Article type: Review

Title: Non-oncotic properties of albumin. A multidisciplinary vision about the implications for critically ill patients

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Acknowledgements: The authors are grateful to Francisco Mota and Jordi Bozzo (Grifols) for their organizational and editorial support in the successful preparation of the manuscript.

Financial and competing interest disclosure: The study and the authors did not receive any financial support. Ricard Ferrer has received payments for consultancies from Grifols. The other authors declare no competing interests.

Abstract

Introduction: Effective resuscitation with human albumin solutions is achieved with less fluid than with crystalloid solutions. However, the role of albumin in today's critical care unit is also linked to its multiple pharmacological effects.

Areas covered: The potential clinical benefits of albumin in select populations of critically ill patients like sepsis seem related to immunomodulatory and anti-inflammatory effects, antibiotic transportation and endothelial stabilization. Albumin transports many drugs used in critically ill patients. Such binding to albumin is frequently lessened in critically ill patients with hypoalbuminemia. These changes could result in sub-optimal treatment. Albumin has immunomodulatory capacity by binding several bacterial products. Albumin also influences vascular integrity, contributing to the maintenance of the normal capillary permeability. Moreover, the albumin molecule encompasses several antioxidant properties, thereby significantly reducing re-oxygenation injury, which is especially important in sepsis. In fact, most studies of albumin administration are a combination of a degree of resuscitation with a degree of maintenance or supplementation of albumin.

Expert commentary: The potential clinical benefits of the use of albumin in selected critically ill patients such as sepsis seem related to its immunomodulatory and anti-inflammatory effects, antioxidant properties, antibiotic transportation and endothelial stabilization. Additional studies are warranted to further elucidate the underlying physiologic and molecular rationale.

Keywords: Albumin, sepsis, critical care, drug transportation, endothelium, immunomodulation, antioxidation

1. Background

Human serum albumin is the most abundant circulating protein in the body. Besides its well-known oncotic function, albumin is known to have many non-oncotic properties (also called pharmacological properties) that may be relevant to its actions under physiological circumstances and in disease [1, 2]. Although therapeutic albumin has been given for many decades in a large number of diseases, and has demonstrated its safety in critically ill patients [3], a debate is still ongoing about the use of albumin in this setting [4]. Albumin administration is not necessary in all critically ill patients and should be reserved for use in specific groups of patients in whom there is evidence of benefit [5]. Recently, the Surviving Sepsis Guidelines suggest the use of albumin in addition to crystalloids for initial resuscitation and subsequent intravascular volume replacement in patients with sepsis and septic shock [6]. On the other hand, a hypotonic albumin solution should be avoided as a resuscitation fluid in patients with traumatic brain injury, based on the results of the SAFE sub-group analysis [7].

Among the multiple physiological functions of albumin, there is the regulation of the oncotic pressure, the transport of multiple substances including multiple drugs, the maintenance of acid-base balance, and others, which are particularly relevant in the critical patient [1]. It is also well established that low albumin levels, a common occurrence in critically ill patients, are associated with worsened outcomes [3, 8]. Therefore, there are quite a few arguments to consider the administration of albumin to those patients.

The objective of this review is to analyze if there is a rationale justifying albumin administration in critically ill patients due to its pharmacological properties beyond its effect as a volume expander. The following key non-oncotic effects associated with the

action of albumin have been explored: drug binding and drug interactions; immunomodulation; anti-inflammation; endothelial stabilization; capillary permeability; antioxidant; hemostasis; and acid-base balance.

2. Non-oncotic properties of albumin

2.1. Albumin drug-binding capacity

Several drugs that are used commonly in critically ill patients like antibiotic, antifungal and anesthetic drugs are transported by albumin. The pharmacokinetics of these drugs could be altered by different conditions like: hypoalbuminemia, hemodyalisis or fever,which, in fact, are also very common in critically ill patients. We analyzed the drug-binding capacities of albumin and the most frequent factors altering binding capacity of each drug.

Albumin is a protein composed of 585 amino acids with a total molecular weight of 66 kDa [9]. This heart-shaped protein contains three domains (I, II and III) located in the vertices which are very similar in structure (Figure 1) [10]. Each domain contains two subdomains (A, and B) [11]. Albumin binds many biological substrates in different *loci*. Drugs have been determined to bind in specific sites [12, 13]. Three main drug-binding sites have been described to date: Albumin drug binding site I, II and III (ADBS-I, ADBS-II, and ADBS-III),

2.1.1 Drugs binding to Albumin

ADBS-I, also known as Sudlow-I, is located in subdomain IIA. Drugs bound to this site are large heterocyclic compounds with a central negative charge in the molecule, as warfarin, phenylbutazone, or furosemide [12, 14]. An inner sub-site that can bind a second drug molecule in the presence of fatty acids has also been described [15].

ADBS-II, also known as Sudlow-II, is located in subdomain IIIA. Drugs bound to this site are lipophilic molecules with a peripherally located electronegative or a polar group, as flufenamic acid, iopanoic acid, or diazepam [12, 16].

ADBS-III is located in subdomain IB [13]. It has a flexible binding capacity. Drugs bound to this site exhibit a wide range of properties, being basic, neutral, or even acidic. Examples of these drugs are digoxin and digitoxin, antineoplastic drugs such as anthracyclines, epipodophyllotoxins, and camptothecins, antibiotic agents such as ampicillin, ceftriaxone, and fusidic acid, and other drugs such as valsartan or carbenoxolone [13].

Albumin of non-human mammals has different binding site characteristics and drug affinities. Thus, studies with animal albumins are difficult to extrapolate to humans [17, 18].

Albumin also has an unspecific esterase activity linked to ADBS-I and, in some extent to ADBS-II [19]. It has been described to hydrolyze aspirin and other xenobiotics as organophosphates, but research on this action on drugs is scarce. Due to the amount of albumin in plasma, this enzymatic activity could be considered of importance for some drugs.

2.1.2 Factors affecting albumin binding capacity

Several factors can affect albumin drug-binding capacity. Free fatty acids are its main physiological ligands. Typically, seven albumin fatty-acid binding sites (AFABS) have been described, three with high affinity (AFABS 2, 4, and 5) and four with low affinity (AFABS 1, 3, 6, and 7) [20, 21]. The presence and type of fatty acids modulated the albumin drug binding capacity in many studies [13, 16, 21]. Glycation is the chemical

reaction between glucose and many proteins present in blood. This process is of significant importance in diabetes. The glycation of albumin affects mainly ADBS-I, but ADBS-II can also be affected. Its effect is difficult to predict, as it depends on the degree and pattern of glycation. Low levels of glycation seem not to affect drug-binding capacity extensively, but highly glycated albumin, as found in diabetes, decrease drug binding [22]. Another disrupting factor can be the presence of high amounts of free amino acids like tryptophan, as occurs during parenteral nutrition. This amino acid inhibits drug binding to ADBS-II [23]. Oxidized albumin increases in many conditions such as renal and liver impairment or diabetes [22, 24, 25]. This form of albumin presents a different binding capacity than non-oxidized albumin, increasing for drugs as verapamil or decreasing for others as cefazolin. This effect can vary depending on the oxidation degree [26]. Albumin is also affected by carboxylation, oxidation, and covalent union to substances present in smokers' blood. These alterations diminished drug binding affinity to both ADBS in a model mimicking albumin of heavy smokers [27]. Drug binding affinity can also be reduced in both ADBS by the kidney impairment resulting from the retention of uremic toxins as creatinine [28]. In addition, urea at supra-physiological concentrations denatures albumin, but urea has also been found to change albumin conformation at concentrations around those found in patients with severe kidney failure [29].

The dynamic structure of albumin is strictly related to its non-oncotic properties. Albumin activity is significantly affected by minimal structural changes, folding, and clearance. Moreover, binding properties of albumin are also affected by structural changes other than oxidation, glycation and carboxylation, such as dimerization. Dimerization of albumin consists of the formation of an inter-molecular disulfide bond at Cys34 as a result of an increased oxidative stress, as it happens in cirrhosis [25].

Albumin dimerization can induce undetermined and perhaps opposite biological consequences. Thus, dimerization reduces the amount of free Cys34 residue, which has a detrimental effect in the albumin antioxidant and binding capacities. Conversely, dimerization doubles the molecular mass and longer plasmatic half-life of albumin, which may improve its plasma-expander capacity and drug transportation [30]. Pharmaceutical-grade albumins may have reduced drug-binding affinity for all ADBS resulting in increased free drug concentration when infused. The stabilizers caprylic acid (octanoate) and N-acetyl-DL-tryptophan [31] and the thermal process [32] used in the manufacturing processes may be responsible for this behavior.

In Table 1, studies on factors altering albumin binding capacity are summarized for antibiotics, antifungals, and anesthetic drugs.

2.2. Immunomodulatory and anti-inflammatory effects of albumin

The vast majority of our knowledge about the role of albumin as an immunomodulatory agent is derived from *in vitro* studies and animal experiments. Clinical studies that confirm or refute these immunomodulatory effects observed in the laboratory are lacking. Several mechanisms have been proposed to explain these immunomodulatory properties of albumin.

2.2.1. Binding of bacterial products: Many of the immunomodulatory effects of albumin rely on its ability to bind a wide range of endogenous and exogenous ligands. Thus, an interesting property of albumin is its capacity to bind several bacterial products such as lipopolysaccharide of the Gram-negative bacilli and other components of the Gram-positive bacteria including lipoteichoic acid and peptidoglycan [33]. Accordingly, albumin is capable of reducing arterial dysfunction induced by lipopolysaccharides (LPS) in a mouse model of endotoxemia [34].

2.2.2. Modulation of functions of antigen presenting cells (APC): T-cells may recognize major histocompatibility complexes (MHCs) on APC surfaces using their T-cell receptors (TCRs). APC process antigens and present them to T-cells. Therapeutic human albumin preparations are able to modulate the MHC II-restricted activation of antigen-specific T cells [35] as well as to upregulate the expression of MHC II and other related genes in APC by mechanisms not fully understood. Human albumin preparations increased T cell activation in a dose-dependent manner. A murine model demonstrated that this effect is mediated by an increase in the expression of MHC II and of two other genes (CIITA and H2-M) involved in antigen presentation [35].

2.2.3. Albumin modulates production of cytokines: Although the beneficial effects of albumin in models of endotoxemia may be in part mediated by its capacity of binding LPS, other mechanisms are also involved. Preconditioning with albumin abrogates LPS-induced tumor necrosis factor (TNF)-α gene expression in macrophages. In mice, exogenous albumin treatment also blunts LPS-mediated TNF-α gene expression *in vivo*. This effect is mediated by the attenuation of nuclear factor kappa B (NF-Kb) activation [35]. Albumin preconditioning elicits a cellular response similar to the phenomenon known as endotoxin tolerance. Thus, albumin preparations significantly inhibit the *in vitro* production of interferon- γ and TNF- α by activated peripheral blood mononuclear cells (PBMCs) and T-lymphocytes. This effect was attributed to the presence of aspartyl-alanyl diketopiperazine (product result of the degradation of the N termini of proteins and peptides) in six commercial preparations analyzed [36]. However, other studies have observed that *in vitro* albumin increases pro-inflammatory gene expression in a NF-κB-dependent manner [37]. In addition, albumin can act as a prostaglandin E2 (PGE2) ligand. Infusion of human albumin may attenuate immune suppression and

reduce the risk of infection in patients with acutely decompensated cirrhosis or end-stage liver disease thorough reduction of circulating PGE2 levels [38].

2.2.4. Other actions: Exogenous albumin decreases hypoxia-inducible factor (HIF)-1 α gene expression as well. In a rat model of endotoxinemia, albumin resuscitation improved the LPS-induced tissue hypoxia and myocardial contractility by ameliorating HIF-1 α gene expression [39]. HIF-1 α is a molecular key player in response to hypoxemic/inflammatory conditions prevailing in sepsis. Immune cells respond to hypoxic conditions by activating the heterodimeric transcription factor complex HIF-1, which is a key regulator of the cellular hypoxia-induced gene expression profile [40]. Finally, there is a possible role of albumin as a transferring tool of the local bactericidal activity of hypochlorite oxidation to the systemic circulation as chloramines [41].

2.3. Albumin: capillary permeability and endothelial stabilization

An intact glycocalyx combined with a minimum concentration of plasma proteins are required for the optimal function of vascular barrier. Albumin is crucially involved in the endothelial surface layer by contributing to vascular integrity, and participating in the maintenance of the normal capillary permeability, through the mechanism of binding the interstitial matrix and interacting with the sub-endothelium space [42, 43].

Therapeutic albumin may also contribute to protecting endothelial cells against oxidant-mediated injury through activation of the oxidant-sensitive transcription of pro-inflammatory proteins. Albumin decreases endothelial nitric oxide synthase (eNOS) activity, nitrosative stress in endothelial cells and increases their gluthatione levels maintaining endothelial cells function. Interestingly, this positive effect was observed with 4% but not with albumin 20%, suggesting a dose-dependent effect [44]. Binding of activated polymorphonuclear leukocytes to endothelial cells was significantly amplified

by hydroxyethyl starch and inhibited by albumin administration [45]. Indirectly, this property may positively influence the vascular integrity.

2.4. Antioxidant activity of albumin

The human body's exposure to free radicals can be regulated by antioxidants, defined as substances that, at low concentrations, have the ability to prevent or avoid the oxidation [46]. The organism has endogenous antioxidants, as albumin, glutathione, transferrin and ceruloplasmin, being endogenous albumin the main extracellular molecule responsible for maintaining the plasma redox state. Moreover, some exogenous substances have antioxidant properties (e.g., vitamin E, vitamin C, carotenoids, selenium, phenol compounds…) [47]. When there is an imbalance between free radicals and antioxidants we refer to "oxidative stress" [48]. The albumin molecule possesses several antioxidant properties, thereby significantly reducing re-oxygenation injury [39, 44, 49]. This action is especially interesting in sepsis, a pathologic condition characterized by a high oxidative stress [50].

The antioxidant properties of albumin rely on the structure of the molecule. Albumin contains a reduced cysteine residue (Cys34), which constitutes the largest pool of thiols in the circulation. Through this cysteine residue, albumin is able to scavenge reactive oxygen species (ROS), nitric oxide and other nitrogen reactive species, as well as prostaglandins [51-54].

The antioxidant properties differentiate albumin from other fluids used for patient resuscitation in clinical practice. Thus, the activation of oxidative and nitric oxide-consuming reactions was inhibited by albumin and augmented by hydroxyethyl starch [45]. Oxygen free radical production was reduced by albumin but not by synthetic

colloids (dextran 40) or crystalloids (Ringer's lactate, normal saline, and hypertonic saline) [55].

The real impact of antioxidant properties of commercial albumin is still being explored. Due to its affinity to a large number of molecules, it is very sensitive to environmental conditions. This can lead to changes in its conformation after exposure to other molecules (e.g., ROS, NOS, Glucose, triglycerides…) or during the process used to purify the molecule [56-60]. The oxidation state of Cys34 in circulating albumin is different to pharmaceutical preparations and this may affect its antioxidant capacity [61-63]. Oxidized cysteine was observed in 23% of human volunteer albumin, whereas in commercial preparations it was up to 60% [62]. Antioxidant effect of albumin seems also to be influenced by the concentration of the albumin infused (stronger antioxidant effect in 4% concentration than in 20%) [44].

Despite the clinical impact of albumin administration on the oxidative processes, the effect is still poorly documented in critically ill patients and mainly evaluated in experimental conditions [64]. Using albumin as a resuscitation fluid could be an opportunity to potentiate endogenous antioxidant protection in critical pathological conditions, while explaining some of the long term benefits observed after albumin administration, as occurred in the ALBIOS trial [65].

2.5. Albumin effects on hemostasis: from in vitro to clinical evidence

In addition to other effects derived from albumin administration in critically ill patients, albumin may have anticoagulant effects similar to those of heparin but much less potent, perhaps due to the similarity of both molecules. It has been described that albumin enhances the neutralization of factor Xa by antithrombin III, inhibits platelet-activating factor-induced responses and slightly reduces levels of fibrinogen.

In humans, albumin is the colloid molecule most representative in the extracellular space. A wide range of published studies concluded that while crystalloids induce a moderate hypercoagulable state with a 10%-30% hemodilution, albumin does not impair hemostasis except with >50% hemodilution [66].

The effects of albumin on hemostasis have been explored in vitro and in animal models, and described in human studies. A recently published in vitro study using rotational thromboelastrometry has shown that fibrinogen activity is more impaired with intense hemodilution with albumin than with hemodilution with normal saline [67]. With the same methodology, another in vitro study showed that hemodilution with gelatin and albumin induced fewer coagulation abnormalities than hydroxyethyl starch [68]. A recent in vitro study performing hemodyalises using blood from healthy donors showed that priming using different heparin-albumin combinations reduced clotting in the circuit allowing hemodyalisis [69]. In this sense, a clinical study showed that raising the extracorporeal circuit with an heparin-albumin solution reduces the need for systemic anticoagulant in hemodyalisis [70].

Among published animal models exploring the effects of synthetic colloids, a piglet model showed that, after a rapid infusion of a moderate volume, hydroxyethyl starch and gelatin impaired blood coagulation (without differences between both artificial colloids) to a larger extent versus albumin or normal saline as assessed by rotation thromboelastrometry [71]. A rabbit model evaluating the effects of synthetic versus natural colloid resuscitation on inducing dilutional coagulopathy and hemorrhage showed that resuscitation with albumin maintained coagulation function, decreased blood loss and improved survival time compared to synthetic colloids [72].

The use of albumin as extracorporeal circuit priming fluid has been shown to prevent platelet adhesion to circuit surfaces, avoiding platelet decrease. Several clinical studies have described the benefits of the use of albumin instead of hydroxyethyl starch in cardiac surgery and in patients undergoing cardiopulmonary bypass [73, 74], situations where the choice of fluids for extracorporeal circuit priming and perioperative volume expansion may modify the risk of excessive coagulopathic bleeding. A meta-analysis including 18 trials with up to 970 patients confirmed an increased blood loss in cardiac surgery with cardiopulmonary bypass among patients receiving hydroxyethyl starch compared with albumin [75].

Excessive postoperative bleeding remains a frequent, serious and unpredictable complication in the previously cited settings. Taking into account that common colloids are albumin and hydroxyethyl starch from the published evidence, the use of hydroxyethyl starch as extracorporeal circuit priming fluid is associated with a dose-dependent increase in hemorrhages that carry additional costs greater than savings afforded by its lower acquisition cost when compared with albumin [75, 76]. Albumin remains the most appropriate control fluid because it is the colloid normally present in circulation and is free of adverse effects on coagulation [75].

2.6. Acid-base balance-related disorders and albumin

Disorders of acid-base balance are common clinical abnormalities in critically ill patients. Acid-base disorders are typically related to clinical outcomes and disease severity, especially for metabolic acidosis [77]. There are currently three methods for the assessment of acid-base disorders: the physiological, the base excess, and the physicochemical approaches [78]. The physiological and the base excess approaches are based on the analysis of plasma concentration of bicarbonate and standard base excess

and plasma anion gap. However, its accuracy in critically ill patients may be limited by assumption of normal plasma protein [79]. Therefore, correction for serum albumin concentration is required for the interpretation of anion gap. Underestimation of anion gap is significant in the presence of hypoalbuminemia, which is frequent in critically ill patients [80].

The mathematical model based on physiochemical principles that determine hydrogen ion concentration and pH in an aqueous solution can be an alternative solution [81]. By this method the clinician can quantify individual components of acid-base abnormalities while providing insight into their pathogenesis. A number of studies have shown that this approach is the most adequate to identify acid-base disorders in critically ill patients, in comparison to traditional approaches. According to this theory, there are three independent variables that determine pH in plasma by changing the degree of water dissociation into hydrogen and hydroxide ions: the partial pressure of carbon dioxide (PCO_2), the concentration of non-volatile weak acids (A_{TOT}) (mainly albumin and phosphate in the extracellular space) and the strong ion difference (SID), defined as the difference between the sum of concentrations of all strong cations (mainly Na^+, K^+, Mg^{2+}, Ca^{2+}) and the sum of concentrations of all strong anions (mainly Cl^- and lactate). Plasma SID is typically much lower in hypoalbuminemic and critically ill patients than in healthy subjects. To conform to the principle of electrical neutrality, positive SID must be balanced by an equal negative charge. Hypoalbuminemic patients also often manifest a reduced A_{TOT}, perhaps as compensation for their reduced SID [82].

The pH i directly affected by variations in these three independent variables. Despite a profound hypoalbuminemia is found in critically ill patients, they are infrequently alkalemic. Although this seems a counterintuitive observation, it can be understood by

the fact that SID and A_{TOT} are best evaluated in relation to one another rather than as absolute values. During fluid infusion, SID and A_{TOT} of plasma tend toward the SID and A_{TOT} of the administered fluid, which can therefore lower, increase, or leave pH unchanged depending on fluid composition.

As a general rule, crystalloids with a SID greater than plasma bicarbonate (HCO_3^-) concentration cause alkalosis (increase in plasma pH), those with a SID lower than plasma HCO_3^- cause acidosis (decrease in plasma pH), while crystalloids with a SID equal to HCO_3^- leave pH unchanged. This can be applied regardless of the extent of the dilution. These rules partially hold true for colloids and blood components, since they are composed of a crystalloid solution as solvent.

SID of commercially available albumin preparations is greater for higher albumin concentrations (20-25%) in comparison to concentrations of 4-5%, due to the increased amount of albumin and resulting increase in negative charges (A^-). The electrolyte composition of the solvent, and therefore its SID of the infusion fluid (SID*inf*) differ considerably between different albumin preparations. The acidifying effect of albumin-containing solutions having a low SID*inf* is easily caused by the decrease of SID and the increase in A_{TOT} decrease plasma pH [83-86].

The use of 5% albumin as replacement fluid in plasma exchange procedures has been associated with a decrease in serum pH and bicarbonate levels in a large cohort of patients [87].

The effect of the administration of 20% albumin on acid-base equilibrium has been recently studied in critically ill patients. The administration of 20% albumin induced an alkalizing effect with an increase in SID due to a decrease in Cl^- concentration, and

conversely an acidification effect by a rise in A^- due to the rise in albumin serum concentration. The pH level was unchanged because SID and A^- increased to almost the same amount [88].

The effect of albumin infusion in Cl^- levels depends on the different preparations. The rise in Cl^- levels seen after 4% albumin infusion is likely a reflection of the larger amounts of Cl^- present in the commercial solution (Cl^- 128 mmol/L), whereas the decline in Cl^- levels seen after 20% albumin infusion reflects the moderate amounts of Cl^- present in the solution (65 mmol/L) [83, 88].

In the same way, the decline in Ca^{2+} concentrations is likely due to binding of free calcium with the infused albumin (both for iso and hyperoncotic) taking into account that albumin solutions have an absence of calcium [83, 88].

The relationship between acid-base abnormalities and inflammation is another issue to consider. Experimental data provide evidence that acidosis increases inflammation. Zampieri *et al*. recently described that acid–base variables on admission to intensive care unit (ICU) are associated with immunological activation. Specifically, albumin was negatively associated with interleukin (IL)6, IL7, IL8, IL10, TNF-α and interferon (IFN)α [89]. Hence, interplay between the level of albumin and acid–base status and inflammation would imply that decreased albumin on admission to the ICU could be associated with immunological activation.

3. Hypoalbuminemia in critically ill patients

Hypoalbuminemia is generally defined as a serum albumin concentration $\leq$30 g/l [8, 90]. Hypoalbuminemia is very common in critically ill patients, and it is typically

caused by increased albumin loss from bleeding and via the gastrointestinal tract [91], by redistribution from the intravascular to the interstitial space due to increased capillary permeability [92], and by dilution from intravenous fluid administration.

Hypoalbuminemic states may be associated with a reduced efficacy of albumin-bound drugs due to increased volume of distribution. Such effect may require dose adjustment due to sub-optimal treatment, particularly for time-dependent drugs. Protein binding of antibacterials such as ceftriaxone, ertapenem, teicoplanin, and aztreonam has been reported to be frequently decreased in critically ill patients with hypoalbuminemia and increased volume of distribution and drug clearance [93].

Patients with hypoalbuminemia show severe deficits in cellular immunity. The correlation between marked oxidative stress and low levels of serum albumin is supported by some clinical studies [94]. The potent antioxidant capacity of albumin administration can explain its beneficial effect. For instance, albumin improves plasma thiol-dependent antioxidant status as well as diminishes the protein oxidative damage in patients with acute lung injury [95]. Although the association between the albumin level and the severity of the damage is clear, whether the effect of hypoalbuminemia on outcome is a cause-effect relationship or whether hypoalbuminemia is rather a “marker” of serious disease, remains uncertain.

Administration of exogenous albumin to target a specific albumin level may help restore or provide additional not only antioxidant capacity, but also transport capabilities and vascular barrier competence. These effects may account for some of the beneficial effects of albumin seen in specific patient populations, although they are rather difficult to differentiate from albumin’s effects on intravascular volume.

4. Conclusions

Human albumin solutions have been demonstrated to provide effective resuscitation with less fluid than that required with crystalloid solutions. However, in our opinion, the role of albumin in today's critical care unit cannot be separated from its multiple pharmacological effects. The potential clinical benefits of the use of albumin in selected populations of critically ill patients like sepsis seem to be related to the immunomodulatory and anti-inflammatory effects, antioxidant properties, antibiotic transportation and endothelial stabilization, in addition to its oncotic properties. Mechanistic studies are warranted to shed light on the molecular and physiologic rationale behind the beneficial effects of albumin as well as to further explore the therapeutic role of albumin's pleiotropic actions in pharmaceutical-grade albumins.

5. Expert Commentary

Although albumin was initially considered mostly as an acute resuscitation fluid, there is currently an increased interest in the use of albumin solutions as a supplement to correct and maintain albumin levels identification, greatly induced by advances in the identification of the adverse outcomes associated with hypoalbuminemia and by a better knowledge about the functioning of vascular barrier. However, to distinguish volume effects from the effects of maintenance of serum albumin is not always trivial, particularly in critically ill patients. Hypoalbuminemia is common in critically ill patients, in whom it is difficult to clearly relate the timing of interventions to the onset of disease. Therefore, the majority of studies of albumin administration are a combination of a measure of resuscitation with a measure of supplementation or maintenance of albumin [96]. Moreover, transport and antioxidant effects of albumin may become important when used as supplementation.

Substitution of synthetic colloids for albumin as part of perioperative fluid therapy has not been very successful. Hence, hydroxyethyl starch (HES) solutions can persist for long periods of time in the skin, liver and most importantly, the kidney [97], which involves not only a potential risk of renal failure but also increased mortality rates in septic patients [98]. On the other hand, gelatin solutions have been less commonly studied, partly because their shorter intravascular persistence and their limited availability in some countries.

6. Five-year view

New knowledge about the non-oncotic properties of serum albumin paves the path for new potential indications in the management of critical patients and in particular of septic patients. The immunomodulatory and anti-inflammatory properties of albumin, as well as their involvement in the pharmacokinetics of various molecules including antibiotics, are of special interest. These new mechanisms of action should be carefully studied and translated into the usual clinical practice. An important innovation could be the development of a new commercial albumin with enhanced non-oncotic properties to be used in some selected groups of patients. Moreover, it is also crucial to study the optimal administration of albumin: bolus versus continuous infusion, dosage, concentration and targets. Future research should also be focused to answer questions about the mechanisms of development of hypoalbuminemia and its consequences in the ICU setting.

7. Key issues

- Human serum albumin is the most abundant circulating protein in the body.

- Hypoalbuminemia is very common in critically ill patients.

- The potential clinical benefits of the use of albumin in selected critically ill patients such as sepsis seem related to the its immunomodulatory and anti-inflammatory effects, antioxidant properties, antibiotic transportation, and endothelial stabilization.

-The main mechanisms of immunomodulatory and anti-inflammatory properties of albumin are: binding of bacterial products such as LPS, modulation of functions of APC, and modulation of synthesis of cytokines.

-Albumin is a crucial part of the endothelial surface and contributes to maintenance of the normal capillary permeability.

-Albumin has antioxidant activity that is especially important in sepsis.

- Albumin has anticoagulants effects: it enhances the neutralization of factor Xa by antithrombin III, inhibits platelet-activating factor-induced responses, and has the capacity of reducing fibrinogen levels.

Table 1 - Factors altering albumin binding capacity for antibiotic, antifungal and anesthetic drugs.

Drug	Binding site	Factor or interfering drug	Result	Clinical relevance	Type of study	Reference
Antibiotic drugs						
Cefazolin	ADBS-I, Bilirubin binding site (ADBS-III?)	Increase in pH (alkalization)	Increase in free drug concentration	Unknown	*In vitro*	[99]
		Mildly oxidized albumin	Increase in free drug concentration	Unknown	*In vitro*	[26]
Cefotaxime	Bilirubin binding site (ADBS-III?), ADBS-II	Ibuprofen	Increase of free drug concentration	Unknown	*In vitro*	[99]
Ceftazidime	ADBS-II (main) ADBS-I (secondary)	Ibuprofen	Increase in free drug concentration	Unknown	*In vitro*	[99]
Cefditoren	Unknown (ADBS-I?)	Ibuprofen	Increase of free drug concentration	Increase in drug bactericidal activity	*In vitro*	[100]
Ceftriaxone	ADBS-I?, ADBS-II?, Bilirubin binding site (ADBS-III?), fatty acid binding sites	Meloxicam	Increase >10% of free drug concentration	Unknown	*In vitro*	[99, 101]
		Valdecoxib	Small decrease <10% of free drug concentration	Unknown	*In vitro*	[99, 101]

Drug	Binding site	Factor or interfering drug	Result	Clinical relevance	Type of study	Reference
Cefuroxime	ADBS-I	Coumarin (warfarin)	Increase of free drug concentration	Unknown	*In vitro*	[99]
Ciprofloxacin		Acetaminophen	Increase in AUC and decrease in half-life of the antibiotic	Unknown. Faster drug clearance?	Randomized, two-way crossover study in healthy volunteers	[102]
		Acetaminophen, cefotaxime, repaglinide (in decreasing order of importance).	Increase in >10% of free drug concentration	Unknown	*In vitro*	[103]
	ADBS-I (main), ADBS-II	Gliclazide, caffeine, ibuprofen (in decreasing order of importance)	Small increase in <10% of free drug concentration	Unknown	*In vitro*	[103]
		Iron (Fe^{3+})	Increase in >10% of free drug concentration	Unknown	*In vitro*	[104]
		Magnesium (Mg^{2+})	Increase in <10% of free drug concentration	Unknown	*In vitro*	[104]
Clarithromycin	Unknown	Hemodialysis	Increase of free drug concentrations after hemodialysis	Increased drug effects including adverse effects?	*In vitro*	[105]
Doxycycline	ADBS-II	Hypoalbuminemia	Increase of free drug concentration	Unknown	*In vitro*	[106]

Drug	Binding site	Factor or interfering drug	Result	Clinical relevance	Type of study	Reference
		Increase in temperature	Increase of free drug concentration	Unknown	*In vitro*	[106]
		Ketoprofen	Increase of free drug concentration	Unknown	*In vitro*	[106]
Fosfomycin	ADBS-I	Sodium (Na^+), potassium (K^+), chloride (Cl^+) (in decreasing order of importance)	Increase of free drug concentration	Faster drug clearance?	*In vitro*	[107]
		Warfarin	Increase of free drug concentration	Faster drug clearance?	*In vitro*	[107]
Imipenem	ADBS-II (main), a specific site in subdomain IIA−IIB (secondary)	High affinity drug – albumin. Inhibition of albumin esterase activity	Stable complex imipenem - albumin	Compromised bioavailability of imipenem to the site of infection?	*In vitro*	[108]
Levofloxacin	ADBS-I (main), ADBS-II (secondary)	Acetaminophen, cefotaxime, caffeine, cefdinir, diclofenac, gliclazide, ibuprofen, repaglinide	Small increase in <10% of drug free concentration	Unknown	*In vitro*	[108]
		Magnesium (Mg^{2+})	Increase <10% of free drug concentration	Unknown	*In vitro*	[104]

Drug	Binding site	Factor or interfering drug	Result	Clinical relevance	Type of study	Reference
		Zinc (Zn^{2+})	Decrease >10% of free drug concentration	Unknown	*In vitro*	[104]
Teicoplanin	Unknown	Continuous veno-venous hemodiafiltration	Increase of free drug concentration	Unknown	Pharmacokinetic study in human patients	[109]
		Hypoalbuminemia	Increase of free drug concentration	Increase in drug adverse effects?	Pharmacokinetic studies in human patients	[110, 111]
Tetracycline	ADBS-I or ADBS-II?	Copper (Cu^{2+})	Decrease of free drug concentration	Increase in drug half-life? Decrease in drug effectiveness?	*In vitro*	[112]
		Zinc (Zn^{2+}), Calcium (Ca^{2+}) (in decreasing order of importance)	Mild increase of free drug concentration	Decrease in drug half-life?.	*In vitro*	[112]
Vancomycin	Unknown	Changes in albuminemia	Unchanged free drug concentration	Albumin appears to be not related to free vancomycin variations	Pharmacokinetic retrospective study in patients with serious acute infections	[113]
		Hypoalbuminemia (<2.5 g/dL)	Increase of plasma drug clearance	Unknown	Pharmacokinetic retrospective study in hospitalized patients	[114]
Antifungal drugs						

Drug	Binding site	Factor or interfering drug	Result	Clinical relevance	Type of study	Reference
Amphotericin B	ADBS-II (main), ADBS-I (secondary)	Bolus or continuous infusion administration	Unaltered free drug concentration	No improvement in antifungal activity	*In vitro*	[115]
		Free fatty acids	Decrease of free drug concentration	Unknown	*In vitro*	[116]
Caspofungin	Unknown	Hypoalbuminemia (<2.36 g/dL)	Decreased drug trough concentrations	Decreased drug tissue distribution?	Pharmacokinetic study in surgical intensive care unit patients	[117]
Itraconazole	Unknown	Decrease in albuminemia	Increase in hepatic clearance of drug and of its active metabolite hydroxy-itraconazole	Unknown	Pharmacokinetic study in immunocompromised patients	[118]
		Hypoalbuminemia (2.8 g/dL)	Decrease in plasma levels of the drug and its active metabolite hydroxy-itraconazole. Increase in free drug concentration?	Increase in drug tissue levels?	Case report	[119]
		Insulin-dependent and non-insulin dependent diabetes mellitus	Increase >25% of free drug concentration	Increase antifungal activity	*In* vitro using serum of patients with diabetes mellitus and healthy controls	[120]

Drug	Binding site	Factor or interfering drug	Result	Clinical relevance	Type of study	Reference
		Cancer patients	Increase >40% of free drug concentration	Unknown	*In vitro* using serum of patients with cancer and healthy controls	[121]
Micafungin	Unknown	Changes in albuminemia	No influence in drug plasma levels	No drug adjustment in hypoalbuminemia	Pharmacokinetic study in patients with hematologic malignancies.	[122]
Anesthetic drugs						
Diazepam	ADBS-II	Tetrahydrocannabinol (THC)	THC does not affect diazepam binding to albumin	Unknown	*In vitro*	[123]
Ketomebidone	Unknown	Changes in albuminemia	Unaltered drug elimination	Unknown	Pharmacokinetic study in critically-ill patients	[124]
Midazolam	ADBS-II	Propofol	Increase in free midazolam concentration	Increased sedative effect of midazolam administered with propofol?	*In vitro*	[125]
Morphine	Unknown	Decrease in albuminemia	Increase of free drug concentration	Unknown	Study in children with cancer and healthy neonates and adults	[126]

Drug	Binding site	Factor or interfering drug	Result	Clinical relevance	Type of study	Reference
Propofol	ADBS-II (main), ADBS-I (secondary)	Increase in plasma pH	Decrease of <10% of free drug concentration	No influence affecting significantly morphine	Study in children with cancer, healthy neonates, and healthy adults	[126]
		Increase in total drug plasma concentration	Increase in free drug concentration. Marked effect at lower concentrations	Unknown	Study in children with cancer, healthy neonates, and healthy adults	[126]
		Isovolemic hemorrhage with crystalloid resuscitation	Increase >60% of free drug concentration	Increase in the hypnotic potency	Pharmacokinetic study in patients during elective surgery	[127]
		Fentanyl, morphine, naloxone (in decreasing order of importance)	Increase in propofol free drug concentration	Unknown	*In vitro*	[128]
		Increase in total drug concentration at low or physiological plasma albumin level	Decrease in free propofol concentration	Increased drug effects at lower doses?	*In vitro* and plasma of patients undergoing elective neurosurgical procedures and anesthetized with propofol	[129]
		Decrease in albuminemia	Increase in free propofol concentration	Increased drug effects in hypoalbuminemia?	*In vitro*	[130]

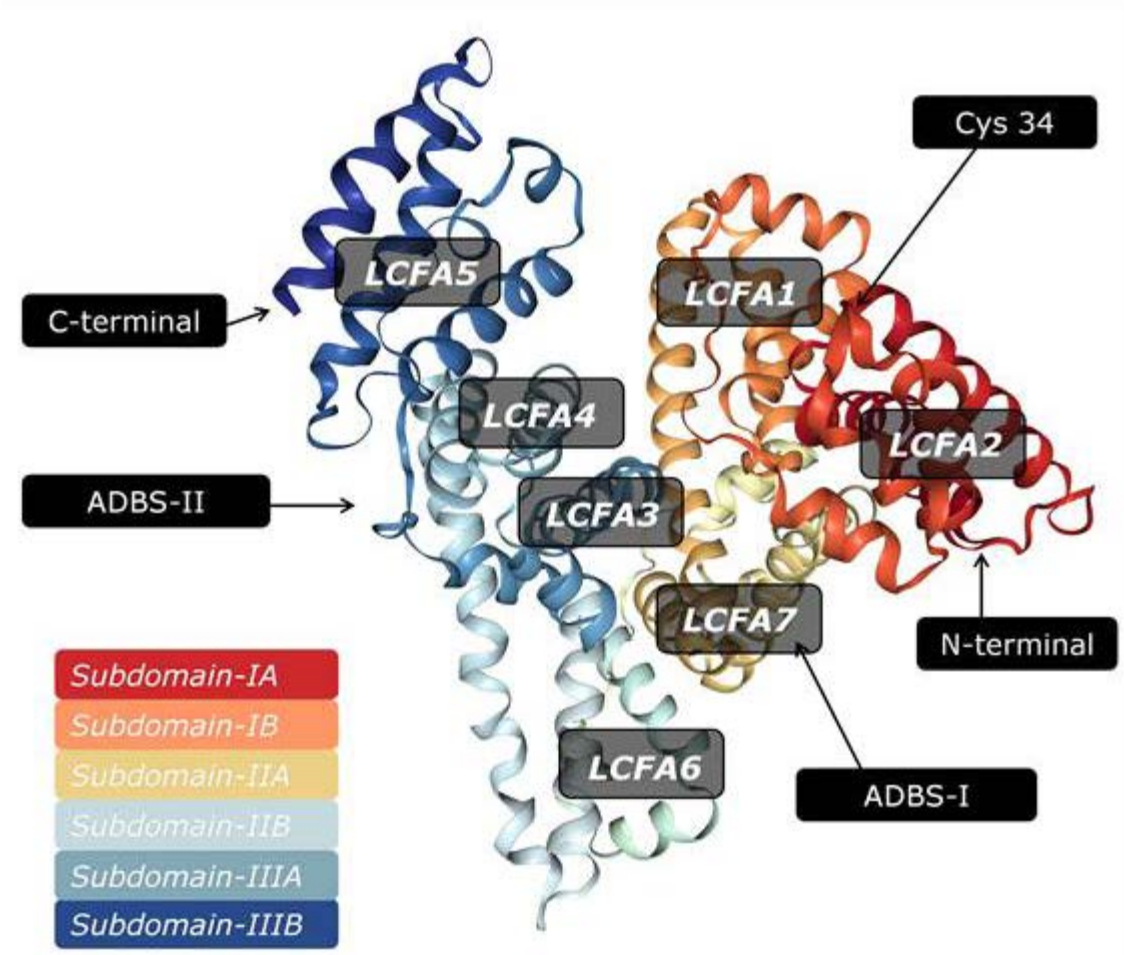
Drug	Binding site	Factor or interfering drug	Result	Clinical relevance	Type of study	Reference
		Decrease in albuminemia	Increase in free propofol concentration	In hypoalbuminemia: Increased adverse effects? Prolongation of effect?	*In vitro* with blood from healthy males	[131]
		Decrease in albuminemia	Increase in free propofol concentration	In critically ill patients: Increase in drug effect?	*In vitro* with blood from healthy volunteers and critically-ill patients	[132]
		Increase in free fatty acids	Increase in free propofol concentration	Unknown	*In vitro*	[133]
		Ibuprofen	Increase in albumin-drug complex stability	Unknown	*In vitro*	[134]
Thiopental	ADBS-I	Copper (Cu^{2+}), iron (Fe^{3+}), calcium (Ca^{2+}) (in decreasing order of importance); increase thiopental - albumin binding	Decrease of free drug concentration	Increased half-life?	*In vitro*	[135]
		Decrease in albuminemia	Increase of free drug concentration	Unknown	*In vitro*	[135]

ADBS: Albumin drug binding site (Sudlow)

Figure 1. Human albumin structure.

ADBS: Albumin drug binding site (Sudlow); LCFA: low chain fatty acid binding site.

Adapted from Protein Data Bank (PDB) ID 1AO6 [10].



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