiments of Kyoto University.

Chemicals

Bovine liver CAT was purchased from Sigma Chemical (St. Louis, MO, USA). CAT was subjected to gel filtration

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ABBREVIATIONS: AUC, the area under the plasma concentration-time curve; BSA, bovine serum albumin; CAT, catalase; C_b, inflow concentration; C_{in}, concentration in the perfusate before passing through the liver; CL_h, hepatic clearance; C_{out}, concentration in the perfusate after passing through the liver; C_s, sinusoidal concentration; CL_{total}, total body clearance; CL_{liver}, hepatic uptake clearance; DTPA, diethylenetriaminepentaacetic acid; E_{ss}, the extraction ratio; Gal-CAT, galactosylated CAT; K, binding constant; k_{int}, internalization rate constant; LDH, lactate dehydrogenase; Man-CAT, mannosylated CAT; NPC, liver nonparenchymal cells; PC, liver parenchymal cells; Q, flow rate; ROS, reactive oxygen species; SOD, superoxide dismutase; Suc-CAT, succinylated CAT; V_s, sinusoidal volume; X, binding amount; X_∞, maximum binding amount.

chromatography and only the fractions containing tetramer of the subunits with a molecular weight of about 240 kDa were used in the experiments (4). Diethylenetriaminepentaacetic acid (DTPA) anhydride was purchased from Dojindo Laboratory, Kumamoto, Japan. ^{111}Indium chloride ($[^{111}In]InCl_3$) was supplied by Nihon Medi-Physics Co., Takarazuka, Japan. All other chemicals were of the highest grade available.

Synthesis and Characterization of CAT Derivatives

Galactosylated (Gal-), mannosylated (Man-) and succinylated (Suc-) CAT derivatives were synthesized by the method reported previously (6). About 70% of the total protein amino groups were used for chemical modification in all derivatives. The enzymatic activity of CAT derivatives was measured by monitoring their ability to degrade hydrogen peroxide (9) and satisfactory enzymatic activity (>86% of each unmodified enzyme; 86, 90 and 97% of the original activity for Suc-CAT, Gal-CAT, and Man-CAT, respectively) was found to remain after each modification. For disposition experiments, CAT derivatives were radiolabeled with ^{111}In using DTPA anhydride as reported previously (10).

In Vivo Disposition Experiment

Rats were anesthetized by intraperitoneal injection of sodium pentobarbital (20 mg/kg). The bile duct and urinary bladder were canulated to allow the collection of bile and urine, respectively. A bolus injection of a solution of ^{111}In-CAT was made into the femoral vein at a dose of 0.1, 1, or 10 mg/kg. At given periods after injection, blood samples (0.2 ml) were withdrawn from the jugular vein over 2 h and centrifuged at 2,000 g for 2 min to obtain plasma. At 2 h after injection, the kidney, liver, spleen, heart, and lung were excised, rinsed with saline and weighed. ^{111}In-radioactivity in the plasma and organs was counted with a well-type NaI scintillation counter (ARC-500, Aloka, Tokyo, Japan). The ^{111}In-radioactivity concentration in plasma was normalized to the % of dose/ml and the area under the plasma concentration-time curve (AUC) for the experimental period or for infinite time was estimated by fitting an equation to the plasma concentration data using the non-linear least-square program MULTI (11). Total body clearance (CL_{total}) and apparent hepatic uptake clearance (CL_{liver}) were calculated by, respectively, dividing the administered dose and the amount in the liver at 2 h after administration by the AUC, as reported previously (12).

Liver Perfusion Experiment

The *in situ* liver perfusion experiments were performed as reported previously (13,14). Briefly, the liver was perfused in a single-pass mode at a flow rate of 13 ml/min with Krebs–Ringer bicarbonate buffer with 10 mM glucose, which was oxygenated with 95% O_2-5% CO_2, adjusted to pH 7.4 and incubated at 37°C. To prevent interactions between CAT derivatives and blood components, perfusate without red blood cells nor albumin was used. After conditioning the perfused liver for 20 min, ^{111}In-CAT derivatives dissolved in the perfusate were continuously infused at concentrations of 0.1, 1, 10 μg/ml. The venous outflow and the bile were collected into weighed tubes at appropriate intervals. The viability of the liver was checked with respect to both the bile flow and the LDH activity in the out flow. In all experiments, perfused livers remained viable during the course of the study. From the venous outflow curves, the extraction ratio (E_{ss}) and the hepatic clearance (CL_h) at steady- state for ^{111}In-CAT derivatives are calculated as follows:

$$E_{ss} = \frac{C_{in} - C_{out}}{C_{in}} \quad (1)$$

$$CL_h = E_{ss} Q \quad (2)$$

where C_{in} and C_{out} are the concentrations of ^{111}In-compounds in the perfusate before and after passing through the liver under steady-state conditions, and Q is the perfusion rate.

Pharmacokinetic Analysis

The outflow curves of ^{111}In-CAT derivatives in the liver perfusion system were analyzed by the one-organ pharmacokinetic model shown as Fig.1 (15). The sinusoidal compartment including the Disse space is assumed to be under well-stirred conditions, and the concentration is assumed to be identical to that in the outflow (C_s). The terms C_s and C_b correspond to C_{out} and C_{in}, respectively, in Eq. (1). The binding compartment is characterized by a maximum binding amount (X_∞) and a binding constant (K), where rapid equilibration is assumed to occur between the sinusoidal and binding compartments. This assumption of rapid equilibrium was confirmed by our previous studies where chemically modified albumin derivatives were used (14,15). V_s represents the sum of the volumes of the sinusoid and Disse spaces and this was estimated to be 0.180 ml/g liver (16).

The internalization process is assumed to follow first-order rate kinetics, the internalization rate (dX/dt) is expressed as a product of a binding amount X and its rate constant (k_{int}). The efflux of radioactivity after internalization was neglected since radioactive metabolites of ^{111}In-labeled ligands using DTPA cannot easily pass through biologic membranes (17,18). Then, in the sinusoidal and binding compartments, a mass-balance equation can be defined as follows:

$$V_s \cdot \left(\frac{dC_s}{dt}\right) + \left(\frac{dX}{dt}\right) = QC_b - QC_s - k_{int}X \quad (3)$$

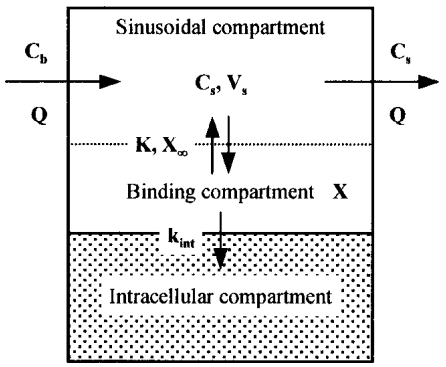

Fig. 1. Physiologic pharmacokinetic model for analyzing the hepatic uptake of ^{111}In-CAT derivatives. Q, flow rate (ml/min); C_b, inflow concentration (μg/ml); C_s, sinusoidal concentration (μg/ml); V_s, sinusoidal volume (ml); X, binding amount (μg/liver); X_∞, maximum binding amount (μg/liver); K, binding constant (ml/μg); k_{int}, internalization rate constant (min^{-1}).

The binding of ^{111}In-CAT derivatives to the cell surface is assumed to be consistent with the Langmuir equation and so the following expression holds:

$$X = \frac{X_\infty KC_s}{1 + KC_s} \tag{4}$$

Differentiated with respect to *t*, Eq. (4) can be rearranged to

$$\frac{dX}{dt} = \frac{X_\infty K}{(1 + KC_s)^2} \cdot \left(\frac{dC_s}{dt}\right) \tag{5}$$

Substituting Eq. 3 with Eq. (5) gives the following equation:

$$\left(V_s + \frac{X_\infty K}{(1 + KC_s)^2}\right)\left(\frac{dC_s}{dt}\right) = QC_b - QC_s - k_{int}\frac{X_\infty KC_s}{1 + KC_s} \tag{6}$$

The differential equations derived from Eq. (6) for various inflow concentrations are numerically solved using the Runge-Kutta-Gill method. An initial condition is $C_s = 0$ when t = 0. Curve fitting of the equations to the outflow curves was conducted using the MULTI(RUNGE) program (19) on a mainframe computer on the Kyoto University Data Processing Center.

Treatment of Perfused Liver with H_2O_2 and CAT Derivatives

The isolated rat liver was perfused in a similar way to that described above. After a stabilization period of 20 min, CAT derivative dissolved in the perfusate (500 U/ml) was infused into the liver for 30 min. The concentration of each derivative ranged from 11.4 to 13.2 μg/ml (11.4, 11.8, 12.6 and 13.2 μg/ml for CAT, Man-CAT, Gal-CAT and Suc-CAT, respectively), calculated based on the remaining CAT activity (6). Then, 0.5 mM H_2O_2 dissolved in the perfusate was continuously infused for 60 min. The venous outflow samples were collected every 10 min and the lactate dehydrogenase (LDH) activity in the outflow samples was determined using a commercial kit (LDH-UV test Wako, Wako Pure Chemicals, Japan).

Statistical Analysis

Differences were statistically evaluated by one-way analysis of variance followed by the Fisher's PLSD test at a significance level of $p < 0.05$.

RESULTS

In Vivo Disposition and Clearances of ^{111}In-CAT after Intravenous Injection in Rats

Figure 2a shows the plasma concentration-time courses of radioactivity after intravenous injection of ^{111}In-CAT into rats at different doses. ^{111}In-CAT was rapidly cleared from plasma after injection at doses of 0.1 and 1 mg/kg. Increasing the dose to 10 mg/kg, however, reduced the elimination rate of ^{111}In-CAT. Fig. 2b. shows the cumulative amounts of radioactivity in tissues, bile and urine after intravenous injection of ^{111}In-CAT. Most of the radioactivity was recovered in the liver, and little radioactivity (less than 2.5% of the dose) was recovered in other samples. Radioactivity excreted in bile gradually increased with time, and increasing the dose to 10 mg/kg resulted in a reduced biliary excretion rate (Fig. 2c).

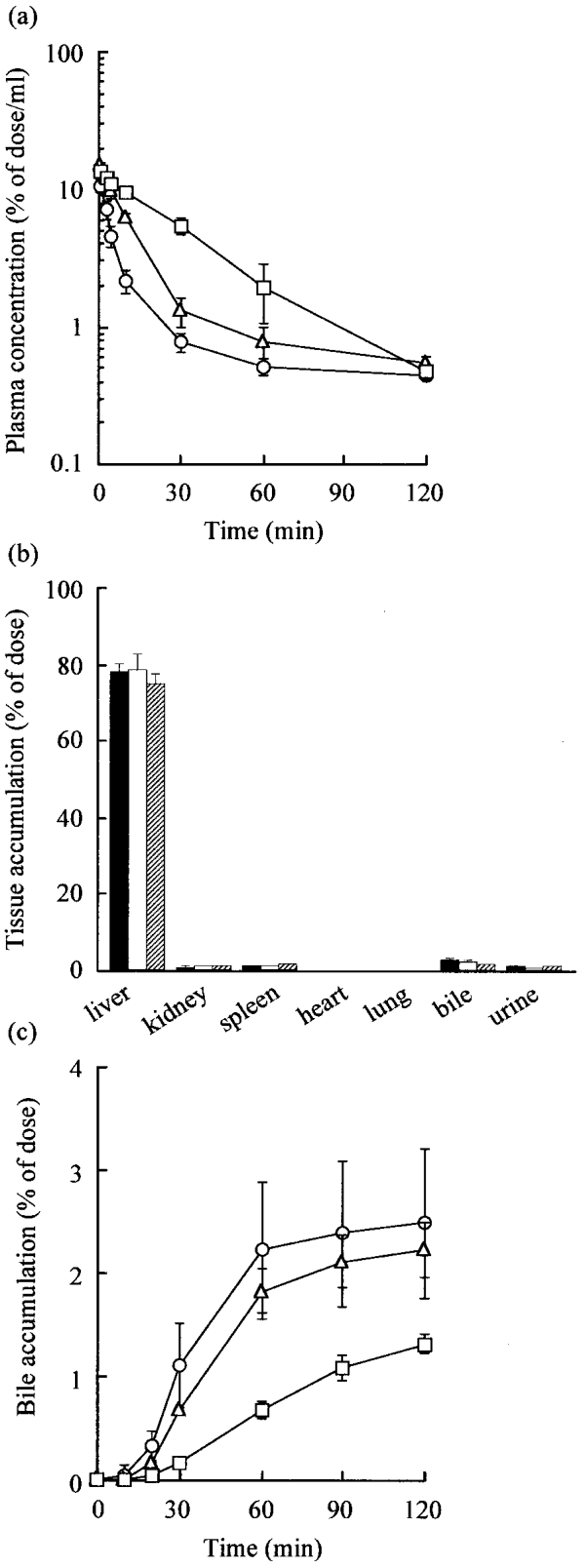

Fig. 2. (a) Plasma concentration of ^{111}In-CAT after intravenous injection in mice at doses of 0.1 mg/kg (○), 1 mg/kg (△) or 10 mg/kg (□). (b) Tissue accumulation of ^{111}In-CAT at 2h after intravenous injection in mice at a dose of 0.1 mg/kg (closed column), 1 mg/kg (open column), and 10 mg/kg (hatched column). (c) Accumulation of radioactivity in the bile after intravenous administration of ^{111}In-CAT to rats at doses of 0.1 mg/kg (○), 1 mg/kg (△) or 10 mg/kg (□). Results are expressed as the mean ± SD of at least three rats.

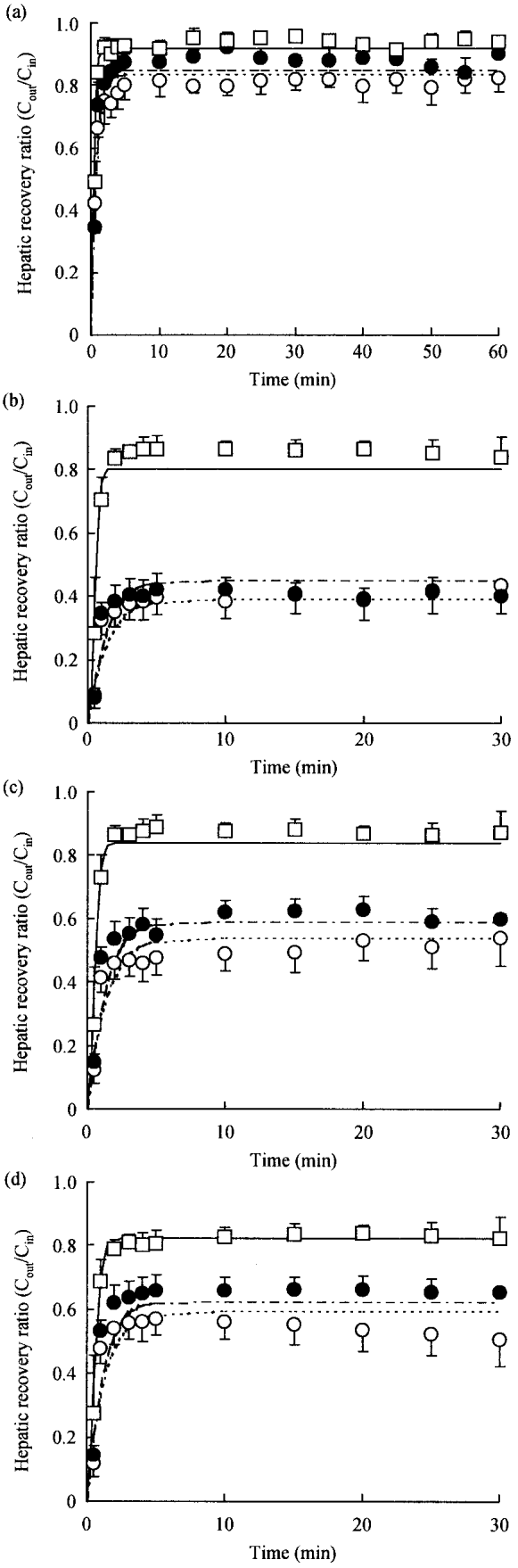

Fig. 3. Hepatic recovery ratio (C_{out}/C_{in})-time profiles of ^{111}In-CAT derivatives in the single-pass liver perfusion experiments at an inflow concentration of 0.1 μg/ml (○), 1 μg/ml (●) or 10 μg/ml (□). Values are expressed as the mean ± SD of at least three experiments. (a) CAT, (b) Gal-CAT, (c) Man-CAT, (d) Suc-CAT. Curves show simulated functions obtained based on the parameters shown in Table I. (dotted line) 0.1 μg/ml, (broken line) 1 μg/ml, (solid line) 10 μg/ml.

At any dose, the hepatic uptake clearance (CL_{liver}) accounted for more than 75% of the total body clearance (CL_{total}) (data not shown), indicating that the liver is the main organ determining the *in vivo* distribution of ^{111}In-CAT. These results are consistent with our previous results obtained in mice (4).

Hepatic Uptake of ^{111}In-CAT Derivatives during Constant Infusion in Perfused Rat Liver

Figure 3 shows the hepatic recovery ratio (C_{out}/C_{in})-time profiles of ^{111}In-CAT derivatives at various inflow concentrations (0.1, 1 and 10 μg/ml). Each ^{111}In-CAT derivative was continuously extracted by the liver during the uptake steady state. The CL_h was calculated from steady-state extraction ratio (E_{ss}) and summarized in Fig. 4. The CL_h of each derivative decreased as the inflow concentration increased. At the highest inflow concentration, the E_{ss} of ^{111}In-Suc-CAT, Gal-CAT and Man-CAT was 2.8, 2.2 and 1.9 times greater than that of ^{111}In-CAT, respectively. Extraction of ^{111}In-CAT by the liver was reduced by reducing the temperature to 4°C (data not shown), suggesting that ^{111}In-CAT was internalized by the liver cells via an energy-dependent mechanism.

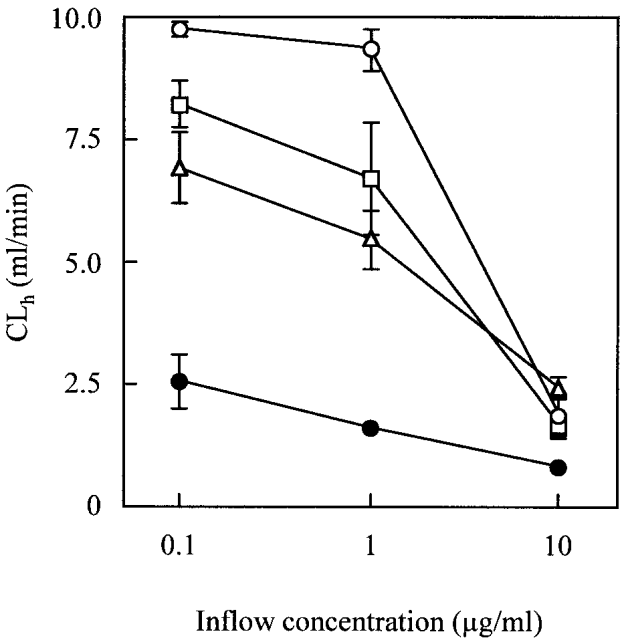

Fig. 4. Hepatic uptake clearance at steady state of ^{111}In-CAT derivatives in the single-pass liver perfusion experiments at various inflow concentrations; ●: CAT, □: Suc-CAT, ○: Gal-CAT, △: Man-CAT. Values are expressed as the mean ± SD of at least three experiments.

Analysis of Outflow Patterns Based on a Pharmacokinetic Model

The pharmacokinetic parameters of each ^{111}In-CAT derivative were estimated by fitting the mass-balance equations to the experimental results of the hepatic recovery ratio at various inflow concentrations (Table I). The maximum association amount (X_∞, μg) of each CAT derivative was relatively large compared with that of bovine serum albumin (BSA) derivatives reported previously (20). However, there were no significant differences among the CAT derivatives. Compared with ^{111}In-CAT, ^{111}In-Gal-, Man-, and Suc-CAT had a binding constant (K) that was 5.3, 3.2, and 2.1 times greater and an internalization rate (k_{int}) 2.0, 1.5, and 1.5 times greater, respectively.

Simulation curves for the hepatic recovery ratio-time profiles of ^{111}In-CAT derivatives were reconstructed employing the estimated parameters listed in Table I, and are shown in Fig. 3. In general, good agreement was observed between fitted curves and experimentally observed data at all inflow concentrations in all derivatives.

Protective Effect of CAT Derivatives against Hepatic Injury

Figure 5 shows the LDH activity in the venous outflow of the rat liver perfused with H_2O_2. When no H_2O_2 was added to the perfusate (control group), little LDH activity was detected in the venous outflow. In contrast, the LDH activity markedly increased, starting 10 min after infusion of H_2O_2-containing perfusate (no treatment group). Pretreatment with CAT resulted in a reduced LDH activity in the venous outflow. The other CAT derivatives also reduced LDH release. This effect was gradually weakened after 30 min of H_2O_2 infusion onward in the groups treated with a CAT derivative. Fig. 6 shows the cumulative LDH activity in the venous outflow for 40 min of H_2O_2 infusion. Of all the derivatives studied, Man-CAT suppressed the LDH release from the liver most efficiently, followed by Suc-CAT.

DISCUSSION

CAT is an enzyme that detoxifies hydrogen peroxide, which is involved in various ROS-mediated injuries. Therefore, treatment with CAT alone or its combination with SOD has been applied to the treatment of ROS-mediated diseases. Our recent studies have demonstrated that bovine liver CAT is rapidly delivered to liver PC (4) after intravenous injection in mice and the tissue disposition of CAT can be controlled by chemical modification (6). Furthermore, it has been shown that targeted delivery of CAT to liver NPC is a promising way to increase its potential for preventing hepatic ischemia/reperfusion injury (6,8). In order for these targeted enzymes to be effective against ROS-mediated injuries, they should be active after delivery to the cells. However, ligands, such as Gal-, Man- and Suc-proteins, internalized by receptor-mediated endocytosis are normally degraded within lysosomes (21,22). To design a theoretical strategy for targeted delivery of CAT to the liver in an attempt to prevent ROS-mediated injuries, the temporal and spatial disposition of CAT derivatives needs to be determined. To this end, the hepatic disposition of CAT derivatives was investigated in the isolated rat livers, because this system can give quantitative information on the binding and internalization characteristics of liver-targeted ligands (15,16,20,23).

For disposition studies, CAT derivatives were radiolabeled with ^{111}In using DTPA anhydride, because radioactive

Table I. Pharmacokinetic Parameters for Hepatic Uptake of ^{111}In-CAT Derivatives Obtained by Model Analysis

Compound	X_∞ (μg)	K (ml/μg)	k_{int} (min^{-1})
CAT	84.4 ± 11.3	0.13 ± 0.02	0.235 ± 0.018
Gal-CAT	64.1 ± 6.0	0.69 ± 0.08	0.470 ± 0.048
Man-CAT	74.8 ± 8.1	0.42 ± 0.06	0.358 ± 0.038
Suc-CAT	94.0 ± 14.4	0.27 ± 0.05	0.357 ± 0.044

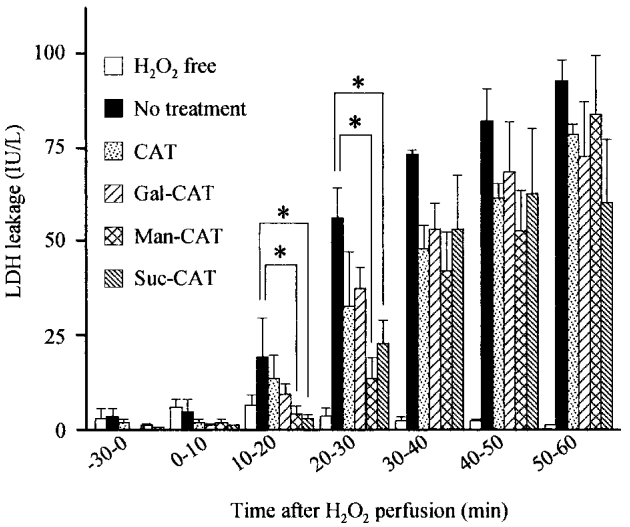

Fig. 5. LDH leakage-time profiles in the venous outflow after H_2O_2 constant infusion. Results are expressed as the mean ± SEM of at least three rats. Significantly different: *, $p < 0.05$.

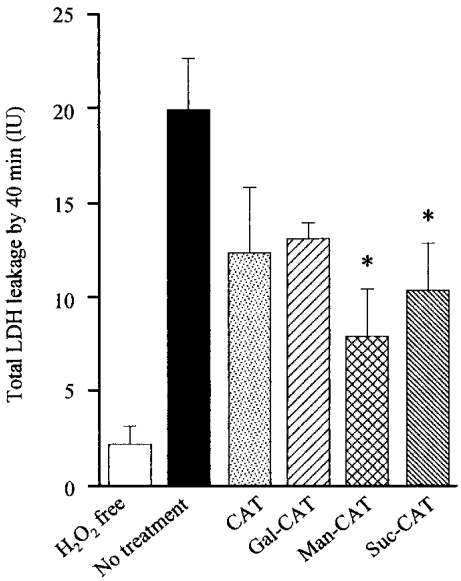

Fig. 6. Effect of pre-infusion of CAT derivatives on the accumulated LDH activity in the venous outflow 40 min after H_2O_2 constant infusion. Results are expressed as the mean ± SEM of at least three rats. Significantly different from no treatment group: *, $p < 0.05$.

metabolites, when produced after cellular uptake, are trapped within the cells where the uptake takes place (17,18). Tissue disposition studies in rats demonstrated that intravenously administered ^{111}In-CAT is rapidly eliminated from plasma and nearly 80% of the radioactivity is recovered in the liver. Increasing the dose to 10 mg/kg, however, reduced the elimination rate of ^{111}In-CAT from plasma, suggesting that CAT is taken up by the liver via a saturable process like that observed in mice (4). To quantitatively study the uptake characteristics of CAT derivatives, pharmacokinetic parameters were calculated based on the models. In the tissue distribution experiments *in vivo*, the hepatic uptake was calculated based on the amount taken up by the liver. On the other hand, the outflow profile was used to calculate the hepatic clearance in the perfusion study. To distinguish the differences, we used the two abbreviations of hepatic clearances, CL_{liver} for the *in vivo* experiments and CL_h for the *in situ* perfusion experiments.

In the perfused liver studies, ^{111}In-CAT was constantly extracted by the liver after its uptake reaching a steady state within 5 min of infusion. Furthermore, part of the radioactivity was recovered in the bile, indicating that ^{111}In-CAT is internalized into cells after binding to the cell surface. Hypoxia of the perfused liver might be concerned based on the slow perfusion of medium containing neither RBC nor albumin. However, no significant injuries caused by the simple perfusion were observed as previous studies (13–16,20,23). The E_{ss} values of ^{111}In-Gal-CAT, Man-CAT and Suc-CAT were 60.6, 50.7 and 45.4%, respectively, at the same concentration, and 2.3 to 3.0 times greater than that of ^{111}In-CAT.

We have used the model shown in Fig. 1 to analyze the pharmacokinetic characteristics of chemically modified bovine serum albumin (BSA) derivatives in the perfused rat livers (13–16,20,23). In these previous studies, the internalization rate constants (k_{int}) of BSA derivatives were experimentally obtained in addition to the model analysis; the surface bound and internalized amounts were separately measured by some means such as EDTA-wash treatment. These calculations clearly showed that the k_{int} values obtained by the model analysis were similar to those obtained experimentally, suggesting the validity of the analysis. By fitting the mass-balance equations to the outflow concentration-time profiles based on the pharmacokinetic model, the k_{int} value for Gal-, Man- and Suc-CAT was estimated to be 0.470, 0.358 and 0.357 min^{-1}, respectively, which was greater than the value for CAT (Table I). It has been reported that galactosylated, mannosylated and succinylated proteins are recognized by the asialoglycoprotein, mannose and scavenger receptors, respectively, on liver cells (24,25). The finding of greater k_{int} values for chemically modified CAT suggests that the receptor-mediated process for the hepatic uptake of modified CAT derivatives is faster than that of unmodified CAT. The k_{int} values for CAT derivatives obtained in this study were slightly different from the reported values for BSA derivatives (14,20,23). Although the reason for this discrepancy remains unknown, the CAT-specific hepatic uptake mechanism and/or differences in size and molecular weight between proteins may influence the internalization process.

The dose of CAT derivatives for the experiment to examine their preventive effects against H_2O_2 injury was adjusted in terms of the enzymatic activity to 500 U/ml, which corresponded to 11.4, 13.2, 12.6 and 11.8 μg/ml for CAT, Suc-CAT, Gal-CAT and Man-CAT, respectively. There were slight differences in the weight-based dose among CAT derivatives, but such differences could hardly affect the pharmacokinetic profiles in the perfused liver since the hepatic uptake of any CAT derivative was highly saturated at these concentrations (Fig. 4). Although pretreatment of each CAT derivative reduced the LDH release from the liver to some extent, the preventive effect did not last over 30 min (Fig. 5). The degradation of internalized ligands in the cells could explain these results. However, a series of our reports using BSA derivatives showed that the surface-bound protein derivatives are rapidly internalized (14,15,20,23). These results indicate that CAT derivatives internalized by liver cells can contribute to their protective effects against H_2O_2 injury in the perfused livers. Although the internalization rate of Suc-CAT and Man-CAT was faster than that of CAT, they were more effective than CAT in the prevention of the hepatic injury, suggesting the importance of the delivery of CAT to liver NPC. These results are comparable with our previous results on the preventive effect of CAT derivatives against hepatic ischemia/reperfusion injury (6,8).

Generally, it is known that extracellular ROS derived from Kupffer cells and polymorphonuclear leukocytes (neutrophils) play important roles in ROS-mediated hepatic injury such as ischemia/reperfusion injury (26, 27). Cellular injury to hepatocytes and endothelial cells seems to be mainly attributed to extracellular ROS rather than its intracellular counterpart. The experimental design of this study, therefore, reflects the phenomena that occur *in vivo*. Although H_2O_2 is initially localized outside cells, it can diffuse into cells to trigger oxidative reactions. Internalized CAT derivatives might prevent the reactions generated by internalized H_2O_2. The activity of endogenous antioxidant enzymes, especially CAT, in NPC is much less than in hepatocytes (28, 29), which suggests that the endothelial cells are much more susceptible to ROS than hepatocytes. Man-CAT or Suc-CAT can supplement CAT activity in hepatic endothelial cells. The present results confirm that targeted delivery of CAT to liver NPC is effective.

In conclusion, the pharmacokinetics of the hepatic uptake of CAT derivatives was determined in perfused rat liver, showing that chemically modified CAT derivatives are more rapidly internalized by liver cells than CAT. CAT derivatives targeted to liver NPC are more effective in preventing H_2O_2-induced hepatic injury. It has been shown that not only the surface- bound CAT derivative but also its counterpart internalized by receptor-mediated endocytosis is effective in preventing injury. These findings provide useful information to assist in the design and application of CAT derivatives to a range of ROS-mediated injuries.

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