Administration of albumin to patients with sepsis syndrome: a possible beneficial role in plasma thiol repletion

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ABSTRACT

1. Albumin is often administered intravenously to critically ill patients as a volume expander, to combat hypoalbuminaemia, and to decrease hyperbilirubinaemia. There is, however, an ongoing debate concerning the therapeutic benefit of the former which is an expensive form of treatment.

2. Albumin has several biological functions, in particular as a ligand binder. It also acts as an extracellular transition metal ion-binding and radical-scavenging antioxidant. These functions are influenced by the presence of an exposed thiol group (cys 34) on the surface of the albumin molecule.

3. The ability of infused albumin to influence the plasma thiol pool, and hence antioxidant potential, was investigated in patients with sepsis syndrome.

4. Plasma thiol levels rose rapidly after albumin infusion and remained elevated even after plasma albumin levels had declined significantly, due to interstitial leakage. Data are suggestive of some form of thiol exchange in the plasma of these patients between albumin and molecules containing oxidized thiol groups.

5. Administration of albumin to patients with sepsis syndrome leads to a sustained increase in plasma thiols. Thiols have several important antioxidant functions, and thiol repletion in these patients, who are known to suffer from oxidative stress, may have beneficial antioxidant effects. Antioxidant repletion may represent an important facet of clinically administered albumin.

INTRODUCTION

Albumin is a 66-kDa protein which comprises around 50% of the total proteins present in normal healthy human plasma [1]. It is a single polypeptide chain containing 585 amino acids, and is unusual among plasma proteins in that it does not contain a carbohydrate moiety. In its reduced form, albumin has one exposed free thiol-containing cysteine at position 34 [2]. Albumin preparations have been used during the past 50 years as 4.5% w/v (isotonic) solutions for intravascular volume maintenance, as 20% w/v solutions for volume expansion, and to treat hypoalbuminaemia and hyperbilirubinaemia. Recently, the necessity of maintaining serum albumin levels, and the clinical benefits of using an expensive resource as a volume replacement, have been discussed.

Key words: albumin, antioxidant, plasma thiols, sepsis syndrome.
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questioned. Several clinical studies have shown no differences in clinical outcome between patients receiving albumin supplementation or non-albumin-containing colloid solutions [3,4]. Most of these, however, have used inappropriate end points, such as mortality; others have used intermediate measures such as duration of stay in intensive care, and have investigated relatively small numbers of patients. Albumin has a number of important properties and functions in both health and disease; for example, it is an important transport vehicle for metals [5], fatty acids (reviewed in [6]), cholesterol [7], nitric oxide [8], bile pigments [9] and drugs. Because of its high concentration in plasma, its highly negative charge and its relatively small size, it is also responsible for approximately 70% of the plasma colloid osmotic pressure and thus plays a pivotal role in fluid distribution between the extracellular compartments [10]. There is also evidence that it is important for maintaining permeability of the capillary membrane [11], and that it has a role in inhibiting platelet aggregation [12]. Finally, albumin contributes to plasma antioxidant defences by binding transition metals, and as a non-specific sacrificial scavenger [13]. Albumin normally exists in an equilibrium of reduced and oxidized forms, with the reduced form predominating, although this balance shifts somewhat in pathological states and during ageing [14].

A key question addressed here is the ability of infused albumin to influence the body’s thiol pool, and hence the balance that normally exists between pro-oxidants and antioxidants.

**MATERIALS AND METHODS**

**Materials**

5,5′-Dithiobis-(2-nitrobenzoic acid) was from Sigma Chemical Co., Poole, Dorset, U.K. Twenty per cent human albumin for clinical use was from Bio Products Laboratory, Elstree, U.K. All other reagents were of the highest grade available and were from BDH (Merck), Poole, Dorset, U.K.

**Patients**

Twenty-eight patients were investigated in this study using the initial protocol (group 1). Three of these patients were investigated in the extended protocol (group 2); only a small number of patients were suitable for inclusion in this study because of ongoing treatment regimes which included subsequent albumin infusions used during the study period on the other patients. All patients were sedated and ventilated, in a mixed surgical and medical intensive care unit, in a steady state and at least 48 h after admission. All patients received fluid resuscitation and inotropic agents sufficient to maintain an oxygen delivery index in excess of 330 ml min⁻¹ m⁻². The average age was 50 ± 20 years, the median APACHE II score was 16 (range 7–36). Patient demographics are shown in Table 1. All procedures were carried out in accordance with the Declaration of Helsinki (1989) of the World Medical Association. Approval for the study was obtained from the Chelsea and Westminster Hospital Ethics Committee. Informed consent is not required in our hospital for taking small volumes of blood (10 ml) from critically ill patients for experimental purposes, provided that indwelling catheters are already in place.

**Sample collection**

Arterial blood was sampled throughout. Baseline blood samples were taken for total protein, albumin, thiol and full blood count measurements, after which each patient received a rapid infusion of 200 ml of 20% (w/v) human albumin. Central venous pressure was monitored continuously to ensure fluid overload did not occur, with the intention of halting the infusion if the central venous pressure rose by 8 mmHg or exceeded an absolute value of 20 mmHg. It was not necessary to stop the study on any occasion. Blood samples were taken 5 min and 4 h

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OSF, organ systems failure.
post albumin infusion in group 1, whereas in group 2 patients sampling continued at 8, 12 and 18 h. No other blood products or albumin infusions were given during the study period, fluid maintenance being achieved with gelatin solutions (haemacel) and dextrose only.

Control subjects
A control group for comparison with group 1 patients comprised six healthy adult volunteers (age range 25–39 years, mean age 32 years). Each of the control subjects lay supine for 15 min before the start of the study. Baseline venous blood samples were taken, and 200 ml of 20% (w/v) albumin was infused through a large bore (14G Y-can) cannula in the antecubital fossa. Additional blood samples were taken from a small (23G Y-can) cannula in the contralateral arm, at the same time intervals as the group 1 patients, i.e. at 5 min and at 4 h.

Measurement of total plasma thiol levels
Total plasma thiol groups were determined using Ellman’s reagent [5,5′-dithiobis-(2-nitrobenzoic acid)]. The following reagents were added to new clean plastic tubes in the order stated: 50 µl of plasma, 550 µl of distilled water, 200 µl of phosphate/saline buffer pH 7.4 (0.1 M sodium phosphate in 0.15 M NaCl) and 200 µl of Ellman’s reagent (10 mM) in buffer. Blanks were established for each sample containing distilled water instead of Ellman’s reagent. The reaction was allowed to proceed for 15 min at room temperature and the resulting chromagen was measured at 412 nm. Values for total plasma thiols were expressed in µM using a molar absorption coefficient of 13600 litre mol⁻¹ cm⁻¹ for the chromagenic anion formed. A 0.1-mM cysteine solution was used as a reference standard each time the assay was performed. Assay precision was ±5% within a batch as ascertained previously [15].

Measurement of low-molecular-mass plasma thiol levels
Low-molecular-mass plasma fractions were obtained by use of ultrafiltration devices (Centricon 10 from Amicon U.K.) in accordance with the manufacturer’s instructions. The thiol content of ultrafiltrates was measured by use of Ellman’s reagent as described above.

Plasma protein and albumin measurements
Plasma total protein was measured using a Biuret reagent, and albumin levels using a bromocresol purple dye-binding technique on a Hitachi 717 multi-channel analyser.

Statistics
Comparisons between variables were made by parametric repeated measures analysis of variance with Tukey–Kramer comparison tests. P < 0.05 was considered significant. Data are represented throughout as means ± S.E.M.

RESULTS

Blood samples were taken from patients with sepsis syndrome before and after the administration of 40 g (200 ml of 20%) of albumin. Normal healthy control subjects were treated in a similar way. Total protein, thiol and albumin levels were immediately measured in the fresh plasma samples obtained. In the first study (group 1 patients) these parameters were measured at three time intervals (pre- and 5 min and 4 h post albumin administration). Group 1 sepsis patients showed significantly increased levels of plasma albumin 5 min after albumin administration compared with the pre-administration levels (pre, 12.62 ± 1.29 mg/ml; 5 min post, 22.35 ± 1.42 mg/ml, P < 0.05). Albumin levels remained significantly increased 4 h after its administration (18.92 ± 1.37 mg/ml, P < 0.05). Comparisons between 5 min and 4 h time points post albumin administration showed there was a significant decrease in plasma
**Figure 2** Plasma albumin levels (A) and total plasma thiols (B) from normal healthy individuals before and after albumin administration

**P < 0.01** compared with pre-albumin administration, *****P < 0.001** compared with pre-albumin administration.

Albumin levels after 4 h (**P < 0.05**). Total plasma thiol levels at the same time intervals showed trends similar to albumin (pre-, 138.40 ± 15.7 µM; 5 min post, 192.18 ± 15.30 µM; 4 h post, 192.26 ± 15.40 µM, **P < 0.05**). Unlike the albumin measurements, however, there were no significant differences between the thiol levels at 5 min and 4 h (**P > 0.05**). Results are shown in Figure 1. The same analytical procedures were carried out in normal healthy control subjects (**n = 6**). When pre-administration levels (albumin 40.55 ± 0.766 mg/ml and thiols 364 ± 22.35 µM) were compared with values at 5 min (albumin 49.11 ± 1.02 mg/ml and thiols 444.66 ± 15.2 µM) and 4 h (albumin 46.55 ± 0.835 mg/ml and thiols 446.00 ± 16.38 µM), significantly (**P < 0.05**) increased values were seen in all cases. No significant differences for either thiols or albumin were seen in the plasma of normal healthy controls at 5-min post administration of albumin compared with 4 h post administration (**P > 0.05**). Results for normal healthy controls are shown in Figure 2.

In the second study, blood samples from three patients with sepsis syndrome (group 2 patients) were collected pre-administration of albumin and at specified time points up to 18 h after administration for the measurement of albumin and total thiol levels (Figure 3). It can be seen clearly that albumin and total thiol levels increase after administration (5 min), whereupon albumin levels show a pronounced and sustained decrease over the next 18 h, but total thiol levels remain elevated for up to 8 h, and only then begin to fall. Although this is a limited study it nevertheless corroborates our initial findings.

Finally, samples from 10 sepsis patients were subjected to ultrafiltration, before and after the administration of albumin (5 min and 4 h), and the low-molecular-mass plasma fraction was analysed for total thiols. Increases in low-molecular-mass thiols were apparent after albumin administration (pre, 10.80 ± 2.91 µM; 5 min post, 17.92 ± 3.08 µM; 4 h post, 18.65 ± 3.56 µM), but overall differences were not significant (**P > 0.05**). Plasma ultrafiltrates from five normal healthy individuals were also analysed for low-molecular-mass thiols but none were measurable in these control samples.

**DISCUSSION**

Albumin in the reduced state contains a single exposed thiol (-SH) group which is easily measurable, and is the major thiol of this type in plasma [2]. The infusion of albumin into patients might therefore be expected to result in an increase in total plasma thiols. This is clearly demonstrated in our results where, immediately after administration, i.e. 5 min, a pronounced and significant (**P < 0.05**) increase in albumin and thiols was seen (Figures 1, 2 and 3).

In normal healthy individuals, albumin is mainly synthesized in the liver at a rate of approximately 12 g per day. There is a total body albumin mass of some 280 g with a metabolic half-life of about 20 days. Catabolism of albumin occurs in the tissues with a miniscule loss through the gut and kidneys [16]. In critically ill patients the balance between synthesis, catabolism and loss no
longer holds. For example, there may be haemorrhagic and exudative losses, and often markedly increased renal and gut losses. However, the predominant mechanism by which serum albumin falls is secondary to increased capillary permeability and redistribution from plasma to interstitium [17]. Decreased hepatic albumin synthesis during the acute phase response may accentuate and prolong the duration of this fall. Plasma albumin levels in critically ill patients are therefore almost always markedly lower than those seen in normal healthy individuals [3].

Albumin is widely administered to critically ill patients with sepsis syndrome for a number of clinical reasons; however, many of these now appear to be increasingly contentious. Increasing the plasma albumin content results in an increase in the transcapillary colloid osmotic pressure gradient, leading to increased fluid resorption from the interstitium to the intravascular compartment. This benefit is only transitory, however, as the administered albumin can leak into the interstitium, potentially negating the beneficial osmotic effects. Indeed, results obtained in this study demonstrated that 33% (16% of absolute levels) of the rise in plasma albumin levels achieved 5 min after its administration had been lost at 4 h (Figure 1). Interestingly, although albumin levels decrease, thiol levels remain elevated and unaltered in these patients at 4 h (Figure 1).

Albumin is a relatively small protein and its increased leakage from the circulation of sepsis patients has been repeatedly demonstrated [16,17]. However, the novel observation that a decrease in plasma total thiols does not accompany the loss of albumin, suggests that a thiol exchange has occurred in plasma. The most likely explanation is that exchange occurs with proteins less susceptible to leakage, and with low-molecular-mass thiols such as cystine and oxidized glutathione. This observation is not seen in normal healthy controls (Figure 2), probably because of the low transcapillary escape of albumin in this group.

We also expressed plasma thiol levels relative to levels of albumin and total protein. When expressed as a factor of albumin, thiol patterns showed pronounced differences between sepsis patients and controls. In sepsis patients pre-administration of albumin, thiol levels were elevated (12.02 ± 0.95 nM/mg albumin) compared with controls (9.02 ± 0.59 nM/mg albumin). After albumin infusion (5 min), thiol levels appeared to drop significantly in sepsis patients (8.80 ± 0.58 nM/mg of albumin, P < 0.05) compared with pre-infusion levels, whereas in controls the levels appeared to rise slightly (9.10 ± 0.38 nM/mg albumin, P < 0.05). After 4 h thiol levels showed some recovery in sepsis patients (10.54 ± 0.68 nM/mg albumin, P 0.05), but levels in controls increased further compared with pre- and 5-min post-infusion levels (9.58 ± nM/mg albumin, P > 0.05). When thiol levels were expressed relative to total plasma proteins similar patterns were observed in sepsis patients and controls, as thiol levels increased significantly at 5 min and 4 h post infusion in both groups (results not shown). The most likely explanation for these results relates to the contribution of albumin to the total plasma thiol pool in these critically ill patients. Under normal circumstances albumin is responsible for 80% of measurable plasma thiols; however, this protein is often down-regulated in critical illness and other thiol-containing proteins may be up-regulated, and levels of low-molecular-mass thiols may also increase [18]. Under such circumstances correcting thiols to albumin levels may lead to an overestimation of albumin-related thiols as appears to be the case here. However, when corrected to total plasma proteins the expected pattern can be seen; therefore we used uncorrected thiol levels for the purposes of this study.

Longer-term changes in plasma albumin and total plasma thiol levels were observed in three patients with sepsis. These patients were given only one dose of albumin over an 18-h period (Figure 3). Trends consistent with a steady and sustained decrease in albumin levels were seen in these patients. Total thiols, however, remained elevated for up to 12 h. For technical reasons we were only able to use three patients for this study; we have therefore not analysed the results statistically. Our results, however, strongly suggest an increase in plasma total thiols associated with albumin administration to patients with sepsis syndrome that is sustained long term compared with the plasma albumin levels, indicative of an albumin-mediated thiol exchange in the plasma of these patients.

When plasma total thiols and albumin levels in patients with sepsis syndrome are monitored for up to 4 h after the administration of albumin, there is a significant positive correlation (P < 0.05) between them, although the r² value of 0.51 is somewhat lower than expected (Figure 4). It is likely that the pharmaceutical albumin

![Figure 4](image)

**Figure 4** Correlation between albumin and thiol levels in plasma from patients with sepsis syndrome 4 h after albumin infusion

r² = 0.51, P < 0.0001.
product used was already partially oxidized since it is known that commercial batches of albumin have variable levels of reduced thiol groups [19]. This may in part explain the lack of correlation observed. However, the albumin used in this study was from the same manufacturer and had a comparable thiol content with a mean value of 1245.3 ± 27.34 µM which for a 200-ml infusion is equivalent to 250 µM. Albumin accounts for most of the total plasma thiol content (80%) in normal healthy individuals; the other 20% is associated mainly with proteins such as γ-globulins [20], caeruloplasmin [21] and fibrinogen [22]. Low-molecular-mass thiols are not major contributors to the extracellular thiol pool [23]: 99% of glutathione in whole blood is located within erythrocytes, whereas 97% of cyst(e)ine is located extracellularly although this is mainly bound (60%) [24]. In pathological states this situation may be altered as it has been shown that cells can release glutathione under oxidizing conditions [18]. Our results showed some evidence of a replenishment of this low-molecular-mass thiol pool after albumin administration although the observed changes did not reach significance. It is interesting that before albumin administration there are measurable low-molecular-mass thiols in plasma from patients with sepsis but none in control. We cannot explain this difference but it may be related to release of cellular glutathione under the oxidizing conditions associated with pathological states [18]. Previous studies in critically ill patients have shown increased evidence of oxidative damage to plasma lipids and proteins including loss of protein thiol groups [15,25,26]. The increased microvascular permeability associated with sepsis, together with impaired lymphatic return of protein from the tissues, results in a large proportion of the intravascular albumin pool escaping into the interstitium. Albumin administered to such patients may, as suggested here, help replenish oxidized thiol groups on such proteins, thereby performing a thiol donor antioxidant function. Previous studies have shown that immunoglobulins contain labile disulphide groups which can be readily reduced to free thiols [20]. It is therefore possible that administered albumin may be reducing these labile groups. An alternative explanation is that thiol levels may remain elevated as albumin levels fall because oxidatively modified albumin preferentially leaks out of the circulation, although there is no evidence to support this at present. Further studies are now required so that the thiol-containing molecules in plasma may be identified and any clinical benefits associated with their reduction due to albumin infusion assessed.

In conclusion, administration of albumin to patients with sepsis results in an increase in plasma thiol levels, which is sustained even when plasma albumin levels fall. Thiols have several important antioxidant functions both directly, and for molecules such as glutathione as cofactors for enzymes [27]. Since oxidative stress is a feature in the pathogenesis of sepsis syndrome [28–30], an elevation in plasma thiols may have beneficial antioxidant effects. This antioxidant effect may turn out to be one of the few truly beneficial effects of albumin administration in patients with sepsis syndrome.

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