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Comparison of antioxidant properties of different therapeutic albumin preparations

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ABSTRACT

Albumin displays several important functions for homeostasis amongst which the maintenance of the plasma redox-state. The study aim was to compare the redox state of pharmaceutical human albumin preparations since it reflects the oxidation-reduction status of the surrounding environment. Using an array of analytical methods, four commercially available albumins were compared with respect to their structural characteristics (cobalt ion binding, glycation, spectrophotometric and fluorometric profiles) and their ability to scavenge hydroxyl, peroxyl or free radicals. The different albumins exhibited a similar structural profile as well as hydroxyl and peroxyl scavenging activities. By contrast, the albumin from LFB (Vialebex®) possessed a significantly higher capacity to transfer electrons to DPPH, as compared with other albumins that was correlated with the level of free cysteine-34.

Commercially available albumins differed for some of their antioxidant properties. The albumin preparation possessing the highest level of free cysteine-34 exhibited the highest antioxidant potential.

1. Introduction

Free radicals are highly reactive molecules produced as the result of normal cellular oxygen metabolism in mammals. At low or moderate levels free radicals exert beneficial effects on cellular responses and immune function whereas at high concentrations, they generate oxidative stress, a deleterious process that can damage all cell structures and cause the onset and/or progression of a growing number of diseases [1–3].

The pivotal role of oxidative stress in pathophysiology, which is elicited by a redox imbalance between free radicals and antioxidants, forms the rationale for antioxidant therapeutic approaches [2–5].

Plasma, a body compartment known to be exposed to continuous oxidative stress, contains a wide range of endogenous antioxidant species among which albumin represents the most abundant and important [6,7]. Therefore, human serum albumin (HSA) used as a resuscitation fluid could represent an opportunity to enhance endogenous antioxidant protection in critical pathological interventions [8]. HSA infusion has been shown to allow for restoring thiol-radical deficient stock during cirrhosis and improving thiol-dependent anti-oxidant protection [9]. In the same way a beneficial effect of HSA administration in patients with acute respiratory distress syndrome was evidenced by the resulting enhancement in plasma thiol-dependent antioxidant status and in the reduction in protein oxidative damage [10]. Kremer et al. showed a protective dose-dependent effect of HSA treatment both on mice survival and endothelial dysfunction by inhibiting inflammatory and oxidative stress pathways induced by endotoxins [11]. Most of the antioxidant properties of albumin can be attributed to its unique biochemical structure [8]. HSA contains 35 cysteine residues of which 34 are involved in intramolecular disulfide bonds thus contributing to overall tertiary structure. One free cysteine residue, Cys-34, is the major extracellular source of redox active thiol group (-SH) which accounts for ≈ 80% of thiols in plasma [7,9]. The thiol moiety of Cys-34 is reactive and is a potent scavenger of reactive oxygen and nitrogen species (ROS/RNS) supporting an important redox regulator role in extracellular compartment [7,12]. Another part of the antioxidant properties of HSA is due to its ligand-binding capacities. The N-terminal part of HSA (N-Asp-Ala-His-Lys) forms a high affinity site for Cu^{2+}, Ni^{2+}

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and Co^{2+} ions [13]. By virtue of its high affinity binding site, HSA limits the potential of cationic metal ions to generate reactive oxygen species (ROS) after a reaction with oxygen and, consequently, to damage other biomolecules. Furthermore, an indirect antioxidant activity of HSA results from its ability to transport bilirubin whereby the albumin-bilirubin complex was shown to act as an inhibitor of lipid peroxidation [14].

Serum albumin is heterogeneous with respect to its reduced thiol content, reported as typically 0.6–0.7 SH/HSA [15,16]. Bioprocess and storage conditions increase heterogeneity of HSA which was shown to be more pronounced in commercially available preparations intended for clinical use [17–22]. A wide range of variable post-translational modifications has been reported in commercially available HSA preparations including glycation, missing terminal amino-acids and cysteinylation and nitrosylation of the free cysteine residue [18,20,22]. This variability may add to the controversy surrounding the administration of albumin and contributes to the conflicting evidence from clinical trials [23]. The administration of commercially available HSA that is largely oxidized might augment the oxidative stress burden on an already oxidatively-challenged critically ill patient [19].

The aim of this study was to analyze several commercially available albumin preparations to assess their antioxidant capacity and, more precisely, the relationship between the antioxidant ability and the level of reduced Cys-34 residues. For this purpose the antioxidant profiles of these therapeutic HSA preparations were compared using an array of antioxidant assays.

2. Materials and methods

2.1. Chemicals

All chemicals were purchased from Sigma–Aldrich (L'Isle d'Abeau, France), VWR (Fontenay sous Bois, France) and Merck (Fontenay sous Bois, France) unless otherwise noted.

Human glycated albumin (GA) ELISA kit (ref. EO997h) was purchased from EIAab (Wuhan, China).

DTNB (5,5′-dithiobis-(2-nitrobenzoic acid) or Ellman's reagent (ref. 22582) was purchased from Pierce (Rockford, USA).

DPPH (2,2-diphenyl-1-picrylhydrazyl) was purchased from Merck Millipore (Darmstadt, Germany).

The ORAC (Oxygen Radical Absorbance Capacity) antioxidant assay kit was purchased from Zen-Bio, Inc (Research Triangle Park, USA).

2.2. Albumin samples

A panel of several commercially available albumin preparations from different suppliers were tested and compared: 3 lots of "Albumine Humaine 20%" from Baxter (Mississauga, Canada); 3 lots of Albuminar or AlbuRx 25%from CSL-Behring (Victoria, Australia); 3 lots of Albunorm 20% from Octapharma (Lachen, Switzerland) and 3 lots of Vialebex® and Ydralbum® from LFB, (Les Ulis, France). The batches were randomly purchased. All the storage buffers contained caprylic acid and sodium chloride. N-Acetyl-tryptophan was also present except to the two products from LFB. All the products were stored as recommended by the manufacturers and used at least 6 months before their expiration dates, except to 1 lot from Baxter at 1 month before. One batch of Vialebex out-of-date was systematically used as reference without demonstrating any difference in the results with the fresh batches (data not shown). Data from this batch were however not aggregated. The albumin preparations (2 μg) were analyzed by 10% SDS- PAGE. The electrophoretic profiles were similar between the different preparations with a purity degree >98% (data not shown).

2.3. Evaluation of the structural quality of human albumin preparations

2.3.1. Albumin binding cobalt assay

The albumin samples were assessed for cobalt binding using a colorimetric assay as described by Bar-Or et al. [24]. Briefly, 50 μL of a solution of 0.1% cobalt chloride in H_2O_2 was added to 200 μL of a serial albumin dilutions (0–60 mg/mL), gently mixed and then incubated for 10 min to allow for cobalt-albumin binding. Dithiothreitol (DTT, 1.5 mg/mL in H_2O, 50 μL) was then added as a colorizing agent and the reaction was quenched 2 min later by adding 1.0 mL of 0.9% NaCl solution. The color development produced by DTT was read at 470 nm using a spectrophotometer (Infinite M200 Pro TECAN). The albumin-cobalt without DTT was used as blank and the results were reported in optical density units (ODU) as a function of albumin concentration.

2.3.2. Evaluation of albumin glycation

A commercial enzyme-linked immunosorbent assay (ELISA) was used to evaluate the glycation level in human albumin preparations. Each albumin sample (100 μL), diluted in provided sample buffer, to a range of concentrations from 0.0016 to 0.1 μg/mL was directly transferred into a 96-well plate and subsequently incubated for 1 h at 37 °C. After a 4-washing step using provided washing buffer (the washing step was repeated after each incubation), wells were saturated using a provided 2% Tween-20 solution for 1 h at 37 °C. The anti-advanced glycated end-products (AGE) antibody (100 μL of 1 μg/mL solution) was added and incubated overnight at 4 °C. Antibody binding was detected using horseradish peroxidase (HRP)-coupled anti-rabbit IgG (100 μL, 0.04 μg/mL) and developed using 0-Phenylenediamine (OPD, 0.8 mg/mL in citrate buffer). The reaction was then stopped by addition of H_2SO_4 2.5 M (50 μL) and the optical density was determined using a microplate reader set at 492 nm. A glycated albumin from Sigma–Aldrich containing 2 hexoses/molecule was used as control.

2.3.3. UV/visible and fluorescence spectrometry of albumins

Albumins were diluted extemporaneously with phosphate-buffered saline (PBS) before the measurement. The UV/visible spectra were obtained by scanning the albumin samples (200 mg/mL) in the wavelength range of 230–600 nm using the Infinite® M200 PRO spectrophotometer (TECAN).

Fluorescence emission measurements were performed using the same spectrofluorometer. The excitation was set at 295 nm and the emission was collected in the range of 325–600 nm. Different concentrations of albumin solutions (12.5, 25 and 50 mg/mL) were analyzed.

2.4. Evaluation of the antioxidant capacity of human albumin preparations

2.4.1. Albumin controls

A reduced albumin solution, or positive control, was prepared as follows: Albumin solution (Vialebex®; 200 mg/mL or 3 mM) was dialyzed against a degassed phosphate buffer 0.1M, 0.3 M NaCl, pH 6.86. A 0.1% DTT solution was extemporaneously prepared and incubated with dialyzed albumin for 45 min at room temperature in a w/w ratio 5:1. The resulting solution was then extensively dialyzed against CH_3COONa 0.1M, pH 5.5 and stored at −80 °C.

An oxidized albumin solution, or negative control, was also prepared. Albumin (Vialebex®; 200 mg/mL) was dialyzed against PBS. A 12.5 mg/mL N-ethylmaleimide (NEM) solution (0.1M) was prepared and incubated for 60 min at 35 °C in a ratio of 200 mg albumin for 1.25 mg NEM. The solution was then extensively