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Altered chain-length and glycosylation modify the pharmacokinetics of human serum albumin

Yasunori Iwao [a], Mikako Hiraike [a], Ulrich Kragh-Hansen [b], Keiichi Kawai [c], Ayaka Suenaga [a], Toru Maruyama [a], Masaki Otagiri [a,*]

[a] Department of Biopharmaceutics, Graduate School of Pharmaceutical Sciences, Kumamoto University, 5-1 Oe-honmachi, Kumamoto 862-0973, Japan
[b] Department of Medical Biochemistry, University of Aarhus, DK-8000 Aarhus C, Denmark
[c] School of Health Sciences, Faculty of Medicine, Kanazawa University, Ishikawa 920-0942, Japan

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ABSTRACT

Human serum albumin with modified plasma half-life will be useful for clinical purposes. Therefore, the pharmacokinetics of three of each of the following types of genetic variants, and of their corresponding normal albumin, were examined in mice: N-terminally elongated, C-terminally truncated and glycosylated albumins. Isoforms differing from the normal protein by three or more amino acids, especially two of the truncated forms, had shorter half-lives. The effect of glycosylation depended on the position of attachment: in domain II it increased half-life, whereas in domain I and III it had no significant effect. Liver, kidney and spleen uptake clearances were also modified. The pronounced changes in half-life of the two truncated variants and the glycosylated isoform could be explained, at least partly, by large changes in organ uptakes; in the remaining six cases, different effects were registered. Such information should be useful when designing therapeutical albumin products for, e.g., drug delivery systems. In addition to various types of cell endocytosis, leading to intracellular destruction or recycling of the proteins, the metabolism of the alloalbumins could be affected by plasma enzymes. No correlation was found between mutation-induced changes in the pharmacokinetic parameters and changes in α-helical content or changes in heat stability as represented by ΔH_v.

1. Introduction

Human serum albumin (HSA) is produced in the parenchymal cells of the liver, and it is the most abundant plasma protein. It is an important circulating depot protein and transport protein for endogenous and exogenous ligands in the blood, and contributes to the maintenance of osmotic pressure, plasma pH and to the Donnan-effect in the capillaries [1,2]. The protein is formed by a single polypeptide chain of 585 amino acids, and it has a molecular mass of approximately 66.5 kDa [2]. According to X-ray crystallographic analyses of HSA and of its recombinant version (rHSA), albumin has about 67% α-helix but no β-sheet. The analyses also showed that the polypeptide chain forms a heart-shaped protein with three homologous domains (I–III), each comprised of two subdomains (A and B) with distinct helical folding patterns that are connected by flexible loops [3,4]. A combined phosphorescence depolarization-hydrodynamic modeling study has proposed that the overall conformation of HSA in neutral solution is very similar to that observed in the crystal form [5].

Clinically, HSA is used for urgent restoration of blood volume, emergency treatment of chock, acute management of burns and other situations associated with hypoproteinemia [2]. To date, albumin has been produced by fractionation of whole blood. However, there is the potential risk of HSA contamination with blood-derived pathogens. In addition, human plasma can be in limited supply. Because of these problems, rHSA, which is highly expressed by *Pichia pastoris*, most probably will be commercially available in the near future [6]. Another benefit of this approach is that protein engineering will enable the creation of rHSAs with modified properties such as extended half-life in the circulation. In this connection HSA dimers seem to be useful candidates. Matsushita et al. [7] found that rHSA dimers had a high retention rate in the circulatory blood and a lower vascular permeability than native rHSA in normal rats and in mice with paw edema. Similar observations have been made by Komatsu et al. [8], who examined the pharmacokinetics of chemically crosslinked rHSA dimers in the rat. On the other hand, recombinant albumin domain(s) are cleared very fast. Sheffield et al. [9] found that recombinant domain I, I+II and III of rabbit serum albumin all had very short mean terminal catabolic half-lives in rabbits due to a fast elimination in the urine.

Abbreviations: HSA, human serum albumin; rHSA, recombinant HSA; Alb, albumin; Alb A, normal (wild-type) albumin; CD, circular dichroism; ΔH_v, van't Hoff enthalpy; RAGE, receptor for advanced glycation end products

* Corresponding author. Tel.: +81 96 371 4150; fax: +81 96 362 7690.
E-mail address: otagirim@gpo.kumamoto-u.ac.jp (M. Otagiri).

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Because of a relatively high in vivo half-life of ca. 19 days [2], HSA is an attractive fusion partner to extend the half-life, and potentially the therapeutic utility, of recombinant peptides and proteins. Among recent examples are rHSA genetically fused to type 1 interferons [10], glucagon-like peptide-1 [11] and interleukin-2 [12]. However, although an extension of the half-life of therapeutic peptides and proteins often is desirable, an extension to that of albumin could be excessive.

Although HSA-preparations with a modified half-life thus could be very useful, not much has been done to design or find such preparations. In our search for useful candidates, we have paid our attention to HSA genetic variants. Until now, 65 inherited variants of HSA, including proalbumin variants, have been identified and structurally characterized [13]. Usually, these genetic variants are expressed in heterozygous form and without any known association to disease [13]. Therefore, unlike lethal mutations, such may occur for hemoglobin and coagulation factors, studying the pharmacokinetic properties of HSA variants is a good way of gaining information which can be used when designing recombinant HSAs, because we can consider the effects of molecular variation without worrying about complications such as antigenic effects.

Recently, we have studied the pharmacokinetic properties in mice of 17 alloalbumins with single-residue mutations [14]. The study showed that, for example, only a few of the variants had a significantly modified half-life in the blood. In an attempt to find genetic variants with a more pronounced impact on pharmacokinetics, we now have expanded that study by determining the plasma half-lives and organ uptakes of three HSAs with a slightly longer chain-length (proalbumin variants), three with a slightly shorter chain-length (C-terminal variants) and three alloalbumins *N*-glycosylated in domain I, II and III, respectively. For being able to make a more detailed comparison between molecular characteristics and pharmacokinetic properties, we have estimated the effect of the molecular modifications on the α-helical content of the alloalbumins by using circular dichroism (CD). Previously, the effect of genetic variation on the thermal stability of HSA has been quantified in terms of, for example, changes in the van't Hoff enthalpy (ΔH_v) [15]. In the present work, the pharmacokinetic results have also been related to changes in ΔH_v.

2. Materials and methods

2.1. Protein samples

The genetic variants of HSA and their normal (wild-type) counterpart (endogenous Alb A) were isolated from serum from heterozygous carriers by ion-exchange chromatography. The locations of the structural changes of the nine variants are indicated in Fig. 1. After isolation, the albumins were checked for homogeneity by native electrophoresis, and no denaturation or significant (no more than 5%) cross-contamination between variant and Alb A was detected. The proteins were donated to us by Drs. M. Galliano and L. Minchiotti, University of Pavia, Pavia, Italy; Dr. S.O. Brennan, Canterbury Health Laboratories, Christchurch, New Zealand; and Dr. D. Donaldson, East Surrey Hospital, Redhill, UK. Before use, the albumins were delipidated by treatment with hydroxyalkoxypropyldextran at pH 3.0, as described in a previous paper [16]. After defatting, the albumins were dialysed extensively against deionized water, lyophilized and stored at −20 °C until used. Thus, the albumins from a donor have been exposed to exactly the same conditions from the time the blood samples were taken until the present experiments were performed.

Fraction V HSA (96% pure), assumed to be Alb A, was donated by the Chemo-Sera-Therapeutic Research Institute (Kumamoto, Japan) and defatted using the charcoal procedure described by Chen [17], deionized, freeze-dried and then stored at −20 °C until used.

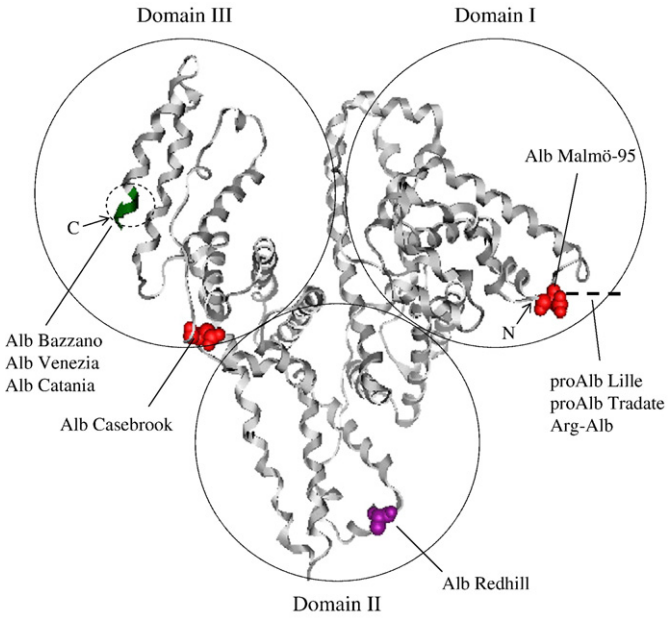

Fig. 1. The crystal structure of HSA indicating the locations of the mutations of the three C-terminal variants and the three proAlb variants used in this study. The locations of the glycosylated 63 Asn (Alb Malmö-95), 318 Asn (Alb Redhill) and 494 Asn (Alb Casebrook) are also shown. The subdivision of HSA into domains is marked; N and C stand for the N-terminal and the C-terminal ends, respectively. The broken, black line added to the N-terminal end indicates the prosegment of HSA.

2.2. Chemicals and animals

$^{111}InCl_3$ (74 Mbq/mL in 0.02 N HCl) was donated by Nihon Medi-Physics (Takarazuka, Japan). All chemicals were of the highest grade commercially available, and all solutions were prepared using deionized, distilled water.

Male ddY mice (26–32 g) were purchased from the Shizuoka Agricultural Cooperative Association for Laboratory Animals (Shizuoka, Japan), and were maintained under conventional housing conditions. All animal experiments were conducted in accordance with the principles and procedures outlined in the National Institute of Health Guide for the Care and Use of Laboratory Animals.

2.3. In vivo experiments

All proteins were radiolabeled with ^{111}In using the bifunctional chelating reagent DTPA anhydride according to the method of Hnatowich et al. [18], as described elsewhere [19]. In previous works, we found no significant differences in pharmacokinetic properties among these albumins, when ^{111}In-labeled mouse, rat, bovine or human serum albumin was administered to mice (unpublished), suggesting that immunogenic behavior does not occur in mice. Therefore, we chose the mouse as a reasonable model for the study of the pharmacokinetics of the HSAs. Mice received tail vein injections of ^{111}In-labeled proteins in saline, at a dose of 0.1 mg/kg and were housed in metabolic cages to allow the collection of urine samples. Urine samples were collected throughout the 120 min of the experimental period. In the early period after injection, the efflux of ^{111}In radioactivity from organs is assumed to be negligible, because the degradation products of ^{111}In-labeled proteins using DTPA anhydride cannot easily pass through biological membranes [20]. This assumption was supported by the fact that no ^{111}In was detectable in the urine after 120 min. At 1, 3, 5, 10, 30, 60, 90 or 120 min after injection, blood was collected from the vena cava under ether anesthesia and plasma was obtained by centrifugation. After blood collection, the animals were sacrificed, organs were excised, rinsed with saline and weighed.