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Pharmacokinetics and Preventive Effects of Sulfo-Albumin as a Novel Macromolecular Hydrogen Sulfide Prodrug on Carbon Tetrachloride-Induced Hepatic Injury

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ABSTRACT

Hydrogen sulfide (H_2S) has been recently recognized as a gaseous signaling molecule that controls various biological activities. In the present study, we developed sulfo-albumin as a macromolecular H_2S prodrug for therapeutic use, in which multisulfide groups (source of H_2S) were conjugated with bovine serum albumin through a covalent linkage. In an *in vitro* study on H_2S release in phosphate buffered saline solution, we found that H_2S was released from sulfo-albumin in the presence of 5-mM glutathione but not in its absence. Furthermore, sulfo-albumin was taken up by RAW 264.7 cells, and it released H_2S in cells but not in plasma. These results indicate that H_2S can be selectively released from sulfo-albumin in cells. ^{111}In-labeled sulfo-albumin predominantly accumulated in the liver, dependent upon the number of sulfide groups, after intravenous injection in mice. In a carbon tetrachloride-induced acute liver injury mouse model, sulfo-albumin significantly suppressed the increase in plasma aspartate aminotransferase and alanine aminotransferase activities, which are indicators of hepatocyte injury, after intravenous injection. These findings indicate that sulfo-albumin is a promising compound for the treatment of hepatic injuries.

Introduction

Although hydrogen sulfide (H_2S) has been considered as a toxic pollutant, it has recently been found that H_2S is endogenously generated from various synthases such as cystathionine β-synthase, cystathionine γ-lyase, and 3-mercaptopyruvate sulfur-transferase in the body.[1,2] H_2S plays an important role in various biological activities, including anti-oxidative stress, anti-inflammation, anti-apoptosis, and vascular smooth muscle relaxation.[3-7] Therefore, H_2S is recognized as a third gaseous signaling molecule in biological systems, following nitric oxide (NO) and carbon monoxide (CO).[7,8] As H_2S has a protective effect against reactive oxygen species (ROS)-mediated diseases including inflammatory and ischemia/reperfusion injuries,[3,4] the delivery of H_2S to the site where ROS are generated has been expected for the treatment of ROS-mediated diseases.

To date, some researchers have attempted the use of H_2S to treat ROS-mediated injury.[9,10] Because it is difficult to handle and administer H_2S directly, H_2S prodrugs that generate H_2S *in vivo* have been developed for efficient administration of H_2S into the body.[11-13] However, the tissue distribution of H_2S prodrugs has not been studied, despite the fact that the tissue distribution of H_2S prodrugs requires optimization to obtain the maximal therapeutic effect of H_2S. Of the various strategies available, the use of macromolecular carriers appears to be a good approach to control the release and distribution of H_2S. This is because the macromolecular carriers have several functional groups whereby sulfide groups (source of H_2S), targeting ligands, and functional moieties can be chemically conjugated. To control the delivery of H_2S, the tissue distribution of the sulfide group-modified macromolecular carrier should be examined.

In the present study, to provide basic information for controlled delivery of H_2S *in vivo*, we developed a novel macromolecular H_2S prodrug and examined its physicochemical properties, pharmacokinetics, and therapeutic potential in the ROS-mediated injury. We

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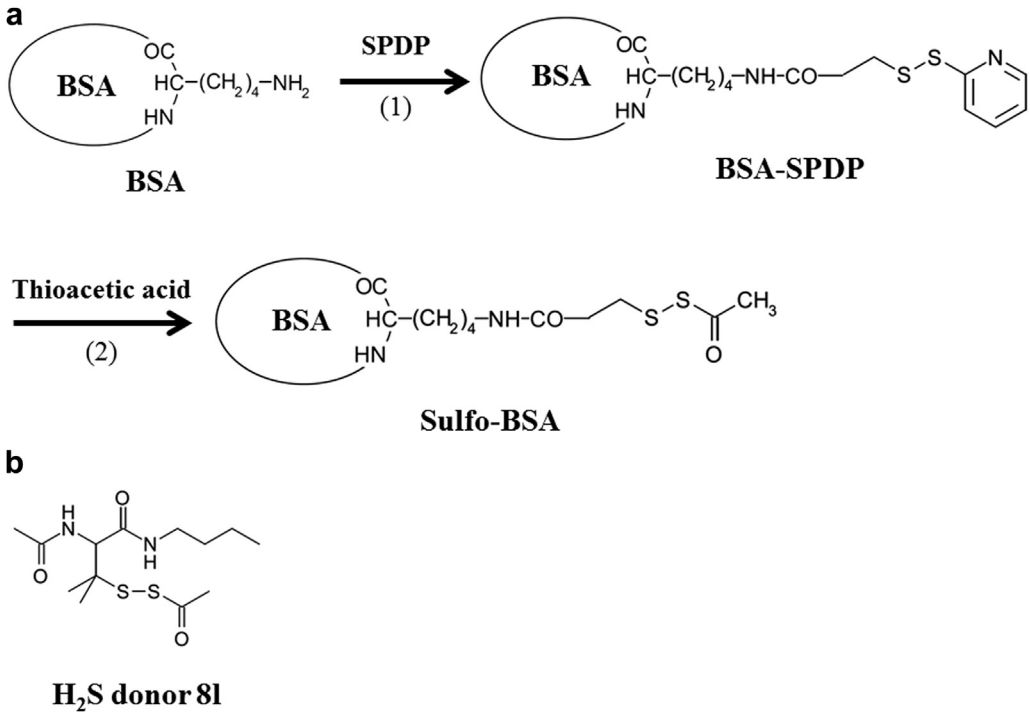

Figure 1. Structures and synthetic routes of sulfo-albumin (Sulfo-BSA) (a) and structure of H_2S prodrug 8l (b).

developed sulfo-albumin, in which multisulfide groups were conjugated to serum albumin through a covalent linkage, as a macromolecular H_2S prodrug (Fig. 1a). Next, the H_2S release and tissue distribution of sulfo-albumin were examined. Finally, the therapeutic effect of sulfo-albumin was investigated in carbon tetrachloride (CCl_4)-induced acute hepatic injury model mice.

Materials and Methods

Chemicals

Bovine serum albumin (BSA) and fluorescein isothiocyanate (FITC) isomer were purchased from Sigma-Aldrich (St. Louis, MO). N-Succinimidyl 3-(2-pyridyldithio) propionate (SPDP), diethylenetriaminepentaacetic acid anhydride, HSip-1, and N-Butyl-N^2-acetyl-S-acetylsulfanyl-DL-penicillamine amide (low-molecular-weight thiol-activated H_2S prodrug 8l; Fig. 1b) were purchased from Dojindo Laboratory (Kumamoto, Japan). Fetal bovine serum was obtained from Biosera (Ringmer, UK). Thioacetic acid, dimethyl sulfoxide, zinc acetate, N,N-dimethyl-*p*-phenylenediamine, and CCl_4 were purchased from Wako Pure Chemical Industries (Osaka, Japan). ^{111}Indium chloride was kindly supplied by Nihon Medi-Physics (Takarazuka, Japan). All other chemicals were of reagent grade.

Animals

Male ddY mice (25 g) were purchased from Japan SLC Inc. (Shizuoka, Japan). Animals were maintained under conventional housing conditions, and all animal experiments were conducted in accordance with principles and procedures outlined in the NIH Guide for the Care and Use of Laboratory Animals. The protocols for animal experiments were approved by the Animal Experimentation Committee of the Kyoto Pharmaceutical University.

Synthesis of Sulfo-Albumin

Sulfo-albumins with one of 3 different degrees of sulfide group modifications (Sulfo (5)-BSA, Sulfo (10)-BSA, and Sulfo (30)-BSA) were prepared by reacting different amounts of SPDP and thioacetic acid with BSA. Typically, to synthesize Sulfo (30)-BSA, 30 mg of BSA was dissolved in 5 mL of 0.1 M phosphate buffer (pH 7.4), and 8.48 mg of SPDP was dissolved in 50 μL of dimethyl sulfoxide and added to the BSA solution. The reaction mixture was stirred at room temperature for 2 h. Then, 9.57 μL of thioacetic acid was added to the reaction mixture, and stirring continued for 1.5 h at room temperature.[11] Next, the mixtures were dialyzed using dialysis membrane (prewetted RC tubing [molecular weight cutoff: 15kD]; Spectrum Laboratories, Inc., Dominguez, CA) against distilled water

Table 1
Physicochemical Properties of Sulfo-albumin

Compound	Molecular Weight[a]	Number of Sulfide Groups[b] (mol/mol)	Diameter (nm)	Zeta Potential (mV)
BSA	66,300	0	7.29 ± 0.42	−12.57 ± 0.12
Sulfo (5)-BSA	67,300	4.83	9.20 ± 0.06	−19.47 ± 0.78
Sulfo (10)-BSA	68,100	9.72	8.24 ± 0.68	−22.23 ± 1.37
Sulfo (30)-BSA	71,900	30.9	7.21 ± 0.70	−30.87 ± 0.75

[a] Molecular weight was measured via MALDI TOF-MS.
[b] The average number of sulfide groups was estimated by measuring the molecular weight via MALDI TOF-MS. Results are expressed as the mean ± SD of 3 experiments.