The Journal of Philadococy and Experimental Therapeutics
The Journal of Philadacococy and Experimental Theoremics
Targeted Delivery of Human Recombinant Superoxide
Dismutase by Chemical Modification with Mono- and **DO22-3665/92/2833-0971830/0**

The Journal of Pharmacology and Experimental Therapeutics

Comptight 6 1992 by The American Society for Pharmacology and Experimental Therapeutics
 Dismutase by Chemical Modification with Mo 0022-3665/92/2633-0971\$3.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Copyright © 1992 by The American Society for Pharmacology and Experimental Therapeutics
POLYSACCHARICE DERIVATIVES
POLYSACCHARICE DE

TOIYSACCHAITIQE DEITVALIVES
TAKUYA FWITA, MAKIYA NISHIKAWA, CHIEKO TAMAKI, YOSHINOBU TAKAKURA, MITSURU HASHIDA and
HITOSHI SEZAKI HITOSHI SEZAKI TAKUYA FUJITA, MAKIYA NISHIKAWA, CHIEKO TAMAKI, YOSHINOBU TAKAKURA, MITSURU HASHIDA a
HITOSHI SEZAKI
*Department of Basic Pharmaceutics, Faculty of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606-01, Japan
A* TAKUYA FUJITA, MAKIYA NISHIKAWA
HITOSHI SEZAKI
Department of Basic Pharmaceutics, Faculty of
Accepted for publication July 28, 1992

ABSTRACT

EXECUTE:
Four types of superoxide dismutase (SOD) derivatives such as of t
SOD-carboxymethyl dextran conjugate, SOD-diethylaminoethyl The **ABSTRACT**
CONFINICT
SOD-carboxymethyl dextran conjugate, SOD-diethylaminoet
dextran conjugate, galactosylated SOD and mannosylated S ABSTRACT
Four types of superoxide dismutase (SOD) derivatives such as of the
SOD-carboxymethyl dextran conjugate, SOD-diethylaminoethyl These
dextran conjugate, galactosylated SOD and mannosylated SOD clearar
were synthesi ABSTRACT
Four types of superoxide dismutase (SOD) derivatives such as of the
SOD-carboxymethyl dextran conjugate, SOD-diethylaminoethyl Thes
dextran conjugate, galactosylated SOD and mannosylated SOD clear
were synthesized Four types of superoxide dismutase (SOD) derivatives such a
SOD-carboxymethyl dextran conjugate, SOD-diethylaminoeth
dextran conjugate, galactosylated SOD and mannosylated SOI
were synthesized and their potential for selec Four types or superoxide distributase (SOD) derivatives such as
SOD-carboxymethyl dextran conjugate, SOD-diethylaminoethyl
dextran conjugate, galactosylated SOD and mannosylated SOD
were synthesized and their potential for SOD-Cancoxymetriyi dextrari Conjugate, SOD-Cherinylaminoetriyi
dextran conjugate, galactosylated SOD and mannosylated SOD clea
were synthesized and their potential for selective targeting to
congans or cells was evaluated Sexual conjugate, galactosylated SOD and manihosylated SOD
were synthesized and their potential for selective targeting to
organs or cells was evaluated in mice by pharmacokinetic analy-
sis. All SOD derivatives retained 5 Were synthesized and their potential for selective targeting to comprograms or cells was evaluated in mice by pharmacokinetic analy-
sis. All SOD derivatives retained 50 to 80% of the original SOD
enzymatic activity and we except the kidney. SoD-carboxymethyl dextran conjugate gave called a long plasma half-life because of impaired solution with mouse tube serum retaining enzymatic activity greater than 80% for 3 hr. diet
After intravenous i enzymatic activity and were stable during includation with modse
serum retaining enzymatic activity greater than 80% for 3 hr. die
After intravenous injection, native SOD was rapidly excreted into
from the and no significa Seithm retaining enzymatic activity greater than 60% for 3 fill. General After intravenous injection, native SOD was rapidly excreted into from urine and no significant accumulation was observed in the organs occurring exc After intravenous injection, native SOD was rapidly excreted intuine and no significant accumulation was observed in the organ except the kidney. SOD-carboxymethyl dextran conjugate gave a long plasma half-life because of drive and no significant accumulation was observed in the organs occurred except the kidney. SOD-carboxymethyl dextran conjugate gave cell
a long plasma half-life because of impaired glomerular filtration con
and tissue in

of the liver, respectively, via receptor-mediated endocytosis.
These uptake processes were nonlinear and hepatic uptake These uptake processes were nonlinear and hepatic uptake
These uptake processes were nonlinear and hepatic uptake
clearance decreased as the dose increased, although almost of the liver, respectively, via receptor-mediated endocytosis.
These uptake processes were nonlinear and hepatic uptake
clearance decreased as the dose increased, although almost
complete extraction was obtained at a dose of the liver, respectively, via receptor-mediated endocytosis.
These uptake processes were nonlinear and hepatic uptake
clearance decreased as the dose increased, although almost
complete extraction was obtained at a dose of the liver, respectively, via receptor-mediated endocytosis.
These uptake processes were nonlinear and hepatic uptake
clearance decreased as the dose increased, although almost
complete extraction was obtained at a dose or the liver, respectively, via receptor-ineutated enlocytosis.
These uptake processes were nonlinear and hepatic uptake
clearance decreased as the dose increased, although almost
complete extraction was obtained at a dose These update processes were norminear and repart update deterance decreased as the dose increased, although almo

complete extraction was obtained at a dose of 0.1 mg/k

Furthermore, the accumulation in kidney of both glyc dietal ance decreased as the dose increased, althought almost
complete extraction was obtained at a dose of 0.1 mg/kg.
Furthermore, the accumulation in kidney of both glycosylated
SODs was drastically decreased due to redu complete extraction was obtained at a cose of 0.1 mg/ky.
Furthermore, the accumulation in kidney of both glycosylated
SODs was drastically decreased due to reduced renal proximal
tubular reabsorption and also enhanced hepa Furthermore, the accumulation in Kinney of both giycosylated
SODs was drastically decreased due to reduced renal proximal
tubular reabsorption and also enhanced hepatic clearance. SOD-
diethylaminoethyl dextran conjugate a SODS was diastically decreased due to reduced renai proximate tubular reabsorption and also enhanced hepatic clearance. SOD-
diethylaminoethyl dextran conjugate also rapidly disappeared
from plasma and distributed into liv diplomar reabsorption and also enhanced nepatic clearance. SOD-
diethylaminoethyl dextran conjugate also rapidly disappeared
from plasma and distributed into liver, but its accumulation
occurred due to electrostatic intera from plasma and distributed into liver, but its accumulation occurred due to electrostatic interaction and was nonspecific in cellular distribution. These results suggest the possibility of controlling the *in vivo* fate o occurred due to electrostatic interaction and was nonspecific in

The toxicity **of reactive oxygen species,** whose production is amplified by pathological events including neutrophil activation, hyperoxia, metabolism of redox-active drugs, radiation The toxicity of reactive oxygen species, whose production is
amplified by pathological events including neutrophil activa-
tion, hyperoxia, metabolism of redox-active drugs, radiation
exposure and ischemia, suggests use of The toxicity of reactive oxygen species, whose production is
amplified by pathological events including neutrophil activa-
tion, hyperoxia, metabolism of redox-active drugs, radiation
exposure and ischemia, suggests use of The toxicity of reactive oxygen species, whose production is
amplified by pathological events including neutrophil activa-
tion, hyperoxia, metabolism of redox-active drugs, radiation
exposure and ischemia, suggests use of amplified by pathological events including neutrophil activation, hyperoxia, metabolism of redox-active drugs, radiation exposure and ischemia, suggests use of antioxidant enzymes such as SOD as therapeutic agents (Fridov tion, hyperoxia, metabolism of redox-active drugs, radiation
exposure and ischemia, suggests use of antioxidant enzymes
such as SOD as therapeutic agents (Fridovich, 1983). Thus, half-li
controlled manipulation of cellula exposure and ischemia, suggests use of antioxidant enzymes
such as SOD as therapeutic agents (Fridovich, 1983). Thus,
controlled manipulation of cellular SOD, an enzyme capable of
eliminating superoxide anion, which exist such as SOD as therapeutic agents (Fridovich, 1983). Thus,
controlled manipulation of cellular SOD, an enzyme capable of
eliminating superoxide anion, which exists in the upper stream
of reactive oxygen metabolism cascade controlled manipulation of cellular SOD, an enzyme capable of
eliminating superoxide anion, which exists in the upper stream
of reactive oxygen metabolism cascade, can help as a defense
mechanism against tissue injury medi eliminating superoxide anion, which exists in the upper stream
of reactive oxygen metabolism cascade, can help as a defense
mechanism against tissue injury mediated by reactive oxygen
species. In practice, SOD is used as a of reactive experimental calculate, can help as a detention-
mechanism against tissue injury mediated by reactive oxygen
species. In practice, SOD is used as a therapeutic agent for
rheumatoid arthritis in humans (Menander Received for publication March 9, 1992.

half-life of only 5 to 10 min following i.v. injection in animal models (Pyatak *et al.*, 1980; Odlind *et al.*, 1988).
Among the various approaches to solving the problems in-

decays alf-life of only 5 to 10 min following i.v. injection in animal odels (Pyatak *et al.*, 1980; Odlind *et al.*, 1988).
Among the various approaches to solving the problems in-
rent to protein and peptide drugs such a half-life of only 5 to 10 min following i.v. injection in animal models (Pyatak *et al.*, 1980; Odlind *et al.*, 1988).
Among the various approaches to solving the problems inherent to protein and peptide drugs such as a half-life of only 3 to 10 mm following i.v. injection in animal
models (Pyatak *et al.*, 1980; Odlind *et al.*, 1988).
Among the various approaches to solving the problems in-
herent to protein and peptide drugs such as a modella or biological characteristics is the problems inherent to protein and peptide drugs such as a short plasma
half-life, chemical modification utilizing moieties with various
physicochemical or biological characterist herent to protein and peptide drugs such as a short plasma
half-life, chemical modification utilizing moieties with various
physicochemical or biological characteristics is the most prom-
ising (Fuertges and Abuchowski 199 half-life, chemical modification utilizing moieties with various
physicochemical or biological characteristics is the most prom-
ising (Fuertges and Abuchowski 1990; Takakura *et al.*, 1989a,b).
In particular, modification physicochemical or biological characteristics is the most pro
ising (Fuertges and Abuchowski 1990; Takakura *et al.*, 1989a,
In particular, modification of proteins with carbohydra
seems to be attractive due to their abili patibilities. In particular, modification of proteins with carbohydrates
seems to be attractive due to their ability to add various
characteristics to proteins and because of their high biocom-
patibilities.
On the other hand, many biol

rheumatoid arthritis in humans (Menander-Huber and Huber,
1977). However, the experimental and therapeutic potentials
of SOD are limited because SOD is rapidly cleared by glomer-
ular filtration in the kidney, leading to a seems to be attractive due to their ability to add various characteristics to proteins and because of their high biocompatibilities.
On the other hand, many biologically active proteins are known to be glycoproteins whose characteristics to proteins and because of their high biocompatibilities.
On the other hand, many biologically active proteins are
known to be glycoproteins whose carbohydrate structures are
important to their *in vivo* be patibilities.

On the other hand, many biologically active proteins are

known to be glycoproteins whose carbohydrate structures are

important to their *in vivo* behaviors and biological activities.

Therefore, from the s On the other hand, many biologically active proteins a known to be glycoproteins whose carbohydrate structures a important to their *in vivo* behaviors and biological activiti Therefore, from the standpoint of physiologic Therefore, from the standpoint of physiological function(s) of a carbohydrate residue and reconstitution of biological potential of glycoproteins from carbohydrate-free recombinant pro-

Downloaded from jpet.aspetjournals.org at ASPET Journals on September 12, 2016

acarbohydrate residue and reconstitution of biological function(s) of
acarbohydrate residue and reconstitution of biological poten-
acarbohydrate residue and reconstitution of biological poten-
dial of glycoproteins from c a carbohydrate residue and reconstitution of biological poter

ial of glycoproteins from carbohydrate-free recombinant pro

ABBREVIATIONS: SOD, superoxide dismutase; DTPA, diethylenetriaminepentaacetic acid; CMD, carboxyme Received for publication March 9, 1992.
 EXECUTE TO FORE SOD, Superoxide dismutase; DTPA, diethylenetriaminepentaacetic acid; CMD, carboxymethyl dextran; SOD-CMD, SOD-carboxymethyl dextran; SOD-CMD, SOD-carboxymethyl dex **ABBREVIATIONS:** SOD, superoxide dismutase; DTPA, diethylenetriaminepentaacetic acid; CMD, carboxymethyl dextran; SOD-CMD, SOD-
carboxymethyl dextran conjugate; DEAED, diethylaminoethyl dextran; SOD-DEAED, SOD-diethylamino **ABBREVIATIONS:** SOD, superoxide dismutase; DTPA, diethylenetriaminepentaacetic acid; CMD, carboxymethyl dextran; SOD-CMD, SOD-carboxymethyl dextran; SOD-CMD, SOD-carboxymethyl dextran conjugate; DEAED, diethylaminoethyl d **Example the internal dextrant sylated SOD; Man-SOI**
bovine serum albumin;
index per unit weight;
polyethylene glycol.

972 Fujita et al.
teins, chemical modification with carbohydrates would be im-
portant. portant. 972 Fujita et al.

teins, chemical modification with carbohydrates would be im-

portant.

In this study, we synthesized four types of recombinant SOD

derivatives modified with sugar moieties and investigated their

teins, chemical modification with carbohydrates would be im-
portant.
In this study, we synthesized four types of recombinant SOD
derivatives modified with sugar moieties and investigated their
potentials in site-specific potentials in site-specific delivery of the enzyme. Tissue distriportant.

In this study, we synthesized four types of recombinant SOD

derivatives modified with sugar moieties and investigated their

potentials in site-specific delivery of the enzyme. Tissue distri-

bution properties In this study, we synthesized four types of recombinant SOD
derivatives modified with sugar moieties and investigated their
potentials in site-specific delivery of the enzyme. Tissue distri-
bution properties of SOD deriva **Material SOD** derivatives were determined compounds and tissue uptake chated for a quantitative evaluation.
 Materials and Methods

Chemicals

Materials and Methods
 Chemicals

Recombinant human SOD (111-Ser) was kindly supplied by Toyo

Jyozo Co., Shizuoka, Japan. Dextran with an average MW of 10 kDa

was purchased from Pharmacia, Uppsala, Sweden. D-Galactos Recombinant human SOD (111-Ser) was kindly supplied by Toyo Jyozo Co., Shizuoka, Japan. Dextran with an average MW of 10 kDa was purchased from Pharmacia, Uppsala, Sweden. D-Galactose and D-mannose were obtained from Wako Jyozo Co., Shizuoka, Japan. Dextran with an average MW of 10 kDa
was purchased from Pharmacia, Uppsala, Sweden. D-Galactose and D-
mannose were obtained from Wako Pure Chemical, Osaka, Japan.
DTPA anhydride was obtained fr was purchased from Pharmacia, Uppsala, Sweden. D-Galactose and D-
mannose were obtained from Wako Pure Chemical, Osaka, Japan. af
DTPA anhydride was obtained from Dojindo Laboratory, Kumamoto,
Japan. ¹¹¹Indium chloride (mannose were obtain
DTPA anhydride was
Japan. ¹¹¹Indium chlori
Medi-Physics Co., Tal
finest grade available. DTPA anhydride was obtained from Doji
Japan. ¹¹¹Indium chloride ([¹¹¹In]InCl₃) wa
Medi-Physics Co., Takarazuka, Japan. Al
finest grade available.
Synthesis of SOD Derivatives
SOD-polysaccharide conjugates. Ty

Medi-Physics Co., Takarazuka, Japan. All other chemicals were of the
finest grade available.
Synthesis of SOD Derivatives
SOD-polysaccharide conjugates. Two types of SOD-polysaccha-
ride conjugates, an anionic CMD conjugat finest grade available.
 Synthesis of SOD Derivatives
 SOD-polysaccharide conjugates. Two types of SOD-polysaccha-

ride conjugates, an anionic CMD conjugate (SOD-CMD) and a cationic

DEAED conjugate (SOD-DEAED) were s Synthesis of SOD Derivatives

SOD-polysaccharide conjugates. Two types of SOD-polysaccha-

ride conjugates, an anionic CMD conjugate (SOD-CMD) and a cationic

DEAED conjugate (SOD-DEAED) were synthesized according to the
 SOD-polysaccharide conjugates. Two types of SOD-polysaccharide conjugates, an anionic CMD conjugate (SOD-CMD) and a cationic DEAED conjugate (SOD-DEAED) were synthesized according to the method reported previously (Fujit accomplete and diethylaminoethyl chloride, respectively, with dextranal diethylaminoethyl chloride, respectively, with dextran under alkaline conditions at 80°C. These dextran derivatives were oxidized alkaline conditions DEAED conjugate (SOD-DEAED) were synthesized according to the
method reported previously (Fujita *et al.*, 1990; Yasuda *et al.*, 1990). In
brief, CMD and DEAED were synthesized by reacting monochloracetic *et al*
acid an brief, CMD and DEAED were synthesized by reacting monochloracetic et al.

acid and diethylaminoethyl chloride, respectively, with dextran under

alkaline conditions at 80°C. These dextran derivatives were oxidized

by sod alkaline conditions at 80°C. These dextran derivatives were oxidized form
by sodium periodate, conjugated with SOD in 50 mM borate buffer (pH 40μ
10.0) for 24 hr at 4°C in the dark, and reduced by 1 mM sodium
borohydri **cm).** Solution periodic, conjugated with SOD in 60 links solute states then 40 μ 1 on 0.0) for 24 hr at 4°C. The obtained compounds were purified by PD-10 definitation chromatography using Sephadex G-75 column $(1 \times 40$ DTPA. borohydride for 2 hr at 4° C. The obtained compounds were purified gel-filtration chromatography using Sephadex G-75 column $(1 \times$ cm).
Glyc-SOD. Introduction of galactose and mannose residues to States are at at accor

gel-filtration chromatography using Sephadex G-75 column $(1 \times 40$ DTPA.

cm).

Glyc-SOD. Introduction of galactose and mannose residues to SOD coupled

was carried out according to the method of Lee *et al.* (1976). Cyan complex details and mannose residues to SOD
complex details at room of galactose and mannose residues to SOD
was carried out according to the method of Lee *et al.* (1976). Cyano-
methyl 1-thiogly coside (220 mg, 0.94 mmo Glyc-SOD. Introduction of galactose and mannose residues to was carried out according to the method of Lee *et al.* (1976). C methyl 1-thioglycoside (220 mg, 0.94 mmol) was treated with 0. sodium methoxide at room tempera was carried out according to the method of Lee *et al.* (1976). Cyanomethyl 1-thioglycoside (220 mg, 0.94 mmol) was treated with 0.01 M column methoxide at room temperature. After 24 hr, the solvent was fracevaporated in methyl 1-thioglycoside (220 mg, 0.94 mmol) was treated with 0.01 M consolum methoxide at room temperature. After 24 hr, the solvent was frae evaporated in vacuo and the resultant syrup of 2-imino-2-methoxy-octhyl-1-thiogl sodium methoxide at room temperature. After 24 hr, the solvent was
evaporated in vacuo and the resultant syrup of 2-imino-2-methoxy-
ethyl-1-thioglycoside was added to SOD (300 mg, 9.4 μ mol) in 15 ml 50
mM borate buffe ethyl-1-thioglycoside was added to SOD (300 mg, 9.4 μ mol) in 15 ml 50 mM borate buffer (pH 10.0). After 5 hr at room temperature, the reaction mixture was concentrated by ultrafiltration and applied to Sephadex G-25 co mM borate buffer (pH 10.0). After 5 hr at room temperature, the mM borate buffer (pH 10.0). After 5 hr at room temperature, the reaction mixture was concentrated by ultrafiltration and applied Sephadex G-25 column $(1 \times 3$ reaction mixture was concentrated by ultrafiltration and applied to Sephadex G-25 column $(1 \times 30 \text{ cm})$ equilibrated with 0.1 M acetate buffer (pH 6.0) to separate the coupled product from the unreacted compound. Purity o buffer (pH 6.0) to separate the coupled product from the unreacted compound. Purity of the products was confirmed by affinity chroma-
tography with Con A-Sepharose (Man-SOD) or agarose-peanut lectin (Gal-SOD). Gal-BSA and buffer (pH 6.0) to separate th
compound. Purity of the produ
tography with Con A-Sepharos
(Gal-SOD). Gal-BSA and Mai
procedure as described above. compound. I urily of the protography with Con A-Seph
(Gal-SOD). Gal-BSA and
procedure as described above
Analytical Methods

al-SOD). Gal-BSA and Man-BSA were synthesized by the same
ocedure as described above.
halytical Methods
Protein concentration was determined by the method of Lowry *et al.*
951) using BSA fraction V as a standard in the **Analytical Methods**

Protein concentration was determined by the method of Lowry *et al.*

(1951) using BSA fraction V as a standard in the range of 0 to 200 μ g/

ml. The degree of modification of amino groups was det **Analytical Methods**

Protein concentration was determined by the method of Lowry *et al.*

(1951) using BSA fraction V as a standard in the range of 0 to 200 μ g/ the

ml. The degree of modification of amino groups was Protein concentration was determined by the method of Lowry *et al.* (1951) using BSA fraction V as a standard in the range of 0 to 200 μ g/ ml. The degree of modification of amino groups was determined by measuring the The degree of modification was determined by the method of LOWI et al.

(1951) using BSA fraction V as a standard in the range of 0 to $200 \mu g/m$

ml. The degree of modification of amino groups with trinitrobenzene sul-

f The degree of modification of amino groups was determined by measuring the amount of free amino groups with trinitrobenzene sulfonic acid using glycine as a standard (Habeeb, 1966). Enzymatic activity of SOD was determine The MW of SOD derivatives was determined by

fonic acid using glycine as a standard (Habeeb, 1966). Enzymatic

activity of SOD was determined by nitroblue tetrazolium reduction

The MW of SOD derivatives was estimated by h chromatography (LC-6A, Shimadzu, Japan) using a Shim-pack Diol-
Chromatography (LC-6A, Shimadzu, Japan) using a Shim-pack Diol-
300 column (inside diameter, 7.5 mm \times 50 cm) eluted with 20 mM
phosphate buffer (pH 7.0) co activity of SOD was determined by inclodue detazonum reduction
method using a SOD test kit (Wako Pure Chemical, Osaka, Japan).
The MW of SOD derivatives was estimated by high-performance liquid
chromatography (LC-6A, Shima The MW of SOD derivatives was estimated by high-performance liquid
chromatography (LC-6A, Shimadzu, Japan) using a Shim-pack Diol-
300 column (inside diameter, 7.5 mm \times 50 cm) eluted with 20 mM
phosphate buffer (pH 7.0 chromatography (LC-6A, Shimadzu, Japan) using a Shim-pack D
300 column (inside diameter, 7.5 mm \times 50 cm) eluted with 20 r
phosphate buffer (pH 7.0) containing 0.2 M sodium sulfate. The
parent MW of SOD derivatives were circularly, $(2e^{-\lambda t})$, Unimated, superfunctions, since a chim place Euclidon (inside diameter, 7.5 mm \times 50 cm) eluted with 20 mM phosphate buffer (pH 7.0) containing 0.2 M sodium sulfate. The apparent MW of SOD deriva phosphate buffer (pH 7.0) containing 0.2 M sodium suparent MW of SOD derivatives were determined by turve obtained from the marker proteins (Gel Filtration cia, Uppsala, Sweden). The physicochemical properties atives teste

TABLE 1 Physicochemical properties **of SOD and SOD derivatives used in TABLE 1**
Physicochemical _|
this study

method.
MW of SOD derivatives were estimated by high-performance liquid chromatog-

Man-SOD 3.8 34,000 65.6 -
 Raphy using Shim-pack Dio-300 column (Shimadzu, Kyoto, Japan).

² Mumbers of amino group were determined by trinitrobenzene sulfonic acid

² MW of SOD derivatives were estimated by high-per

method.
^d Net electric charge of SOD derivatives was confirmed by a batch method using **Solution** Solution and the method.
 S Network confirmed by the method charge of MW of SOD derivatives were estimated by high-performance liquid chromatography using Shim-pack Diol-300 column (Shimadzu, Kyoto, Japan).

S ger. ^o SOD enzymatic activity was assayed by nitroblue tetrazolium

^o Net electric charge of SOD derivatives was confirmed by a batch me

a DEAE-Sephadex A-50 anion exchanger and CM-Sephadex C-50 catic

ger.
 Stability of

a DEAE-Sephadex A-50 anion exchanger and CM-Sephadex C-50 cation exchanger.
 Stability of SOD Derivatives in Mouse Serum

One hundred units of SOD derivatives were incubated at 37°C in 1

ml of phosphate buffered saline **Stability of SOD Derivatives in Mouse Serum**
One hundred units of SOD derivatives were incubated at 37° C in 1
ml of phosphate buffered saline (pH 7.4) containing mouse serum
(20%). Aliquots of the incubation medium One hundred units of SOD derivatives were incubated at
ml of phosphate buffered saline (pH 7.4) containing mov
(20%). Aliquots of the incubation medium were taken at van
intervals and assayed for remaining SOD enzymatic ac

(20%). Aliquots of the incubation medium were taken at various time
intervals and assayed for remaining SOD enzymatic activity.
Radiolabeling of SOD Derivatives
 111 In labeling of SOD derivatives was performed using **Radiolabeling of SOD Derivatives**
¹¹¹In labeling of SOD derivatives was performed using the bifunc-
tional chelating agent DTPA anhydride by the method of Hnatowich
et al. (1982). In brief, SOD derivatives equivalent madiolabelling of SOD derivatives was performed using the bifunctional chelating agent DTPA anhydride by the method of Hnatowich *et al.* (1982). In brief, SOD derivatives equivalent to 10 mg SOD were dissolved in 1 ml of tional chelating agent DTPA anhydride by the method of Hnatowich *et al.* (1982). In brief, SOD derivatives equivalent to 10 mg SOD were dissolved in 1 ml of 0.1 M 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid buffe dissolved in 1 ml of 0.1 M 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid buffer (pH 7.0) and the twice molar of DTPA anhydride in 40 μ l of Me₂SO was added. The mixture was then stirred for 30 min at room temper DTPA. Forty microliters of 111 InCl₃ solution (74 MBq/ml) was added to 40 μ l of 1 M sodium acetate buffer (pH 6.0), and then 80 μ l of DTPA-coupled conjugate solution was added to the mixture. After 30 min, the from the coupled conjugate solution was added to the mixture. After 30 min, the coupled conjugate solution was added to the mixture. After 30 min, the mixture was purified by gel-filtration chromatography using PD-10 colu mixture was purified by gel-filtration chromatography using PD-10 column eluted with 0.1 M acetate buffer (pH 6.0), and conjugate fractions were collected and concentrated by ultrafiltration. The radiochemical purities of cm). by gel-filtration chromatography using Sephadex G-75 column (2 × 40 cm).
Procedure of Animal Experiment

by gel-filtration chromatography using Sephadex G-75 column $(2 \times 40$
cm).
 Procedure of Animal Experiment

Male ddY mice $(20-25 \text{ g})$ were injected into the tail vein with 0.15

M sodium chloride solution containing r **Procedure of Animal Experiment**
Male ddY mice (20–25 g) were injected into the tail vein with 0.15
M sodium chloride solution containing radiolabeled SOD or SOD
derivatives and housed in metabolic cages for subsequent col **Procedure of Animal Experiment**
Male ddY mice $(20-25 g)$ were injected into the tail vein with 0.15
M sodium chloride solution containing radiolabeled SOD or SOD
derivatives and housed in metabolic cages for subsequent c Male ddY mice $(20-25 g)$ were injected into the tail vein with 0.15
M sodium chloride solution containing radiolabeled SOD or SOD
derivatives and housed in metabolic cages for subsequent collection of
urine samples. For t M sodium chloride solution containing radiolabeled SOD or SOD
derivatives and housed in metabolic cages for subsequent collection of
urine samples. For the competitive experiment, ¹¹¹In-labeled Gal- or
Man-SOD (0.1 mg/kg derivatives and housed in metabolic cages for subsequent collection of
urine samples. For the competitive experiment, ¹¹¹In-labeled Gal- or
Man-SOD (0.1 mg/kg) was injected into mice simultaneously with cold
Gal-BSA or M Gal-BSA or Man-BSA at a dose of 20 mg/kg. At an appropriate time
period after administration, blood was collected from the vena cava
under ether anesthesia and centrifuged at 3000 rpm for 2 min to obtain
the plasma sample. Gal-BSA or Man-BSA at a dose of 20 mg/kg. At an appropriate time
period after administration, blood was collected from the vena cava
under ether anesthesia and centrifuged at 3000 rpm for 2 min to obtain
the plasma sample. under ether anesthesia and centrifuged at 3000 rpm for 2 min to obtain
the plasma sample. Organs such as the heart, liver, spleen, intestines,
kidney and muscle were excised, rinsed in cold saline, weighed and
subjected t the plasma sample. Organs such as the heart, liver, spleen, intestines, kidney and muscle were excised, rinsed in cold saline, weighed and subjected to assay. The 111 In radioactivities of organ samples were counted wi kidney and muscle were excised, rinsed in cold saline, weighed and
subjected to assay. The ¹¹¹In radioactivities of organ samples were
counted with a well NaI-scintillation counter (ARC-500, Aloka, Japan).
Plasma volume counted with a well NaI-scintillation counter (ARC-500, Aloka, Japan).
Plasma volume of each organ was determined from the distribution
data of [¹¹¹In]BSA at 10 min after i.v. injection and used for the
correction of the Plasma volume of each organ was determined from the distribution data of [¹¹¹In]BSA at 10 min after i.v. injection and used for the correction of the tissue concentration. Cellular localization of SOD derivatives in the

Data **Analysis**

parenchymal and nonparenchymal cells after collagenase perfusion.
 Data Analysis

Tissue distribution patterns of SOD derivatives were evaluated ac-

cording to the method reported previously (Takakura *et al.*, 1987). T

1991
dose/ml and analyzed by a biexponential function using the nonlinear
legat grupps program MU TI (Yemsele et al. 1991). 1991
dose/ml and analyzed by a biexponential function usin
least-square program MULTI (Yamaoka *et al.*, 1981):

$$
Cp(t) = Ae^{-\alpha t} + Be^{-\beta t}
$$
 (1)

least-square program MULTI (Yamaoka *et al.*, 1981):
 $Cp(t) = Ae^{-\alpha t} + Be^{-\beta t}$ (1)

CL_{total} was calculated by dividing the injected dose by the area under

the plasma concentration-time curve extrapolated to infinite time.
 $Cp(t) = Ae^{-at} + Be^{-pt}$ (1)
CL_{total} was calculated by dividing the injected dose by the area under
the plasma concentration-time curve extrapolated to infinite time.
Under the assumption of negligible efflux, CLI_i was calcul

$$
CLI_i = C_i/ AUC_{0-i}
$$
 (2) 2

the plasma concentration-time curve extrapolated to infinite time.
Under the assumption of negligible efflux, CLI_i was calculated from,
CLI_i = C_i/AUC₀ - t (2)
where C_i was the concentration of ¹¹¹In-radioactiv $CLI_i = C_i/AUC_0$ - t
where C_i was the concentration of 111 In-radioactivity in each
time, t. Then the apparent $CL_{i,ver}$ was expressed as follows:

$$
CLliver = CLliver W
$$
 (3)

where C_i was the concentration of 111 In-radioactivity in each organ at time, t. Then the apparent CL_{liver} was expressed as follows:
 $CL_{\text{liver}} = CLI_{\text{liver}}W$ (3)

where W (g) is the total wet weight of the liver. $CL_{\$ time, t. Then the apparent CL_{liver} was expressed as follows:
 $CL_{\text{liver}} = CL_{\text{liver}}W$ (3)

where W (g) is the total wet weight of the liver. CL_{urine} was estimated

from Eq. (2) using the cumulative amount of SOD deriva where W
from Eq. (
in urine.

Results

In urine.

Physicochemical characteristics of SOD derivatives.

Four types of SOD derivatives listed in table 1 were prepared S

and tested. The covalent attachment of dextran derivatives to a **Alternal characteristics of SOD derivatives.** The covalent attachment of dextran derivatives to and tested. The covalent attachment of dextran derivatives to and SOD increases its molecular size but the molecular sizes of **SOD increases is molecular size of SOD** derivatives. \pm Four types of SOD derivatives listed in table 1 were prepared SO
and tested. The covalent attachment of dextran derivatives to an
SOD increases its molecular size Four types of SOD derivatives listed in table 1 were prepared
and tested. The covalent attachment of dextran derivatives to
SOD increases its molecular size but the molecular sizes of the
glycosylated SODs were essentially Four types of SOD derivatives listed in table 1 were prepared and tested. The covalent attachment of dextran derivatives to SOD increases its molecular size but the molecular sizes of the glycosylated SODs were essentially and tested. The covalent attachment of dextran derivatives to SOD increases its molecular size but the molecular sizes of the glycosylated SODs were essentially unchanged. The enzymatic activities of SOD-polysaccharide con SOD increases its molecular size but the molecular sizes of the glycosylated SODs were essentially unchanged. The enzymatic C activities of SOD-polysaccharide conjugates and glycosylated as SODs were retained at about 50 a glycosylated SODs were essentially unchanged. The enzymatic
activities of SOD-polysaccharide conjugates and glycosylated
SODs were retained at about 50 and 70%, respectively. The net
electric charge of SOD derivatives exce

electric charge of SOD derivatives except SOD-DEAED was
negative, similar to that of SOD itself.
Stability of SOD derivatives in mouse serum. SOD was
stable in mouse serum and 87% of the original activity remained
at 3 h negative, similar to that of SOD itself.
 Stability of SOD derivatives in mouse serum. SOD was

stable in mouse serum and 87% of the original activity remained

at 3 hr after the start of the incubation (data not shown). **Stability of SOD derivatives in mouse serum.** SOD was stable in mouse serum and 87% of the original activity remained at 3 hr after the start of the incubation (data not shown). Al SOD derivatives were also stable in mous at 3 hr after the start of the incubation (data not shown). All
SOD derivatives were also stable in mouse serum and kept
their initial activities at similar extents. After 3 hr incubation,
incubation medium was subjected t SOD derivatives were also stable in mouse serum and kept SOD derivatives were also stable in mouse serum and kept
their initial activities at similar extents. After 3 hr incubation,
incubation medium was subjected to gel-filtration chromatog-
raphy with Sephadex G-75 to examine eir initial activities at similar extents. After 3 hr incubation,
cubation medium was subjected to gel-filtration chromatog-
phy with Sephadex G-75 to examine metabolic degradation,
cut no degradation products were detecte

incubation medium was subjected to gel-filtration chromatog-
raphy with Sephadex G-75 to examine metabolic degradation, cum
but no degradation products were detected in any test samples.
Plasma clearance and tissue distr raphy with Sephadex G-75 to examine metabolic degradation,
but no degradation products were detected in any test samples.
Plasma clearance and tissue distribution of $[1^{11} \text{In}]$ SOD
derivatives. Radioactivity concent but no degradation products were detected in any test samples. adm
Plasma clearance and tissue distribution of $[1^{11} \text{In}]$ SOD BS.
derivatives. Radioactivity concentrations of $[1^{11} \text{In}]$ SOD in
almomen, liver, an derivatives. Radioactivity concentrations of $[111]$ SOD in administrated with Gal-BSA, the liver accumulation of $[111]$ n plasma, liver, and kidney following i.v. injection into mice at Gal-SOD was reduced from 71.2 to 11 plasma, liver, and kidney following i.v. injection into mice at G 0.1 and 10 mg/kg are shown in fig. 1 A, B and C. $[$ ¹¹¹In]SOD af was rapidly cleared from blood circulation and accumulated in livel kidney at both dos 0.1 and 10 mg/kg are shown in fig. 1 A, B and C. $[$ ¹¹¹In]SOD aft was rapidly cleared from blood circulation and accumulated in liver the kidney at both doses. However, the normalized amount of Microacentration of $[$ ¹ was rapidly clear
the kidney at bot
radioactivity in th
concentration of
doses (fig. 1B).
In fig. 2 A, B is exidency at both doses. However, the normalized amount of dioactivity in the kidney was lower at 10 mg/kg (fig. 1C). The ncentration of $[$ ¹¹¹In]SOD in the liver was negligible at both uses (fig. 1B).
In fig. 2 A, B radioactivity in the kidney was lower at 10 mg/kg (fig. 1C). The concentration of $[111]$ SOD in the liver was negligible at both doses (fig. 1B).
In fig. 2 A, B and C, $[111]$ SOD-CMD showed a prolonged plasma retention

concentration of $[111]$ SOD in the liver was negligible at doses (fig. 1B).
In fig. 2 A, B and C, $[111]$ SOD-CMD showed a prologram retention and reduced renal accumulation and a s
increase in hepatic uptake. On the other doses (fig. 1B). of ¹
In fig. 2 A, B and C, $[111]SOD-CMD$ showed a prolonged table
plasma retention and reduced renal accumulation and a slight but
increase in hepatic uptake. On the other hand, $[111]SOD-$ the
DEAED was r

Targeted Delivery of SOD 973
the liver. Reflecting the increased accumulation in the liver,
the concentration in the kidney was markedly decreased. Targeted Delivery of SOD
the liver. Reflecting the increased accumulation in the
the concentration in the kidney was markedly decreased.
More rapid disappearance from the blood circulation

Targeted Delivery of SOD 973

ear the liver. Reflecting the increased accumulation in the liver,

the concentration in the kidney was markedly decreased.

More rapid disappearance from the blood circulation and

liver a the liver. Reflecting the increased accumulation in the liver,
the concentration in the kidney was markedly decreased.
More rapid disappearance from the blood circulation and
liver accumulation were observed after i.v. inj compounds, hepatic uptake was marked well-eased.
More rapid disappearance from the blood circulation and liver accumulation were observed after i.v. injection of $\binom{111}{11}$ Gal-SOD and $\binom{111}{11}$ Man-SOD (fig. 3 A, Five radius appearance from the blood circulation and
liver accumulation were observed after i.v. injection of $[1^{11} \text{In}]$
Gal-SOD and $[1^{11} \text{In}]$ Man-SOD (fig. 3 A, B and C). In both
compounds, hepatic uptake was nonl The first accumulation were observed atternation of $\begin{bmatrix} 1 & 0 \\ 1 & 0 \end{bmatrix}$

Cal-SOD and $\begin{bmatrix} 1^{11} \text{In} \end{bmatrix}$ Man-SOD (fig. 3 A, B and C). In both

compounds, hepatic uptake was nonlinear and the percent

amount of can sold and ["Injinian-Sold (iig. 5 A, B and C). In social compounds, hepatic uptake was nonlinear and the percent amount of radioactivity in the liver at 1 mg/kg dose was about 20% lower than that at 0.1 mg/kg. Due to h nount of radioactivity in the liver at 1 mg/kg dose was about % lower than that at 0.1 mg/kg. Due to high accumulation the liver, $[$ ¹¹¹In]Gal-SOD and $[$ ¹¹¹In]Man-SOD were not ken up by the kidney. In this experiment

(3) 20% lower than that at 0.1 mg/kg. Due to high accumulation

in the liver, $[$ ¹¹¹In]Gal-SOD and $[$ ¹¹¹In]Man-SOD were not

taken up by the kidney.

In this experiment, urine samples were collected for 2 hr

($[$ ¹¹ $([111]SOD, [111]Ga1-SOD and [111]Man-SOD)$ or 24 hr is the amount of radioactivity of $(1^{11} \text{In} | \text{SOD}, [1^{11} \text{In} | \text{Gal-SOD} \text{ and } [1^{11} \text{In} | \text{Man-SOD}) \text{ or } 24 \text{ hr}$
($[1^{11} \text{In} | \text{SOD}-\text{CMD} \text{ and } [1^{11} \text{In} | \text{SOD}-\text{DEAED})$ after i.v. administration. At 0.1 mg/kg dose, the amo ([m]SOD, [m]Mal-SOD and [m]Mal-SOD) or 24 in ([m]SOD-CMD and [m]Nal-SOD) after i.v. administration. At 0.1 mg/kg dose, the amount of radioactivity of [m]SOD, [m]n]Gal-SOD and [\text respectively and $\left[\begin{array}{c} \text{m} \text{J} \text{SOD}-\text{CMD} \text{ and } \text{l} \end{array}\right]$ and $\left[\begin{array}{c} \text{m} \text{J} \text{SOD} \text{ of } \text{R}} \text{ and } \text{l} \text{ of } \text{l} \text{ and } \text{l} \text{ of } \text{l} \text{ and } \text{l} \text{ of } \text{l} \text{$ atitation. At 0.1 mg/kg dose, the amount of radioactivity of ["In]SOD, ["In]Gal-SOD and ["In]Man-SOD excreted in urine within 2 hr were 11.7 \pm 4.0%, 3.8 \pm 0.5% and 7.2 \pm 2.0%, respectively (mean \pm S.D.; N = 3) urine within 2 hr were 11.7 \pm 4.0%, 3.8 \pm 0.5% and 7.2 \pm 2.0%,
respectively (mean \pm S.D.; N = 3). However, at 10 mg/kg, the
amount of $[$ ¹¹¹In]SOD excreted in urine was increased to 36.7
 \pm 4.4%. The radio respectively (mean \pm S.D.; N = 3).
amount of $[$ ¹¹¹In]SOD excreted in u
 \pm 4.4%. The radioactivities of $[$ ¹¹¹
SOD-DEAED excreted in urine with
and 19.5 \pm 4.1% of dose, respectivel
Cellular distribution of SO \pm 4.4%. The radioactivities of $\binom{111}{11}$ SOD-CMD and $\binom{111}{11}$ SOD-DEAED excreted in urine within 24 hr were 21.5 \pm 2.5% and 19.5 \pm 4.1% of dose, respectively.
Cellular distribution of SOD derivatives in th

SODs were retained at about 50 and 70%, respectively. The net shown in fig. 4. Although all three compounds significantly electric charge of SOD derivatives except SOD-DEAED was accumulated in the liver, the cellular loca \pm 4.4%. The Taubactivities of \pm Injecto-CMD and \pm Injecto-CMD and \pm 1.9% and 19.5 \pm 4.1% of dose, respectively.
Cellular distribution of SOD derivatives in the liver.
Cellular distributions of ¹¹¹In-label and 19.3 \pm 4.1% of dose, respectively.
Cellular distribution of SOD derivatives in the liver.
Cellular distributions of 111 In-labeled SOD-DEAED, Gal-SOD
and Man-SOD after i.v. injection at a dose of 0.1 mg/kg are
s **Cellular distribution of SOD derivatives in the liver.**
Cellular distributions of 111 In-labeled SOD-DEAED, Gal-SOD
and Man-SOD after i.v. injection at a dose of 0.1 mg/kg are
shown in fig. 4. Although all three compo centual distributions of In-labeled SOD-DEAED, Gal-SOD
and Man-SOD after i.v. injection at a dose of 0.1 mg/kg are
shown in fig. 4. Although all three compounds significantly
accumulated in the liver, the cellular localiza and whan-SOD after i.v. injection at a dose of 0.1 mg/kg are
shown in fig. 4. Although all three compounds significantly
accumulated in the liver, the cellular localization patterns were
rather different: $\binom{111}{11}$ Gal accumulated in the liver, the centuar localization patterns were
rather different: [¹¹¹In]Gal-SOD predominantly accumulated in
the parenchymal cells in the liver, whereas [¹¹¹In]Man-SOD
was recovered in the nonparenchy rather unferent. [Thigaa-SOD predominantly accumulated in
the parenchymal cells in the liver, whereas [¹¹¹In]Man-SOD
was recovered in the nonparenchymal cell fraction after colla-
genase perfusion. [¹¹¹In]SOD-DEAED was fractions, probably in proportion to their total cell surface area. amount of $\{^{111}\text{H}\}\text{SOD}$ excreted in urine was increased to 36.7
 \pm 4.4%. The radioactivities of $\{^{111}\text{H}\}\text{SOD}-\text{CDH}\text{ABCD}$ and $\{^{111}\text{H}\text{SO}-\text{CDH}\text{ABCD}$ and 19.5 \pm 4.1% of dose, respectively.

Cellular

was recovered in the nonparenchymal cell fraction after colla-
genase perfusion. [¹¹¹In]SOD-DEAED was recovered in both
fractions, probably in proportion to their total cell surface area.
Competition of hepatic uptake o cumulation of Gal-SOD or Man-SOD (0.1 mg/kg) after single **Competition of hepatic uptake of glycosylated SOD by**
co-administration with glycosylated BSA. The liver ac-
cumulation of Gal-SOD or Man-SOD (0.1 mg/kg) after single
administration or co-administration with Gal-BSA and co-administration with glycosylated BSA. The liver accumulation of Gal-SOD or Man-SOD (0.1 mg/kg) after single administration or co-administration with Gal-BSA and Man-BSA (20 mg/kg) is shown in fig. 5. When $\left[1^{11}\text{In}\$ administration or co-administration with Gal-BSA and Man-BSA (20 mg/kg) is shown in fig. 5. When [¹¹¹In]Gal-SOD was administrated with Gal-BSA, the liver accumulation of [¹¹¹In]Gal-SOD was reduced from 71.2 to 11.5% of Man-BSA. al-SOD was reduced from 71.2 to 11.5% of dose at 30 min
ter injection. Similarly, the uptake of $[$ ¹¹¹In]Man-SOD by the
rer was significantly inhibited by co-administration with
an-BSA.
Pharmacokinetic analysis of SOD d

after injection. Similarly, the uptake of $[111]$ Man-SOD by the
liver was significantly inhibited by co-administration with
Man-BSA.
Pharmacokinetic analysis of SOD derivatives. Areas
under the curve, CL_{total}, CL_{live} liver was significantly inhibited by co-administration with

Man-BSA.
 Pharmacokinetic analysis of SOD derivatives. Areas

under the curve, CL_{total}, CL_{liver}, CL_{urine} and CLI_i for six organs

of ¹¹¹In-labeled S Man-BSA.
 Pharmacokinetic analysis of SOD derivatives. Areas

under the curve, CL_{total}, CL_{liver}, CL_{urine} and CLI_i for six organs

of ¹¹¹In-labeled SOD and SOD derivatives are summarized in

table 2. Native [¹¹ **FIRM INCONTRETT:** analysis of SOD derivatives. Areas under the curve, CL_{total}, CL_{liver}, CL_{urine} and CLI_i for six organs of ¹¹¹In-labeled SOD and SOD derivatives are summarized in table 2. Native [¹¹¹In]SOD ha under the curve, CL_{total}, CL_{liver}, CL_{urine} and CLI_i for six organs
of ¹¹¹In-labeled SOD and SOD derivatives are summarized in
table 2. Native $[$ ¹¹¹In]SOD has large CL_{urine} and CLI_{kidney} values,
but other by the dimension and SOD derivatives are summarized in table 2. Native $[111]$ SOD has large CL_{urine} and CL_{kidney} values, but other CLI values were low. These results suggested that the elimination of $[111]$ SOD fro

Fig. 1. A, Plasma concentration; B, liver
accumulation C, kidney accumulation of ra-Fig. 1. A, Plasma concentration; B, liver
accumulation C, kidney accumulation of ra-
dioactivity of ['''In]SOD following i.v. injec-Fig. 1. A, Plasma concentration; B, liver
accumulation C, kidney accumulation of ra-
dioactivity of [¹¹¹ln]SOD following i.v. injec-
tion into mice. Results are expressed as **Fig. 1.** A, Plasma concentration; B, liver
accumulation C, kidney accumulation of ra-
dioactivity of $[1^{11} \ln]$ SOD following i.v. injec-
tion into mice. Results are expressed as
mean \pm S.D., N = 3. \bullet , 0.1 mg/kg do **Fig. 1.** A, Plasma concentration; B, liver accumulation C, kidney accumulation of radioactivity of [¹¹¹ln]SOD following i.v. injection into mice. Results are expressed as mean \pm S.D., N = 3. \bullet , 0.1 mg/kg dose; O, **Fig. 1.** A, Plasma concomulation C, kidne
dioactivity of $[$ ¹¹¹ln]SC
tion into mice. Resul
mean \pm S.D., N = 3.
O, 10 mg/kg dose.

Vol. 263
 Fig. 2. A, Plasma concentration; B, liver

accumulation and C, kidney accumulation Fig. 2. A, Plasma concentration; B, liver
accumulation and C, kidney accumulation
of radioactivity of ['''In]SOD-polysaccha-Fig. 2. A, Plasma concentration; B, I
accumulation and C, kidney accumula
of radioactivity of [¹¹¹In]SOD-polysacc
ride conjugates following i.v. injection Fig. 2. A, Plasma concentration; B, liver
accumulation and C, kidney accumulation
of radioactivity of [¹¹¹ln]SOD-polysaccha-
ride conjugates following i.v. injection into
mice at 0.1 mg/kg. Results are expressed **Fig. 2.** A, Plasma concentration; B, liver accumulation and C, kidney accumulation of radioactivity of $[111n]SOD-polysaccha-
ride conjugates following i.v. injection into mice at 0.1 mg/kg. Results are expressed as mean \pm S.D., N = 3. O, $[111n]SOD-$$ **Fig. 2.** A, Plasma concentration; B, live accumulation and C, kidney accumulatio of radioactivity of $[111n]SOD-polysaccha$ ride conjugates following i.v. injection int mice at 0.1 mg/kg. Results are expresse as mean \pm S.D. **CMD; Δ, [¹¹¹ln]SOD-DEAED.**

Fig. 3. A, Plasma concentration; B, liver Fig. 3. A, Plasma concentration; B, liver
accumulation and C, kidney accumulation
of radioactivity of [¹¹¹ln]glycosylated SOD **Fig. 3.** A, Plasma concentration; B, liver
accumulation and C, kidney accumulation
of radioactivity of [¹¹¹ln]glycosylated SOD
derivatives following i.v. injection into mice.
Results are expressed as mean \pm S.D., N **Fig. 3.** A, Plasma concentration; B, liver
accumulation and C, kidney accumulation
of radioactivity of [''In]glycosylated SOD
derivatives following i.v. injection into mice.
 $Results$ are expressed as mean \pm S.D., N
= 3. **Fig. 3.** A, Plasma concentration; B, liver
accumulation and C, kidney accumulation
of radioactivity of [¹¹¹ln]glycosylated SOD
derivatives following i.v. injection into mice.
Results are expressed as mean \pm S.D., N
= accumulation and C, kidney accumulation
of radioactivity of $[111]$ n]glycosylated SOD
derivatives following i.v. injection into mice.
Results are expressed as mean \pm S.D., N
= 3. O, $[111]$ n]Gal-SOD (0.1 mg/kg); \bullet , of radioactiv
derivatives f
Results are
= 3. O, [''
mg/kg; \triangle , ['
1 mg/kg.

Fig. 4. Cellular localization of ¹¹¹In-labeled
glycosylated SOD derivatives and SOD-Fig. 4. Cellular localization of ¹¹¹In-labeled
glycosylated SOD derivatives and SOD-
DEAED after i.v. injection in mice at a dose of
0.1 mg/kg. \Box , Parenchymal cells; \Box , nonpa-
renchymal cells. Results are expressed **Fig. 4.** Cellular localization of 111 In-labeled glycosylated SOD derivatives and SOD-DEAED after i.v. injection in mice at a dose of 0.1 mg/kg. \Box , Parenchymal cells; \Box , nonparenchymal cells. Results are expressed mean \pm S.D., N = 3. * P < .01; ** P < .001.

Amount Recovery (% of dose
sorption in the kidney. In contrast, $[$ ¹¹¹In]SOD-CMD showed
suppressed CLI_{kidney} which resulted in very small CL_{total}. $[$ ¹¹¹In]
SOD-DEAED showed a relatively high CL_{liver}. $[$ ¹¹¹In] **Amount Recovery (% of dose)**
sorption in the kidney. In contrast, $[$ ¹¹¹In]SOD-CMD showed compressed CLI_{kidney} which resulted in very small CL_{total}. $[$ ¹¹¹In] Y
SOD-DEAED showed a relatively high CL_{liver}. $[$ ¹ sorption in the kidney. In contrast, $[111]$ SOD-CMD showed
suppressed CLI_{kidney} which resulted in very small CL_{total}. $[111]$ SOD-DEAED showed a relatively high CL_{liver}. $[111]$ Gal-SOD
showed a large CL_{liver} value sorption in the kidney. In contrast, $[$ ¹¹¹In]SOD-CMD showed compressed CLI_{kidney} which resulted in very small CL_{total}. $[$ ¹¹¹In] SOD-DEAED showed a relatively high CL_{liver}. $[$ ¹¹¹In]Gal-SOD to showed a large suppressed CLI_{kidney} which resulted in very small CL_{total}. $[111]$ In SOD-DEAED showed a relatively high CL_{liver}. $[111]$ In JGal-SOD showed a large CL_{liver} value at 0.1 mg/kg which approximately corresponds to th SOD-DEAED showed a relatively high CL_{live} . [¹¹¹In]Gal-SOD tions showed a large CL_{live} value at 0.1 mg/kg which approximately teins corresponds to the hepatic plasma flow rate. [¹¹¹In]Man-SOD moietishowed a large showed a large
corresponds to
showed a large (
the CLI values
were decreased.

Discussion

Piscussion

We have previously studied the *in vivo* pharmacokinetic

operties of several model proteins modified with mono- or **Discussion** the model properties of several model proteins modified with mono- or depolysaccharide derivatives such as glycosylated albumin (Ni-**Discussion**
We have previously studied the *in vivo* pharmacokinetic
properties of several model proteins modified with mono- or
polysaccharide derivatives such as glycosylated albumin (Ni-
shikawa *et al.*, 1992) and so shikawa *et at.*, 1992) and soybean trypsin inhibitor and uricase the solution of a polysaccharide derivatives such as glycosylated albumin (Nishikawa *et al.*, 1992) and soybean trypsin inhibitor and uricase the solution

8 IO

e/10⁷ cells)

conjugated with dextran derivatives (Takakura *et al.*, 1989a,b;

Yasuda *et al.*, 1990; Fujita *et al.*, 1990, 1991). These investigae/10⁷ cells)
conjugated with dextran derivatives (Takakura *et al.*, 1989a,b;
Yasuda *et al.*, 1990; Fujita *et al.*, 1990, 1991). These investiga-
tions demonstrated that the pharmacokinetic behavior of pro-
teins can b conjugated with dextran derivatives (Takakura *et al.*, 1989a,b;
Yasuda *et al.*, 1990; Fujita *et al.*, 1990, 1991). These investiga-
tions demonstrated that the pharmacokinetic behavior of pro-
teins can be controlled by Yasuda *et al.*, 1990; Fujita *et al.*, 1990, 1991). These investigations demonstrated that the pharmacokinetic behavior of proteins can be controlled by chemical modification with sugar moieties having appropriate physic tions demonstrated that the pharmacokinetic behavior of proteins can be controlled by chemical modification with sugar moieties having appropriate physicochemical or biological properties. The purpose of the present study findings. oieties having appropriate physicochemical or biological
operties. The purpose of the present study was to develop
DD derivatives useful for targeted delivery based on these
dings.
Primarily, an appropriate synthetic metho

properties. The purpose of the present staty was to develop
SOD derivatives useful for targeted delivery based on these
findings.
Primarily, an appropriate synthetic method, which can retain
the enzymatic activity of SOD, findings. Primarily, an appropriate synthetic method, which can retain
the enzymatic activity of SOD, should be selected in order to
accomplish the development of SOD derivatives applicable to
therapeutics. For polysacchar the enzymatic activity of SOD, should be selected in order to accomplish the development of SOD derivatives applicable to therapeutics. For polysaccharide modification, periodate oxidation method was used based on our pre the enzymatic activity of SOD, should be selected in order to
accomplish the development of SOD derivatives applicable to
therapeutics. For polysaccharide modification, periodate oxi-
dation method was used based on our pr decomplish the development of SOD derivatives applicable to
therapeutics. For polysaccharide modification, periodate oxi-
dation method was used based on our previous studies (Tak-
akura *et al.*, 1989a; Yasuda *et al.*,

Fig. 5. Competition of hepatic uptake of ¹¹¹In-labeled glyco-
sylated SOD by co-administration with glycosylated BSA after
i.v. injection in mice. Glycosylated SOD (0.1 mg/kg) was **ig. 5.** Competition of hepatic uptake of '''In-labeled glycosylated SOD by co-administration with glycosylated BSA after i.v. injection in mice. Glycosylated SOD (0.1 mg/kg) was injected with cold glycosylated BSAs (20 mg

**AUCS, Clearances, and tissue uptake clearance indices of ¹¹¹In-labeled SOD and SOD derivatives
AUCs, clearances, and tissue uptake clearance indices of ¹¹¹In-labeled SOD and SOD derivatives**

Compound	Dose	AUC [*]	Clearance ^p			Tissue uptake clearance index ⁵			
			CL_{max}	$a_{\bullet \bullet \bullet}$	CL_{max}	CLL	CL_{mean}	CLANOW	CL_{mass}
	mg/kg	% dose hr/ml	μl/hr			µl/hr/g			
SOD	0.1	9.49	10.500	42.3	1230.0	26.2	20.7	23,700.0	31.0
	10.0	9.81	10.200	52.1	3750.0	33.5	14.9	21.500.0	54.2
SOD-CMD	0.1	315.0	317	21.8	68.4	21.0	17.4	344.0	1.9
SOD-DEAED	0.1	34.8	2.870	1.440.0	561.0	1.440.0	216.0	46.3	12.0
Gal-SOD	0.1	1.05	95,200	95,000.0	5630.0	78.300.0	54.0	596.0	158.0
	1.0	3.67	28,600	16,300.0	658.0	12,300.0	33.0	354.0	72.8
Man-SOD	0.1	1.67	72.900	53.400.0	3400.0	37,100.0	9790.0	0.080. ا	67.3
	1.0	5.06	19,800	11,900.0	5210.0	8.360.0	4010.0	861.0	97.5

^{1.0} 3.00 19,000 11,900.0

* AUC, area under curve; calculated by fitting the plasma concentration curve to a biext

et al., 1981).

² Calculated at 0 to 2 hr (SOD, glycosylated SOD and SOD-DEAED) or 0 to 24 hr (SOD

me ⁴ AUC, area under curve; calculated by fitting the plasma concentration curve to a biological activities. A satisfactory level of remaining enzymatic activities of all SOD derivatives were obtained by remaintain biologi ^e Calculated at 0 to 2 hr (SOD, glycosylated SOD and SOD-DEAED) or 0 to 24 hr (SOD-
method of Lee *et al.* (1976) since the method was reported to suff
maintain biological activities. A satisfactory level of remaining an method of Lee *et al.* (1976) since the method was imaintain biological activities. A satisfactory level of enzymatic activities of all SOD derivatives were of employing these synthetic methods (see table 1). The present exteed of Lee *et al.* (1976) since the method was reported to signition biological activities. A satisfactory level of remaining a approximation activities of all SOD derivatives were obtained by mploying these synthetic

maintain biological activities. A satisfactory level of remaining
enzymatic activities of all SOD derivatives were obtained by
employing these synthetic methods (see table 1).
The present study demonstrated that the *in v* ification with various sugars. We evaluated pharmacokinetic employing these synthetic methods (see table 1). d
The present study demonstrated that the *in vivo* fate of li
human recombinant SOD can be controlled by chemical mod-
ification with various sugars. We evaluated pharmacok The present study demonstrated that the *in vivo* fate of like
human recombinant SOD can be controlled by chemical mod-
ification with various sugars. We evaluated pharmacokinetic
characteristics of SOD and SOD derivative human recombinant SOD can be controlled by chemical mod-
ification with various sugars. We evaluated pharmacokinetic
characteristics of SOD and SOD derivatives after i.v. injection
using ¹¹¹In-labeled compounds since ¹¹ ification with various sugars. We evaluated pharmacokinetic
characteristics of SOD and SOD derivatives after i.v. injection
using ¹¹¹In-labeled compounds since ¹¹¹In is reported to be
accumulated in the organ by the ex characteristics of SOD and SOD derivatives after i.v. injection with using 111 In-labeled compounds since 111 In is reported to be for accumulated in the organ by the exchange into an iron-binding shoprotein (Brown using 1^{11} In-labeled compounds since 1^{11} In is reported to be accumulated in the organ by the exchange into an iron-binding protein (Brown *et al.*, 1987) after intracellular degradation of proteins. The radioactivi accumulated in the organ by the exchange into an iron-binding
protein (Brown *et al.*, 1987) after intracellular degradation of
proteins. The radioactivity was retained for a relatively long
time even in the liver and thi protein (Brown *et al.*, 1987) after intracellular degradation of sproteins. The radioactivity was retained for a relatively long stime even in the liver and this enabled us to estimate the net \sim organ uptake clearance proteins. The radioactivity was retained for a relatively long
time even in the liver and this enabled us to estimate the net
organ uptake clearance. However, at the later phase of experi-
ment, the radioactivity of the li time even in the liver and this enabled us to estimate the net Thorgan uptake clearance. However, at the later phase of experi-
ment, the radioactivity of the liver gradually decreased (data con
not shown), suggesting a sl organ uptake clearance. However, at the later phase of experi-
ment, the radioactivity of the liver gradually decreased (data concentration), suggesting a slight release of 111 In from the tissue.
Therefore, the pharmaco ment, the radioactivity of the liver gradually decreased (not shown), suggesting a slight release of ¹¹¹In from the ti
Therefore, the pharmacokinetic analysis was performed
relatively early period when the plasma concent not shown), suggesting a slight release of ¹¹¹In from the tissue.
Therefore, the pharmacokinetic analysis was performed at a
relatively early period when the plasma concentration had
become less than 1% of dose/ml, but t Therefore, the pharmacokinetic analysis was performed at a relatively early period when the plasma concentration had phacome less than 1% of dose/ml, but the efflux of 111 In-radio-activity from the liver was negligib relatively early period when the plasma concentration had ported by several investigators (Pyatak *et al.*, 1980; Fuertges become less than 1% of dose/ml, but the efflux of ¹¹¹In-radio-
and Abuchowski, 1990). On the oth

supponential equation using the nonlinear least squares program MULTI (Yamaoka
SOD-CMD).
sufficiently stable in plasma, as shown in the stability study,
and we obtained plasma concentration-time profiles of enzyiexponential equation using the nonlinear least squares program MULTI (Yamao)
SOD-CMD).
sufficiently stable in plasma, as shown in the stability study
and we obtained plasma concentration-time profiles of enzy
matic activi SOD-CMD).
sufficiently stable in plasma, as shown in the stability study,
and we obtained plasma concentration-time profiles of enzy-
matic activity of SOD almost identical to those of radioactivity
determination after i.v sufficiently stable in plasma, as shown in the stability study, and we obtained plasma concentration-time profiles of enzy-
matic activity of SOD almost identical to those of radioactivity
determination after i.v. injectio sufficiently stable in plasma, as shown in the stability study, and we obtained plasma concentration-time profiles of enzy-
matic activity of SOD almost identical to those of radioactivity determination after i.v. injectio and we obtained plasma concentratio
matic activity of SOD almost identical
determination after i.v. injection in m
likely that disposition of SOD deriva
flected by ¹¹¹In-radioactivity counting.
The CL_{liver} and CL_{urine} atic activity of SOD almost identical to those of radioactivity
termination after i.v. injection in mice. Consequently, it is
rely that disposition of SOD derivatives was accurately re-
cted by ¹¹¹In-radioactivity counti

determination after i.v. injection in mice. Consequently, it is likely that disposition of SOD derivatives was accurately re-
flected by ¹¹¹In-radioactivity counting.
The CL_{liver} and CL_{urine} of the four SOD derivativ likely that disposition of SOD derivatives was accurately re-
flected by ¹¹¹In-radioactivity counting.
The CL_{liver} and CL_{urine} of the four SOD derivatives together
with those of polyethylene glycol-conjugated SOD (PE flected by 111 In-radioactivity counting.
The CL_{liver} and CL_{urine} of the four SOD derivatives together
with those of polyethylene glycol-conjugated SOD (PEG-SOD)
for comparing their basic *in vivo* disposition char The CL_{uiver} and CL_{urine} of the four SOD derivatives together
with those of polyethylene glycol-conjugated SOD (PEG-SOD)
for comparing their basic *in vivo* disposition characteristics are
shown in fig. 6. SOD itself is with those of polyethylene glycol-conjugated SOD (PEG-SOD) for comparing their basic *in vivo* disposition characteristics are shown in fig. 6. SOD itself is characterized by a large CL_{urine} since it is a small protein h for comparing their basic in vivo disposition characteristics are shown in fig. 6. SOD itself is characterized by a large CL_{unine} since it is a small protein having a MW of 32 kDa which is susceptible to glomerular fil shown in fig. 6. SOD itself is characterized by a large CL_{urine}
since it is a small protein having a MW of 32 kDa which is
susceptible to glomerular filtration (Taylor and Granger, 1984).
The CL_{uiver} was markedly incre since it is a small protein having a MW of 32 kDa which is
susceptible to glomerular filtration (Taylor and Granger, 1984).
The CL_{liver} was markedly increased by conjugating with
DEAED, but CL_{urine} was also reduced in The CL_{liver} was markedly increased by conjugating with DEAED, but CL_{urine} was also reduced in this case. In contrast, DEAED, but CL_{urine} was also reduced in this case. In contrast, conjugation of CMD to SOD reduced both CL_{urine} and CL_{urine} and thus resulted in prolonged plasma retention of enzymatic activity. Similar results were ob conjugation of CMD to SOD reduced both CL_{liver} and CL_{urine}
and thus resulted in prolonged plasma retention of enzymatic
activity. Similar results were obtained for PEG-SOD, as re-
ported by several investigators (Pyata and thus resulted in prolonged plasma retention of enzymatic activity. Similar results were obtained for PEG-SOD, as reported by several investigators (Pyatak *et al.*, 1980; Fuertges and Abuchowski, 1990). On the other ha activity. Similar results were obtained for PEG-SOD, as reported by several investigators (Pyatak *et al.*, 1980; Fuertgen and Abuchowski, 1990). On the other hand, by introducing monosaccharides to SOD (Gal-SOD and Man-SO ported by several investigators (Pyatak *et al.*, 1980; Fuertges and Abuchowski, 1990). On the other hand, by introducing monosaccharides to SOD (Gal-SOD and Man-SOD), the value of CL_{liver} was 100 to 1000 times increased

Fig. 6. Hepatic and urinary clearances of SOD derivatives Fig. 6. Hepatic and urinary clearances of SOD derivatives
in mice. Hepatic plasma flow rate (Gerlowski and Jain,
1983), rate of fluid-phase endocytosis of liver (Munniksma
et al., 1980) and glomerular filtration rate (Dedr Fig. 6. Hepatic and urinary clearances of SOD derivatives
in mice. Hepatic plasma flow rate (Gerlowski and Jain,
1983), rate of fluid-phase endocytosis of liver (Munniksma
et al., 1980) and glomerular filtration rate (Dedr g.

CLATTE (EDIT)
In addition to the hepatic plasma flow rate, suggesting (PEG-
In addition to the altered pharmacokinetic properties of SOD SOD ϵ
rivatives, interesting results were obtained in renal disposi-
 ϵt al.,

were nearly equal to the hepatic plasma flow rate, suggestial
most complete uptake of Gal-SOD and Man-SOD in the liver
In addition to the altered pharmacokinetic properties of SC
derivatives, interesting results were obtai almost complete uptake of Gal-SOD and Man-SOD in the liver.
In addition to the altered pharmacokinetic properties of SOD
derivatives, interesting results were obtained in renal disposi-
tion of native SOD in which marked a almost complete uptake of Gal-SOD and Man-SOD in the liver.
In addition to the altered pharmacokinetic properties of SOD
derivatives, interesting results were obtained in renal disposi-
tion of native SOD in which marked a In addition to the altered pharmacokinetic properties of SOD
derivatives, interesting results were obtained in renal disposi-
tion of native SOD in which marked accumulation of radioac-
ativity was observed after i.v. inj tivity was observed after i.v. injection. We have demonstrated
that this accumulation of $[$ ¹¹¹In]SOD occurred via proximal
tubular reabsorption after glomerular filtration in the rat kid-
ney perfusion experiment (unpu that this accumulation of $[111]SOD$ occurred via proximal
tubular reabsorption after glomerular filtration in the rat kid-
ney perfusion experiment (unpublished data). The ratios of
accumulated amount of $[111]SOD$ in the tubular reabsorption after glomerular filtration in the rat kid-
ney perfusion experiment (unpublished data). The ratios of
accumulated amount of $[{}^{111}In]SOD$ in the kidney to total in
amount recovered in the kidney and u accumulated amount of $[111]SOD$ in the kidney to total amount recovered in the kidney and urine were 0.64 (0.1 mg/kg) and 0.24 (10 mg/kg) after i.v. injection in mice (see fig. 1) suggesting saturation in the reabsorption cumulated amount of $[$ ¹¹¹In]SOD in the kidney to total nount recovered in the kidney and urine were 0.64 (0.1 mg/) and 0.24 (10 mg/kg) after i.v. injection in mice (see fig. 1) ggesting saturation in the reabsorption p amount recovered in the kidney and urine were 0.64 (0.1 n kg) and 0.24 (10 mg/kg) after i.v. injection in mice (see fig. suggesting saturation in the reabsorption process.
In contrast, $[$ ¹¹¹In]Gal-SOD and $[$ ¹¹¹In]Ma

kg) and 0.24 (10 mg/kg) after i.v. injection in mice (see fig. 1)
suggesting saturation in the reabsorption process.
In contrast, $[$ ¹¹¹In]Gal-SOD and $[$ ¹¹¹In]Man-SOD were not
accumulated in the kidney. The values of suggesting saturation in the reabsorption process.
In contrast, $[$ ¹¹¹In]Gal-SOD and $[$ ¹¹¹In]Man-SOD were no
accumulated in the kidney. The values of CLI_{kidney} for monos
saccharide derivatives were 1 to 2 orders of In contrast, $[111]$ Gal-SOD and $[111]$ Man-SOD were not on
accumulated in the kidney. The values of CL_{kidney} for mono-
in
saccharide derivatives were 1 to 2 orders of magnitude smaller
than that for $[111]$ SOD, suggesti accumulated in the kidney. The values of CLI_{kidney} for mono-
saccharide derivatives were 1 to 2 orders of magnitude smaller
than that for $[111]$ SOD, suggesting that monosaccharide mod-
ification drastically reduced the than that for [¹¹¹In]SOD, suggesting that monosaccharide modification drastically reduced the tubular reabsorption of the protein. A recent study reported that glycosylated albumin was virtually excluded from reabsorptio ification drastically reduced the tubular reabsorption of the reprotein. A recent study reported that glycosylated albumin was sivirtually excluded from reabsorption process in the proximal helibule using a micropuncture protein. A recent study reported that glycosylated albumin was
virtually excluded from reabsorption process in the proximal
tubule using a micropuncture technique (Kowluru *et al.*, 1992).
We have also confirmed that the virtually excluded from reabsorption process in the proximal
tubule using a micropuncture technique (Kowluru *et al.*, 1992).
We have also confirmed that the renal tubular reabsorption of
 $[$ ¹¹¹In]Gal-SOD and $[$ ¹¹¹In tubule using a micropuncture technique (Kowluru *et al.*, 1992
We have also confirmed that the renal tubular reabsorption (111 In]Gal-SOD and 111 In]Man-SOD was dramatically suppressed in comparison with that of nati is the detailed mechanism of the renal tubular reabsorption of fer-
 ${}^{1}\text{In}$ Gal-SOD and $[{}^{111}\text{In}]$ Man-SOD was dramatically sup-

essed in comparison with that of native $[{}^{111}\text{In}]$ SOD using rat as t
 situ isolat $[111]$ Gal-SOD and $[111]$ Man-SOD was dramatically suppressed in comparison with that of native $[111]$ SOD using rat *in situ* isolated perfusion kidney system (unpublished data). The detailed mechanism of cellular toxici

pressed in comparison with that of native $[111]$ SOD using rat as *in situ* isolated perfusion kidney system (unpublished data). of The detailed mechanism of cellular toxicity mediated by the reactive oxygen species inclu in situ isolated perfusion kidney system (unpublished data). The detailed mechanism of cellular toxicity mediated by
reactive oxygen species including superoxide anion is unknown.
Therefore, an ideal cellular delivery mode The detailed mechanism of cellular toxicity mediated by the
reactive oxygen species including superoxide anion is unknown.
Therefore, an ideal cellular delivery mode of SOD has not been or
established although various appr reactive oxygen species including superoxide anion is unknown.
Therefore, an ideal cellular delivery mode of SOD has not been
established although various approaches have been reported for
improved therapeutic efficacy of Therefore, an ideal cellular delivery mode of SOD has not been
established although various approaches have been reported for
improved therapeutic efficacy of SOD. It is generally known
that oxidative stress occurring insi established although various approaches have been reported for
improved therapeutic efficacy of SOD. It is generally known
that oxidative stress occurring inside the cell may be minimized,
since most cells are highly enric improved therapeutic efficacy of SOD. It is generally known SOD
that oxidative stress occurring inside the cell may be minimized, and s
since most cells are highly enriched with antioxidative system. sugar
From this reason

(PEG-SOD) and polystyrene-co-maleic acid to SOD reported (PEG-SOD) and polystyrene-co-maleic acid to SOD reported
to result in prolonged plasma or extracellular space level of
SOD and subsequent improved effect (Pyatak *et al.*, 1980; Inoue
et al., 1989; Watanabe *et al.*, 198 (PEG-SOD) and polystyrene-co-maleic acid to SOD reported
to result in prolonged plasma or extracellular space level of
SOD and subsequent improved effect (Pyatak *et al.*, 1980; Inoue
et al., 1989; Watanabe *et al.*, 198 to result in prolonged plasma or extracellular space level of SOD and subsequent improved effect (Pyatak *et al.*, 1980; Inoue *et al.*, 1989; Watanabe *et al.*, 1989; Fuertges *et al.*, 1990). In addition, SOD having a C to result in prolonged plasma or extracement space level of SOD and subsequent improved effect (Pyatak *et al.*, 1980; Inoue *et al.*, 1989; Watanabe *et al.*, 1989; Fuertges *et al.*, 1990). In addition, SOD having a C-t sob and subsequent improved effect (*F* yatak *et al.*, 1980, mode *et al.*, 1989; Watanabe *et al.*, 1989; Fuertges *et al.*, 1990). In addition, SOD having a C-terminal heparin-binding domain has been genetically constru dition, SOD having a C-terminal heparin-binding domain
s been genetically constructed and proved to bind the surface
endothelial cells and show inhibitory effects against oxygen
xicity (Inoue *et al.*, 1990).
On the other

has been genetically constructed and proved to bind the surface of endothelial cells and show inhibitory effects against oxygen-
toxicity (Inoue *et al.*, 1990).
On the other hand, several reports showed the importance
in of endothelial cells and show inhibitory effects against oxygen
toxicity (Inoue *et al.*, 1990).
On the other hand, several reports showed the importance of
intracellular level of SOD in protecting cells from oxygen-
media On the other hand, several reports showed the importance of intracellular level of SOD in protecting cells from oxygen-
mediated toxicity (Freeman *et al.*, 1983; Kyle *et al.*, 1988;
Beckman *et al.*, 1988; Liu *et al.*, intracellular level of SOD in protecting cells from oxygen-
mediated toxicity (Freeman *et al.*, 1983; Kyle *et al.*, 1988;
Beckman *et al.*, 1988; Liu *et al.*, 1989). Augmentation of cellular
uptake and pharmacological a inediated toxicity (Freeman et al., 1983, Kyle et al., 1986,
Beckman et al., 1988; Liu et al., 1989). Augmentation of cellular
uptake and pharmacological activities of SOD have been dem-
onstrated through liposomal entrapm beckman et at., 1966, Lid et at., 1969). Augmentation of centual
uptake and pharmacological activities of SOD have been dem-
onstrated through liposomal entrapment and PEG conjugation
in cultured endothelial cells. However

onstrated through hposomal entrapment and FEG conjugation
in cultured endothelial cells. However, the intracellular fate of
enzyme was not shown in these studies.
Generally, many glycoproteins are known to bind specific
re enzyme was not shown in these studies.

Generally, many glycoproteins are known to bind specific

receptors on the cell surface and be internalized. In the present

study, two receptor systems, the asialoglycoprotein recep Generally, many glycoproteins are known to olnu specific
receptors on the cell surface and be internalized. In the prese
study, two receptor systems, the asialoglycoprotein receptor of
hepatocyte (Ashwell and Morell, 1974; study, two receptor systems, the asialoglycoprotein receptor on
hepatocyte (Ashwell and Morell, 1974; Kawasaki and Ashwell,
1976) and the mannose/N-acetylglucosamine receptor on Kupf-
fer and endothelial cells in the liver study, two receptor systems, the asialoglycoprotein receptor on
hepatocyte (Ashwell and Morell, 1974; Kawasaki and Ashwell,
1976) and the mannose/N-acetylglucosamine receptor on Kupf-
fer and endothelial cells in the liver hepatocyte (Ashwen and Moren, 1974, Kawasaki and Ashwen, 1976) and the mannose/N-acetylglucosamine receptor on Kupf-
fer and endothelial cells in the liver (Brown *et al.*, 1978; Schles-
inger *et al.*, 1978; Magnusson an 1370) and the mannose/14-acetygudosamme receptor on Kupi-
fer and endothelial cells in the liver (Brown *et al.*, 1978; Schles-
inger *et al.*, 1978; Magnusson and Berg, 1989) were evaluated
as targets for site-specific d inger *et al.*, 1976, Magnusson and Berg, 1989) were evaluated
as targets for site-specific delivery of enzymes. The advantages
of these systems arise from: 1), high affinity of the receptor to
the ligand; 2), rapid recyc as targets for site-specific delivery of enzymes. The advantages
of these systems arise from: 1), high affinity of the receptor to
the ligand; 2), rapid recycling of the receptor molecules (Mag-
nusson and Berg, 1989) and of these systems arise from: 1), high affinity of the recept
the ligand; 2), rapid recycling of the receptor molecules (
nusson and Berg, 1989) and 3), specific expression of rece
on a certain cell type. After i.v. inject the ligand; 2), rapid recycling of the receptor molecules (Mag-
nusson and Berg, 1989) and 3), specific expression of receptors
on a certain cell type. After i.v. injection, $[^{111}In]Gal-SOD$ ac-
cumulated in the hepatic paren nusson and Berg, 1989) and 3), specific expression of receptors
on a certain cell type. After i.v. injection, $[$ ¹¹¹In]Gal-SOD ac-
cumulated in the hepatic parenchymal cells while $[$ ¹¹¹In]Man-
SOD was taken up by the on a certain cell type. After i.v. injection, $[111]$ In]Gal-SOD acumulated in the hepatic parenchymal cells while $[111]$ In]MaSOD was taken up by the nonparenchymal cells in the livend spleen (see fig. 4). Furthermore, s cumulated in the hepatic parenchymal cells while [¹¹¹In]Man-SOD was taken up by the nonparenchymal cells in the liver and spleen (see fig. 4). Furthermore, specificity of the attached sugar moieties was also confirmed by SOD was taken up by the nonparenchymal cells in the liver
and spleen (see fig. 4). Furthermore, specificity of the attached
sugar moieties was also confirmed by the competition experi-
ments using glycosylated BSA derivati

SODs as well as albumin derivatives (Nishikawa *et at.,* 1992) 1991
SODs as well as albumin derivatives (Nishikawa *et al.*, 1992)
suggesting the generality of this approach in a delivery of $\frac{1}{100}$ FOODs as well as albumin derivatives (Nishikawa *et al.*, 1992)
suggesting the generality of this approach in a delivery of B_1
enzymes to intracellular space.
It is noteworthy that small proteins like SOD, which underg

SODs as well as albumin derivatives (Nishikawa *et al.*, 1992)
suggesting the generality of this approach in a delivery of $\frac{600}{BC}$
enzymes to intracellular space.
It is noteworthy that small proteins like SOD, which u suggesting the generality of this approach in a delivery of penzymes to intracellular space.
It is noteworthy that small proteins like SOD, which undergo rapid glomerular filtration, can be targeted to these cells by modi enzymes to intracellular space.
It is noteworthy that small proteins like SOD, which undergo
rapid glomerular filtration, can be targeted to these cells by
modifying with monosaccharides without a significant increase
in M rapid glomerular filtration, can be targeted to these cells by modifying with monosaccharides without a significant increase in MW. It is also an interesting issue to clarify the relationship between the receptor-mediated modifying with monosaccharides without a significant increase modifying with monosaccharides without a significant increase

in MW. It is also an interesting issue to clarify the relationship

between the receptor-mediated endocytosis and the molecular

size of glycoproteins. In our between the receptor-mediated endocytosis and the molecular between the receptor-mediated endocytosis and the molecular
size of glycoproteins. In our preliminary study, we showed that
¹¹¹In-labeled glycosylated IgGs (MW: 150 kDa) synthesized by
the same method as glycosylated SOD size of glycoproteins. In our preliminary study, we showed that $\frac{PEDKER}{FUME, L}$, $\frac{1}{11}$
In-labeled glycosylated IgGs (MW: 150 kDa) synthesized by $\frac{PIDKER}{NUME, L}$, $\frac{1}{11}$
the same method as glycosylated SODs were the same method as glycosylated SODs were rapidly accumulated in the liver after i.v. injection in mice. Therefore, it was suggested that proteins with a relatively wide range of MW (35-150 kDa) can be recognized and endoc lated in the liver after i.v. injection in mice. Therefore, it was suggested that proteins with a relatively wide range of MW (35-150 kDa) can be recognized and endocytosed by asialogly-coprotein receptor or mannose/N-acet suggested that proteins with a relatively wide range of MW (35-150 kDa) can be recognized and endocytosed by asialogly-
coprotein receptor or mannose/N-acetylglucosamine receptor
of the liver.
In contrast, $[$ ¹¹¹In]SODggested that proteins with a relatively wide range of MW
5-150 kDa) can be recognized and endocytosed by asialogly-
protein receptor or mannose/N-acetylglucosamine receptor
the liver.
In contrast, $[^{111}In]SOD-DEAED$ demonstr

(35-150 kDa) can be recognized and endocytosed by asialogly-

coprotein receptor or mannose/N-acetylglucosamine receptor

of the liver.

In contrast, $[$ ¹¹¹In]SOD-DEAED demonstrated extensive he-

patic uptake presumabl coprotein receptor or mannose/N-acetylglucosamine receptor
of the liver.
In contrast, $\begin{bmatrix}1^{11}\text{In}|\text{SOD-DEAED}\end{bmatrix}$ demonstrated extensive he-
patic uptake presumably due to electrostatic interaction with
both parenchy of the liver.
In contrast, $[111]$ SOD-DEAED demonstrated extensive he-
patic uptake presumably due to electrostatic interaction with
both parenchymal and nonparenchymal cells depending on the
percentages of their surface a In contrast, $\left\{\right.$ In 1990). DERIED demonstrated extensive ne-
patic uptake presumably due to electrostatic interaction with
both parenchymal and nonparenchymal cells depending on the
percentages of their surface area both parenchymal and nonparenchymal cells depending on
percentages of their surface area ratio (73:27) (Nishida *et*
1991). We have elucidated hepatic disposition profiles of
ionized and glycosylated BSA in details by a co 1991). We have elucidated hepatic disposition profiles of cat-

ionized and glycosylated BSA in details by a computer simu-

inization of cationized BSA adsorbed on the cell surface compared

ization of cationized BSA ads ionized and glycosylated BSA in details by a computer simu-

lation in a previous paper (Nishida *et al.*, 1992). Slow internal-

ization of cationized BSA adsorbed on the cell surface compared

with glycosylated BSA was o is expected to be retained on the surface of the liver cells for a
is expected to be retained on the surface of the liver cells for a
is expected to be retained on the surface of the liver cells for a
considerably long pe In the discussive of the calculation of calculated DSA ausobbed on the central ecompared
the glycosylated BSA was observed there and SOD-DEAED
expected to be retained on the surface of the liver cells for a
miderably long

portant, especially in the case of clinical application. Fiume *et*

aconsiderably long period.

Immunological properties of SOD derivatives are very im-

portant, especially in the case of clinical application. Fiume *et* considerably long period.

Immunological properties of SOD derivatives are very im-

portant, especially in the case of clinical application. Fiume et

al. (1982, 1986) demonstrated that a drug-conjugated neogly-

coprote Immunological properties of SOD derivatives are very important, especially in the case of clinical application. Fiume *et* al. (1982, 1986) demonstrated that a drug-conjugated neogly-
coprotein, lactosaminated albumin, wa portant, especially in the case of clinical application. Fiume *et al.* (1982, 1986) demonstrated that a drug-conjugated neogly-coprotein, lactosaminated albumin, was not immunogenic when prepared with homologous albumin coprocem, accosaminated anomini, was not immunogenic wi
prepared with homologous albumin. Dextran conjugation v
also reported to reduce immunogenicity of proteins (Yasuda
al., 1990). Although further studies are required o also reported to reduce immunogenicity of pal., 1990). Although further studies are requinogenicity/antigenicity of SOD derivatives cation of human recombinant SOD with morides might be advantageous in the aspect.
In concl 1990). Although further studies are required on the immugenicity/antigenicity of SOD derivatives, chemical modifition of human recombinant SOD with mono- or polysacchales might be advantageous in the aspect.
In conclusion,

requirity/antigenicity of SOD derivatives, chemical modification of human recombinant SOD with mono- or polysaccharides might be advantageous in the aspect.
In conclusion, we demonstrated that targeted delivery of recombin Eation of numan recombinant SOD with mono- of polysactia-
rides might be advantageous in the aspect.
In conclusion, we demonstrated that targeted delivery of
recombinant SOD can be achieved by chemical modification
utilizi In conclusion, we demonstrated that targeted delivery of recombinant SOD can be achieved by chemical modification utilizing sugar moieties. The derivatives developed in this study are classified as follows: 1), long-circul recombinant SOD can be achieved by chemical modification ^F
utilizing sugar moieties. The derivatives developed in this study
are classified as follows: 1), long-circulating type (SOD-CMD);
2), cell-surface targeting type are classified as follows: 1), long-circulating type (SOD-CMD);

2), cell-surface targeting type (SOD-DEAED); 3), intracellular

targeting type (Gal-SOD for hepatocyte and Man-SOD for

resident macrophages and endothelial targeting type (Gal-SOD for hepatocyte and Man-SOD for resident macrophages and endothelial cells in the liver and spleen). These compounds would be useful for not only thera**of** relationship between cellular localization and biological ef-

peutics of superoxide-related diseases but also for basic analysis

of relationship between cellular localization and biological ef-

fects of SOD. In par spleen). These compounds would be useful for not only there
peutics of superoxide-related diseases but also for basic analysi
of relationship between cellular localization and biological ef
fects of SOD. In particular, Gal of relationship between cellular localization and biological effects of SOD. In particular, Gal-SOD and Man-SOD seem to be unique and effective, although they might be rapidly inactivated inside the cell due to the lysosom of relationship between cellular localization and biological effects of SOD. In particular, Gal-SOD and Man-SOD seem t
be unique and effective, although they might be rapidly inac
tivated inside the cell due to the lysosom iects of SOD. In particular, Gar-SOD and Man-SOD seem to
be unique and effective, although they might be rapidly inac-
tivated inside the cell due to the lysosomal degradation path-
ways for glycoproteins. We have started tivated inside the cell due to the lysosomal degradation pathways for glycoproteins. We have started pharmacological studies of these SOD derivatives using a rat hepatic ischemia-
reperfusion model and promising therapeuti report.

References

- The resolution of the resolution of the resolutions of the report.
 References

ASHWELL, G. AND MORELL, A. G.: The role of surface carbohydrates in the

hepatic recognition and transport of circulating glycoproteins. Adv hepatic recognition and transport of circulating glycoproteins. Adv. Enzymol. 47: **99-128, 1974. BECKMAN,** J. S., MINOR, R. L., WHITE, C. W., REPINE, J. E., ROSEN, *G.* M. AND **FREEMAN,** B. A.: Superoxide dismutase and catalase conjugated polyethylene
-

Targeted Delivery of SOD 977
glycol increased endothelial enzyme activity and oxidant resistance. J. Biol.
BROWN, B. A., COMEAU, R. D., JONES, P. L., LIBERATONE, F. A., NEACY, W. P.,

- **Targeted Delivery of SOD** 977
glycol increased endothelial enzyme activity and oxidant resistance. J. Biol.
Chem. 263: 6884-6892, 1988.
BROWN, B. A., COMEAU, R. D., JONES, P. L., LIBERATONE, F. A., NEACY, W. P.,
SANDS, H. glycol increased endothelial enzyme activity and oxidant resistance. J. Biol.
Chem. 263: 6884-6892, 1988.
BROWN, B. A., COMEAU, R. D., JONES, P. L., LIBERATONE, F. A., NEACY, W. P.,
SANDS, H. AND GALLAGHER, B. M.: Pharmac
- antibody B72.3 and its fragments labeled with either ¹²⁵I or ¹¹¹In. Cancer Res.
47: 1149-1154, 1987.
BROWN, T. L., HENDERSON, L. A., THORPE, S. R. AND BAYNES, J. W.: The
effect of mannose terminal oligosaccharides on t
-
- B by nonparenchymal cell by rat liver. Arch. Biochem. Biophys. 188: 418–428, 1978.

EDRICK, R. L.: Animal scale-up. J. Pharmacokinet. Biopharm. 1: 435–461, 1973.

UME, L., BASSI, B., BUSI, C., MATTIOLI, A. AND SPINOSA, G.: in antiviral chemotherapy: A chemically stable conjugate of 9- β -D-arabinofur-
anosyl-adenine-5'-monophosphate with lactosaminated albumin accomplishes
a selective delivery of the drug to liver cells. Biochem. Pharmacol
- of adenosine-9- α -D-arabinofuranoside monophosphate (ara-AMP) with lacto-
saminated homologous albumin are not immunogenic in the mouse. Experientia
(Basel) 38: 1087–1089, 1982.
termAN, B. A., YOUNG, S. L. AND CRAPO J. D
- FREEMAN, B. A., YOUNG, S. L. AND CRAPO J. D.: Liposome-mediated augmentation of superoxide dismutase in endothelial cells prevents oxygen injury. J.
Biol. Chem. 258: 12534-12542, 1983.
FRIDOVICH, I.: Superoxide radical: An
- Biol. Chem. 258: 12534-12542, 1983.
Hol. Chem. 258: 12534-12542, 1983.
IDOVICH, I.: Superoxide radical: An endogenous toxicant. Ann. Rev. P.
JENCESE, F. AND ABUCHOWSKI, A.: The clinical efficacy of poly
Express, F. AND ABU
-
- FRIDOVICH, I.: Superoxide radical: An endogenous toxicant. Ann. Rev. Pharmacol.

Toxicol. 23: 239–257, 1983.

FUERTGES, F. AND ABUCHOWSKI, A.: The clinical efficacy of poly(ethylene

glycol)-modified proteins. J. Controlle ERITGES, F. AND ABUCHOWSKI, A.: The clinical efficacy of poly(ethylene glycol)-modified proteins. J. Controlled Release 11: 139-148, 1990.
JJITA, T., YASUDA, Y., TAKAKURA, Y., HASHIDA, M. AND SEZAKI, H.: Alteration of biop glycol)-modified proteins. J. Controlled Release 11: 139–148, 1990.
FUJITA, T., YASUDA, Y., TAKAKURA, Y., HASHIDA, M. AND SEZAKI, H.: Alteration
- distribution of ¹¹¹In-labeled uricase conjugates with charged dextrans and macromolecules: Uricase-dextran conjugates. J. Controlled Release 11: 149-
154, 1990.
FUJITA, T., YASUDA, Y., TAKAKURA, Y., HASHIDA, M. AND SEZAKI, H.: Tissue
distribution of ¹¹¹In-labeled uricase conjugates with charged HABEEB, A. F. S. A.: Determination of free amino groups in the harged dextrans and polyethylene glycol. J. Pharmacobio-Dyn. 14: 623-629, 1991.
GERLOWSKI, L. E. AND JAIN, R. K.: Physiological pharmacokinetic modeling:
Princ
-
- GERLOWSKI, L. E. AND JAIN, R. K.: Physiological pharmacokinetic modeling:

Principle and application. J. Pharm. Sci. 72: 1103-1126, 1983.

HABEEB, A. F. S. A.: Determination of free amino groups in proteins by trinitro-

b Principle and application. J. Pharm. Sci. 72: 1103-1126, 1983.
ABEEB, A. F. S. A.: Determination of free amino groups in proteins by trini
benzenesulfonic acid. Anal. Biochem. 14: 328-336, 1966.
NATOWICH, D., LAYNE, W. W.
-
- HABEEB, A. F. S. A.: Determination of free amino groups in proteins by trinitro-
benzenesulfonic acid. Anal. Biochem. 14: 328–336, 1966.
HNATOWICH, D., LAYNE, W. W. AND CHILDS, R. L.: The preparation and labeling
of DTPA-c HNATOWICH, D., LAYNE, W. W. AND CHILDS, R. L.: The preparation and labeling of DTPA-coupled albumin. Int. J. Appl. Radiat. Isot. 12: 327-332, 1982. INOUE, M., EBASHI, I., WATANABE, N. AND MORINO, Y.: Synthesis of a superox
- of DTPA-coupled albumin. Int. J. Appl. Radiat. Isot. 12: 327-332, 1982.

INOUE, M., EBASHI, I., WATANABE, N. AND MORINO, Y.: Synthesis of a superoxide

dismutase derivatives that circulates bound to albumin and accumulates
- INOUE, M., WATANABE, N., MORINO, Y., TANAKA, Y., AMACHI, T. AND SASAKI, J.: Inhibition of oxygen toxicity by targeting superoxide dismutase to endothe-
hial cell surface. FEBS Lett. 269-92, 1990.
KAWASAKI, T. AND ASHWELL,
-
- KAWASAKI, T. AND ASHWELL, G.: Chemical and physical properties of a hepatic
membrane protein that specifically binds asialoglycoproteins. J. Biol. Chem.
251: 1296-1302, 1976.
KOWLURU, A., KOWLURU, R. A., SOLOMON, S. AND MA cytotoxicity. Effects upon protein recognition by the proximal tubule. Life Sci.
50: 281-286, 1992.
KYLE, M. E., NAKAE, D., SAKAIDE, I., MICCADEI, S. AND FARBER, L.: Endocytosis
of superoxide dismutase is required in order KYLE, M. E., NAKAE, D., SAKAIDE, I., MICCADEI, S. AND FARBER, L.: Endocytosis
of superoxide dismutase is required in order for the enzyme to protect hepato-
cytes from the cytotoxicity of hydrogen peroxide. J. Biol. Chem.
- cytes from the cytotoxicity of hydrogen peroxide. J. Biol. Chem. 263: 3784–3789, 1988.

1789, 1988.

LEE, Y. C., STOWELL, C. P. AND KRANTZ, M. J.: 2-Imino-2-methoxyethyl 1-

thioglycoside: New reagents for attaching sugars
- 3789, 1988.
EE, Y. C., STOWELL, C. P. AND KRANTZ, M. J.: 2-Imino-2-methoxyethyl 1-
thioglycoside: New reagents for attaching sugars to proteins. Biochemistry 15:
1956–3963, 1976.
U., T. H., BECKMAN, J. S., FREEMAN, B. A., 3956-3963, 1976.

LIU, T. H., BECKMAN, J. S., FREEMAN, B. A., HOGAN, E. L. AND HSU, C. Y.:

Polyethylene glycol-conjugated superoxide dismutase and catalase reduce is-

chemic brain injury. Am. J. Physiol. 256: H589-H593, LIU, T. H., BECKMAN, J. S., FREEMAN, B. A., HOGAN, E. L. AND HSU, C. Y.:

Polyethylene glycol-conjugated superoxide dismutase and catalase reduce is-

chemic brain injury. Am. J. Physiol. 256: H589-H593, 1989.

LOWRY, O. H
-
- chemic brain injury. Am. J. Physiol. 256: H589-H593, 1989.
LOWRY, O. H., ROSENBERG, N. J., FARR, A. L. AND RANDALL, R. J.: Protein
measurement with folin phenol reagent. J. Biol. Chem. 28: 265-275, 1951.
MAGNUSSON, S. AND
- mannose receptor of sinusoidal endothelial rat liver cells. Biochem. J. 257:
651-656, 1989.
ENANDER-HUBER, K. B. AND HUBER, W.: Orgotein, the drug version of bovine
Cu-Zn superoxide dismutases. II. A summary of clinical tr MENANDER-HUBER, K. B. AND HUBER, W.: Orgotein, the drug version of bovine
Cu-Zn superoxide dismutase. II. A summary of clinical trials in man and
animals. In Superoxide and Superoxide Dismutases, ed. by A. M. Michelson,
J. Cu-Zn superoxide dismutase. II. A summary of clinical trials in man and
animals. In Superoxide and Superoxide Dismutases, ed. by A. M. Michelson,
J. M. McCord and I. Fridovich, pp. 537-556, Academic Press, London, 1977.
UN
- animals. *In* Superoxide and Superoxide Dismutases, ed. by A. M. Michelson, J. M. McCord and I. Fridovich, pp. 537-556, Academic Press, London, 1977.
UNNIKSMA, J., NOTEBORN, M., KOOISTRA, S., BOUMA, J. M. W., GRUBER, A., D MUNNIKSMA, J., NOTEBORN, M., KOOISTRA, S., BOUMA, J. M. W., GRUBER, A., DALEN, D. P. V. AND KNOOK, D. L.: Fluid endocytosis by rat liver and spleen.
Experiments with ¹²⁸1-labeled poly(vinylpyrrolidone) in vivo. Biochem.
- DALEN, D. P. V. AND KNOOK, D. L.: Fluid endocytosis by rat liver and spleen.
Experiments with ¹²⁵I-labeled poly(vinylpyrrolidone) *in vivo*. Biochem. J. 192:
613–621, 1980.
ISHIDA, K., MHARA, K., TAKINO, T., NAKANE, S., 613–621, 1980.

NISHIDA, K., MIHARA, K., TAKINO, T., NAKANE, S., TAKAKURA, Y., HASHIDA,

M. AND SEZAKI, H.: Hepatic disposition characteristics of electrically charged

macromolecules in rat in vivo and in the perfused liv
- M. AND SEZAKI, H.: Hepatic disposition characteristics of electrically charged macromolecules in rat in vivo and in the perfused liver. Pharm. Res. 8: 437-444, 1991.

SHIDA, K., TAKINO, T., EGUCHI, Y., YAMASHITA, F., HASHI NISHIDA, K., TAKINO, T., EGUCHI, Y., YAMASHITA, F., HASHIDA, M. AND SEZAKI, H.: Pharmacokinetic analysis of uptake process of lactosaminated albumin in the rat liver constant infusion experiment. Int. J. Pharmaceut. 80: 10
-

⁹⁷⁸ Fujfta **et al** Vol. 263

**F., HASHIDA, M. AND SEZAKI, H.: Pharmacokinetics of receptor-mediated

F., HASHIDA, M. AND SEZAKI, H.: Pharmacokinetics of receptor-mediated

endocytosis of glycosylated albumin in mice. Int. J. Pharmaceut. in press, 199 ODURING, SCILL SET ALL SET ALL SET ALL SET ALL SET ALL SET AND SEZAKI, H.: Pharmacokinetics of receptor-media endocytosis of glycosylated albumin in mice. Int. J. Pharmaceut. in press, 19

ODLIND, B., APPELGREN, L. E., B**

- F., HASHIDA, M. AND SEZAKI, H.: Pharmacokinetics of receptor-mediated Tendocytosis of glycosylated albumin in mice. Int. J. Pharmaceut. in press, 1992.
DLIND, B., APPELGREN, L. E., BAYTAI, A. AND WOLGAST, M.: Tissue distri F., HASHIDA, M. AND SEZA
endocytosis of glycosylated al
DLIND, B., APPELGREN, L. E.
tion of 125-11abeled bovine si
Toxicol. **62:** 95-100, 1988.
ATAK, P. S., ABUCHOWSKI, A
- F., HASHIDA, M. AND SEZAKI, H.: Pharmacokinetics of receptor-mediated
endocytosis of glycosylated albumin in mice. Int. J. Pharmaceut. in press, 1992.
ODLIND, B., APPELGREN, L. E., BAYTAI, A. AND WOLGAST, M.: Tissue distri ODLIND, B., APPELGREN, L. E., BAYTAI, A. AND WOLGAST, M.: Tissue distribution of 125-I labeled bovine superoxide dismutase (SOD) in the rat. Pharmacol. Toxicol. **82:** 95-100, 1988.
PYATAK, P. S., ABUCHOWSKI, A. AND DAVIS F
- VATAK, P. S., ABUCHOWSKI, A. AND DAVIS F. F.: Preparation of a polyethylene glycol: Superoxide dismutase adduct, and an examination of its blood circulating life and anti-inflammatory activity. Res. Commun. Chem. Pathol. P macol. **29:** 113-127, 1980.
 **ERENGER, P. H., DOEBBER, T. W., MANDELL, B. F., WHIT

SCHRYJVER, C., BORDMAN, J. S., MILLER, M. J. AND STAHLE, P.

clearance by glycoproteins with terminal mannose and N-acetylg

by liver non-**
- SCHRYJVER, C., BORDMAN, J. S., MILLER, M. J. AND STAHLE, P. D.: Plasma

clearance by glycoproteins with terminal mannose and N-acetylglucosamine

of pharmaceutical properties of society. H.: Biochem-

TAKAKURA, Y., FUJITA, SCHRYJVER, C., BORDMAN, J. S., MILLER, M. J. AND STAHLE, P. D.: Plasma

clearance by glycoproteins with terminal mannose and N-acetylglucosamine

by liver non-parenchymal cells. Biochem...J. 176: 103-109, 1978.

by liver n
- of pharmaceutical properties of soybean trypsin inhibitor by conjugation with dextran and polyethylene glycol. Chem. Pharm. Bull. 38: 2053-2056, dextran II. Biopharmaceutical and pharmacological properties. J. Pharm. Sci.
- Vol. 263

TAKAKURA, Y., TAKAGI, A., HASHIDA, M. AND SEZAKI, H.: Disposition and tumor

localization of mitomycin C-dextran conjugates in mice. Pharm. Res. 4: 293-

300, 1987.
- TAKAKURA, Y., TAKAGI, A., HASHIDA, M. AND SEZAKI, H.: Disposition and tumor
localization of mitomycin C-dextran conjugates in mice. Pharm. Res. 4: 293-
300, 1987.
TAYLOR, A. E. AND GRANGER, N. D.: Exchange of macromolecule Society, Bethesda, Md., 1984.

Society, Bethesda, Md., 1987.

Society, Bethesda, Md., 1984.

W. E. Michael, pp. 467-520, American Physiology: The Cardiovascular System I

ed. by E. M. Renkin and C. C. Michael, pp. 467-520, microcirculation. In Handbook of Physiology: The Cardiovascular System IV, ed. by E. M. Renkesia, Md., 1984.

Worker, Brethesda, M. And MoRINO, Y.: Inhibition of postischemic reper-

fusion arrhythmias by an SOD derivative
- fusion arrhythmias by an SOD derivative that circulates bound to albumin
- YAMAOKA, K., TANIGAWARA, Y., TANAKA, H. AND UNO, Y.: A pharmacokinetic analysis program (MULTI) for microcomputer. J. Pharmacobio-Dyn. 4: 879–885. 1981. **YASUDA, Y., FUJITA, T., TAKAKURA, Y., HASHIDA, M. AND SEXAKURA, H.**
 YANAOKA, K., TANIGAWARA, Y., TANAKA, H. AND UNO, Y.: A pharmacokine
 YAMAOKA, K., TANIGAWARA, Y., TANAKA, H. AND UNO, Y.: A pharmacokine

analysis p
- with photogeal in Vive hand the Euclemant Franchical costs own-school space.
MAOKA, K., TANIGAWARA, Y., TANAKA, H. AND UNO, Y.: A pharmacobinetic
analysis program (MULTI) for microcomputer. J. Pharmacobio-Dyn. 4: 879-
885,