Journal of Pharmaceutical Sciences 105 (2016) 1043–1049

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Drug Discovery—Development Interface

Comparison of Posttranslational Modification and the Functional Impairment of Human Serum Albumin in Commercial Preparations

Shigeyuki Miyamura [1], Tadashi Imafuku [1], Makoto Anraku [2, 3], Kazuaki Taguchi [2], Keishi Yamasaki [2, 3], Yuna Tominaga [1], Hitoshi Maeda [1], Yu Ishima [1, 4], Hiroshi Watanabe [1, 4], Masaki Otagiri [2, 3], Toru Maruyama [1, 4, *]

[1] Department of Biopharmaceutics, Graduate School of Pharmaceutical Sciences, Kumamoto University, Kumamoto 862-0973, Japan
[2] Faculty of Pharmaceutical Sciences, Sojo University, Kumamoto 860-0082, Japan
[3] DDS Research Institute, Sojo University, Kumamoto 860-0082, Japan
[4] Center for Clinical Pharmaceutical Sciences, Kumamoto University, Kumamoto 862-0973, Japan

ARTICLE INFO

Article history:
Received 6 August 2015
Revised 11 December 2015
Accepted 15 December 2015

Keywords:
albumin preparation
ESI-TOFMS
antioxidative activity
cysteine 34
cysteinylation
ligand binding

ABSTRACT

On account of its long circulating half-life, human serum albumin (HSA) is susceptible to posttranslational modifications that can alter its functions. Here, we comprehensively compared the degree of posttranslational modifications with the functional impairment of HSA derived from 5 different commercially available albumin preparations and clarified their relationships. We used electrospray ionization–time of flight mass spectrometry to evaluate the degree of posttranslational modification of the entire HSA molecule that was associated with disease development and found that the fraction of Cys34-cysteinylated HSA (Cys-Cys34-HSA), a major form of oxidative modification, varied substantially among the albumin preparations. Meanwhile, no remarkable difference was found in the degree of glycated or N-terminal truncated HSA among the preparations tested. The nonosmotic pressure maintenance functions of HSA, such as its antioxidative and ligand-binding activities significantly differed among the preparations. Interestingly, the alternations of these functions showed a significantly negative correlation only with the Cys-Cys34-HSA fraction. These findings suggest that the Cys-Cys34-HSA fraction, as estimated by electrospray ionization–time of flight mass spectrometry can be used as a predictive marker for the functional impairment of albumin preparations and that it would be preferable to use albumin preparations with higher contents of functionally effective albumin that correspond to a lower degree of cysteinylation of Cys34 in clinical practice.

Introduction

Human serum albumin (HSA) is a major component of the circulating blood, accounting for approximately 60% of plasma protein; it contributes to the maintenance of vascular osmotic pressure.[1] Thus, albumin preparations have long been used as a multipurpose plasma substitute in clinical practice, such as the emergency treatment for shock, the restoration of blood volume, the acute management of burns, and in clinical situations associated with hypoproteinemia.[2] In addition to its oncotic effect, HSA also confers various nononcotic effects.[1] For instance, HSA shows a high affinity for many endogenous as well as exogenous substances, such as drugs and toxins, and thereby affects their pharmacokinetics and consequently their physiological activity and drug efficacy.[3,4] HSA also plays an important role as a major antioxidant in plasma and extracellular compartments, where the cysteine 34 (Cys34) of HSA accounts for approximately 70% of all free sulfhydryl (SH) groups in plasma.[5] These findings suggest that HSA has an important role in the maintenance of biological hemostasis. Considering these features of HSA, there are ongoing attempts to use albumin preparations for purposes other than its oncotic effect in clinical practice. For example, taking advantage of its superior ligand-binding properties, “Albumin dialysis” in which the dialysate contains HSA to remove the protein bound toxic substances would be expected as the next generation of blood purification methodology.[6,7] In addition, a phase II clinical trial in which albumin is used for the treatment of Alzheimer disease, based on the hypothesis that HSA serves as a peripheral amyloid β inactivator is currently ongoing.[8-11]

Abbreviations: HSA, human serum albumin; ROS, reactive oxygen species; DPPH, 1, 1'-diphenyl-2-picrylhydrazyl; ESI-TOFMS, electrospray ionization–time of flight mass spectrometry; SH, sulfhydryl; Cys, cystein.

This paper is dedicated to Distinguished Professor Emeritus Ronald T. Borchardt at The University of Kansas-Lawrence.

Shigeyuki Miyamura and Takashi Imafuku contributed equally to this work.

* *Correspondence to:* Toru Maruyama (Telephone/Fax: +81-96-371-4150).
E-mail address: tomaru@gpo.kumamoto-u.ac.jp (T. Maruyama).

http://dx.doi.org/10.1016/j.xphs.2015.12.015
0022-3549/

On account of its long circulating half-life, however, HSA is subject to a variety of posttranslational modifications *in vivo* as a result of various stress conditions, such as oxidation and glycation and so forth.[1,12] Thus, albumin products prepared from such pooled plasma sources can be posttranslationally modified.[13] In addition, during its preparation from pooled plasma and subsequent storage, HSA can also be modified posttranslationally. Such modifications often compromise albumin's functions, other than its osmotic pressure maintaining function. In fact, in our recent clinical study in 226 patients with chronic oxidative stress-related diseases, we found that Cys34 was increasingly susceptible to oxidative modification as the disease progressed and that the predominant modification involved cysteinylation (cysteine attached to Cys34 by a disulfide bond), as determined by electrospray ionization–time of flight mass spectrometry (ESI-TOFMS).[14] We also found that the cysteinylation of Cys34 (Cys-Cys34-HSA) resulted in reduced antioxidative and ligand-binding activities of HSA. Interestingly, other groups have also reported that HSA is functionally impaired in a disease state and that this alteration is associated with disease progression[15,16] or suggests the potential value of posttranslational modification as a prognostic marker, for instance, the extent of oxidized and N-terminal truncated HSA were independently associated with ascites, renal impairment, and bacterial infections in hospitalized patients with chronic liver failure.[12] Therefore, having albumin preparations in which the HSA function is preserved with minimal posttranslational modifications would be highly desirable. However, this issue has not been comprehensively examined, that is, the nature and extent of posttranslational modification of albumin preparations or the functional difference between the modified preparation and natural albumin remain unknown.

In the present study, we estimated the degree of posttranslational modifications and the differences in the function of HSA molecules derived from 5 kinds of albumin preparations that are currently commercially available in Japan, to examine the relationship between posttranslational modifications and the functional impairments of HSA. In addition, the modification that is responsible for the functional alterations found in albumin preparations was explored as a quality measure.

Materials and Methods

Materials

Five kinds of albumin products can currently be supplied from several companies all over the world. The product numbers [lot numbers] of the HSAs used were as follows: A (Kenketsu Albumin 20 “KAKETSUKEN”) [3 lots; SAN1401, SAN1404, SAN1405], B (Albumin 25% “BENESIS”) [3 lots; V405PX, V406PX, V407PX], C (Albuminar 25% “CSL Behring”) [3 lots; 3M313B, 3M314B, 3M315B], D (KENKETSU Albumin 25 “NICHIYAKU”) [3 lots; N202N, N203N, N204N], and E (Sekijuji Albumin 25% “Japan Blood Products Organization”) [3 lots; 2F178, 2F181, 2F182]. These were prepared from normal human serum by a multistep process. The 1,10-diphenyl-2-picrylhydrazyl (DPPH) was supplied by Nacalai Tesque (Kyoto, Japan). Potassium warfarin (Eisai Co., Tokyo, Japan) was obtained as pure substances from the manufacturers. All other chemicals were of the highest grade commercially available, and all solutions were prepared using deionized and distilled water.

Solid Phase Extraction and ESI-TOFMS Measurement of HSA From Albumin Products

A 5 μL of albumin product sample (20%) was added to 495 μL of 50-mM sodium phosphate buffer (pH 6.0). A solid phase extraction column (Bond Elute-C18 EWP 200 mg/3cc, Varian, Inc., CA) was initialized with 10/90 water/acetonitrile containing 0.1% formic acid, and equilibration was then performed with water (1 mL). The previously mentioned diluted albumin product sample was applied to the equilibrated solid phase extraction column. The column was washed with 10% acetonitrile (1.5 mL) containing 0.1% formic acid, and the albumin was eluted with a 90% aqueous acetonitrile solution containing 0.1% formic acid. A 2-μL aliquot of the eluent was flow injected into the ESI-TOFMS (microTOF®; Bruker Daltonics Inc.) at a flow rate of 15 μL/min with 10/90 water/acetonitrile containing 0.1% formic acid using the auto sampler of Ultimate 3000 (Dionex, Idstein, Germany).

The data were acquired by the MicroTOF® software (Bruker-Daltonics) and processed for Maxent deconvolution using DataAnalysis® software (Bruker-Daltonics). The deconvolution mass range was set to be from 66,000 to 68,000 Da. The mass peak of HSA and related modified molecules such as Cys-Cys34-HSA, glycated HSA, and N-terminal truncated HSA were automatically assigned and converted to an output text file using script with a resolving power of 10,000 m/dm and an absolute intensity threshold of 1000. The fraction of modified HSA (%) was calculated by (modified HSA/[modified HSA + unmodified HSA]) ×100 as previously reported.[14,17]

Reactivity of Thiols in Albumin Product With 5,5′-Dithiobis (2-nitrobenzoic acid) (DTNB)

An albumin product sample (2.0×10^{-4} M) was preincubated at 37°C. The increase in absorbance at 405 nm was monitored against time after the addition of DTNB (final concentration 5.0×10^{-4} M).[18,19] The thiol contents of HSA (%) were calculated by using standard curve of glutathione on concentration-dependent manner as previously reported.[14,19]

Determination of the Binding Parameters of Warfarin to Purified HSAs

HSA was purified from albumin products by a previously reported method using a Blue Sepharose 6 Fast Flow column (GE Healthcare, Tokyo, Japan).[18,20] The samples were then dialyzed against deionized water for 48 h at 4°C, followed by lyophilization. The binding parameters of warfarin to purified HSA were estimated as previously reported.[21] Briefly, the fractions of warfarin bound were calculated using the fluorescence intensities of given concentration of warfarin in a solution of low HSA concentration (2 μM) and in a solution without HSA, and the fluorescence intensities of the same concentration of fully bound warfarin. After values for the fraction of bound warfarin had been found for all points along the titration curve. Binding parameters were estimated by fitting the experimental data to the following equation using GraphPad PRISM® Version 4 (GraphPad Software, Inc, CA)

$$r = \frac{[D_b]}{[P_t]} = \frac{nK\left[D_f\right]}{1 + K\left[D_f\right]}$$

where r is the number of moles of warfarin bound per mole HSA. $[P_t]$ is the HSA concentration, and $[D_b]$ and $[D_f]$ are the bound and unbound warfarin concentrations, respectively. K and n are the binding constant and the number of binding sites for the primary binding site. Here, we assumed the number of binding sites for primary warfarin-binding site is 1 to compare the binding affinity between albumin products.