Original Article

Comparison of quality of human serum albumin preparations in two pharmaceutical products

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Aim: Human serum albumin (HSA) is known for its multiple functions, such as maintenance of colloid osmotic pressure, transport of endogenous and exogenous substances, and antioxidation. The aim of this study was to measure the redox state and concentrations of β-D-glucan and endotoxin to compare the quality of 5% HSA preparations from two different manufacturers.

Methods: The quality of 5% HSA preparations in two different pharmaceutical products (groups A and B) was compared in terms of the percentage of reduced and oxidized albumin and the contaminant level of β-D-glucan and endotoxin.

Results: The percentage of human mercaptoalbumin in group A was significantly higher than that in group B ($P < 0.01$), whereas that of human non-mercaptoalbumin-2 in group A was significantly lower ($P < 0.01$). The concentration of β-D-glucan in group A was significantly lower than in group B ($P < 0.01$).

Conclusions: The present findings indicate the need for quality control of HSA preparations in applications involving the use of large volumes.

Key words: Endotoxin, mercaptoalbumin, non-mercaptoalbumin, β-D-glucan

INTRODUCTION

HUMAN SERUM ALBUMIN (HSA) is known for its multiple functions, such as maintenance of colloid osmotic pressure, transport of endogenous and exogenous substances, and antioxidation. Human serum albumin is a protein composed of 585 amino acids; the amino residue at position 34 from the N-terminus is a cysteine with a mercapto group (sulfhydryl group). The mercapto group deoxidizes other substances according to the degree of surrounding oxidative stress, and is itself oxidized. From the viewpoint of the cysteine residue, HSA is a mixture of human mercaptoalbumin (HMA), in which the mercapto group is not oxidized, human non-mercaptoalbumin (HNA)-1, in which the disulfide bond is reversibly oxidized by either cysteine or glutathione, and HNA-2, which is strongly oxidized and becomes sulfenic, sulfinic, or sulfonic. In healthy adults, approximately 75% of serum albumin exists in reduced form (HMA) and 25% in oxidized form (HNA). The relative proportion of HNA reportedly increases in various clinical conditions.[1–3] The use of high-quality HAS preparations is important for applications involving the use of large volumes of this protein such as plasma exchange and treatment of hypovolemic shock.

To our knowledge, for the last 10 years, there has been no study of the difference in percentages of MNA between commercially available brands of HSA.[4,5] Therefore, the aim of this study was to undertake measurements of the redox state and the concentrations of β-D-glucan (BDG) and endotoxin (ETX) in order to compare the quality of 5% HSA preparations from two different manufacturers.

The study was conducted with the approval of Akita University Graduate School of Medicine (registration no. 1226).

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METHODS

Preparation of HSA samples

TEN DIFFERENT lot-numbered 5% HSA preparations, within the validity period, were purchased from two different manufacturers. The serial number of each HSA preparation was as follows: group A, W260RX, W262RX,

X266RX, X268RX, X269RX; and group B, 5K133D, 5K135D, 5K136D, 5L138D, 5L139D. No significant difference in the duration from the manufacturing date to the test date was observed between the two groups: group A, 389 days (344–576 days) versus group B, 400 days (399–430 days) ($P = 0.5268$).

The HSA preparations (200 μL) were filtered using a Millex-HV syringe-driven filter unit (pore size 0.45 μm, hydrophilic polyvinylidene difluoride membrane; Merck Millipore Corporation, Darmstadt, Germany) and used as sample solutions.

Measurement of the redox status of HSA

High performance liquid chromatography (HPLC) was used for analysis of HMA, HNA-1, and HNA-2.[1,6] The HPLC system used in the present study consisted of a Model LC-20A system and a Model RF-10AXL fluorescence detector (excitation wavelength, 280 nm; emission wavelength, 340 nm), all from Simadzu Corporation (Kyoto, Japan). A Shodex-Asahipak ES-502N column (100 × 0.76 cm I.D., diethylaminoethanol-form for ion-exchange HPLC; Showa Denko, Tokyo, Japan) (column temperature, 35.0°C) was used. Elution was carried out with a linear gradient of increasing ethanol concentration, from 0% to 10%, in 0.05 M sodium acetate–0.40 M sodium sulfate (pH 4.85) (acetate–sulfate buffer) at a flow rate of 1.0 mL/min. The following ethanol concentration gradient was applied: 0–5 min, 0%; 5–55 min, linear increase from 0% to 10%; 55–55.1 min, linear increase from 10% to 30%; 55.1–60 min, 30%; 60–65 min, linear decrease from 30% to 0%; and 65–75 min, 0%. Samples were injected using an autosampler with fixed volume of 1.0 μL. In order to determine the value for each albumin fraction, the HPLC profiles obtained were subjected to numerical curve fitting; each peak shape was approximated by a Gaussian function using PeakFit software (Hulinks, Tokyo, Japan).

Measurement of BDG and ETX concentrations

The concentration of BDG in the HSA preparations was measured using the synthetic chromogenic substrate method (Fungitec G test MK II "Nissui") from Nissui Pharmaceutical Co., Ltd. (Tokyo, Japan) with a Wellreader SK603 (Scinics Corporation, Tokyo, Japan). The cut-off value was set at 20.0 pg/mL.[7]

The ETX concentration of the HSA preparations was measured using high-sensitivity kinetic turbidimetric Limulus test (ETX single test) from Wako Pure Chemical Industries, Ltd (Osaka, Japan) with an MT-6500 Toxinometer (Wako Pure Chemical Industries, Ltd.). The cut-off value was set at 1.0 pg/mL.[8]

Statistical analysis

Data for the parameters are expressed as median values with their ranges. Differences were evaluated for significance by using the Mann–Whitney *U*-test. A *P*-value <0.05 was considered significant.

RESULTS

Redox status of HSA preparations

THE PERCENTAGE OF HMA in group A was significantly higher than that in group B (group A, 48.03% [46.04–49.67%] versus group B, 38.18% [22.67–39.30%]; $P = 0.0090$, Fig. 1].

No significant difference was observed in the percentage of HNA-1 between the two groups (group A, 38.69% [37.76–39.13%] versus group B, 36.79% [36.20–41.09%]; $P = 0.1172$).

The percentage of HNA-2 in group A was significantly lower than that in group B (group A, 13.20% [12.34–14.89%] versus group B, 25.07% [24.51–36.24%]; $P = 0.0090$).

Concentrations of BDG and ETX of HSA preparations

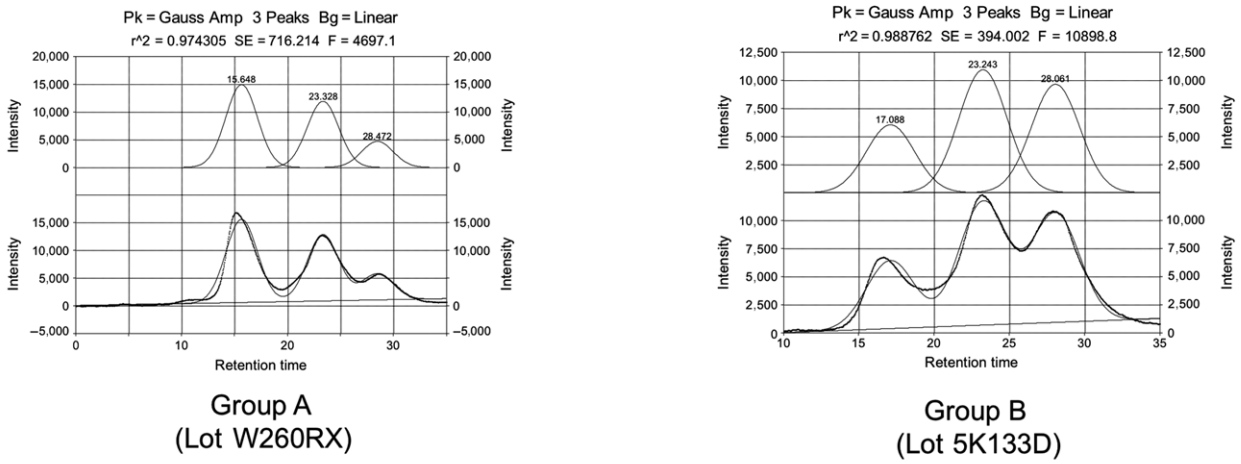
The concentration of BDG in group A was significantly lower than that in group B (group A, 10.5 pg/mL [8.4–11.0 pg/mL] versus group B, 86.8 pg/mL [70.6–102.0 pg/mL]; $P = 0.0090$).

Endotoxins were not detected in either group.

DISCUSSION

THE PROPORTION OF HMA was found to decrease with increasing time, whereas that of HNA was observed to increase.[2] In this study, there was no difference in terms of the duration from the time of manufacture to testing between the two groups; however, the proportion of HMA in group A was significantly higher than that in group B, whereas that of HNA-2 in group A was significantly lower than that of group B. These findings suggest that the redox state of the HSA preparations differed according to the manufacturer.

The rate of HMA is known to decrease and, in contrast, that of HNA has been reported to increase in various clinical



Comparison of redox status of human serum albumin preparations

	Group A	Group B	*P* value
HMA	48.03% (46.04–49.67%)	38.18% (22.67–39.30%)	0.0090
HNA-1	38.69% (37.76–39.13%)	36.79% (36.20–41.09%)	0.1172
HNA-2	13.20% (12.34–14.89%)	25.07% (24.51–36.24%)	0.0090

Fig. 1. Representative chromatograms and comparison of redox status of human serum albumin preparations according to the manufacturer. The three peaks represent human mercaptoalbumin (HMA), human non-mercaptoalbumin (HNA)-1, and HNA-2 (from left to right).

conditions or during aging. In addition, oxidized albumin possesses lower antioxidant levels and lower binding properties.[1–3] Blood purification techniques, such as hemodialysis and albumin dialysis, increased the rate of HMA and decreased the rate of HNA.[9–11] Oral branched-chain amino acid supplementation improved the oxidized/reduced albumin ratio in patients with liver cirrhosis.[12] The addition of Stronger Neo-Minophagen (Minophagen Pharmaceutical Co., Ltd, Tokyo, Japan), which contains 1 mg/mL L-cysteine, to HSA preparations improves their redox state.[13] However, the addition of antioxidant substances to HSA preparations in the clinical setting is not feasible due to hygiene-related and economic considerations. Sakata *et al.*[14] have reported that elevated levels of oxidized albumin is related to disease progression and water retention in patients with chronic liver disease, and that the administration of preparations with a low percentage of oxidized albumin was more effective in terms of alleviating symptoms in these patients for chronic liver disease with ascites than the administration of preparations with a high percentage of oxidized albumin. Anraku *et al.*[15] suggested the oxidized albumin ratio (HMA/HNA) might be a useful marker for systemic redox states, for the evaluation of disease progression and therapeutic efficacy.[15] In short, the redox state of HSA preparations is of importance in applications requiring large volumes, such as plasma exchange and management of hypovolemic shock.

The quality standards for biological products requires the purity of HSA preparations to be over 96%; however, contaminants may still be present.[16] A BDG-containing cellulose membrane is used for ethanol fractionation during HSA production. β-D-glucans from the cellulose membrane are known to mix into HSA preparations.[17–19] These BDGs do not aggravate medical conditions; however, they may affect the concentration of BDG in the blood. In the present study, the concentration of BDG in group B was significantly higher than that in group A. The use of ion-exchange chromatography for the manufacture of HSA preparations in group A may have accounted for the lower concentrations of BDG in this group relative to group B.[20]

This is a small group study that tested several samples from a large number of commercially available products. Further large-scale research might be necessary. It is still unclear whether these results would affect clinical conditions. The influence of the HMA/HNA ratio and BDG concentration on clinical outcome should be examined to distinguish the quality of those products in the future.

CONCLUSION

WE FOUND DIFFERENCES in the redox state and BDG concentration in 5% HSA preparations from different manufacturers. A clinical study should be carried out to reveal how the current result is relevant to the use of albumin in the future. The quality of HSA preparations must be carefully monitored and controlled in applications requiring large volumes of this protein.

CONFLICT OF INTEREST

NONE DECLARED.

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