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A Pilot Study on Continuous Infusion of 4% Albumin in Critically Ill Patients: Impact on Nosocomial Infection via a Reduction Mechanism for Oxidized Substrates

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Drs. Hellé and Strub disclosed government work. Dr. Corti disclosed that he is a co-inventor of the vasostatin-I assay (patent application). The remaining authors have disclosed that they do not have any potential conflicts of interest.

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Objective: Care-related infections affect up to 11% of ICU patients. Running therapeutic albumin is sometimes associated to less infection: whether a specific method of its infusion is of any interest to modulate innate defense is unknown. Our objectives were: 1) to test whether the method for albumin infusion is important to prevent care-related infections and 2) to analyze in vitro the antioxidative role of albumin on host defense proteins during shock (using vasostatin-I as an example).

Design: In a prospective, randomized, open-label trial, shock patients were allocated to receive either continuously 4% albumin or intermittently 20% albumin, as long as they were infused with norepinephrine. A translational study including in vivo and in vitro analyses of albumin-vasostatin-I interactions is reported.

Setting: A tertiary ICU caring for 1,000 patients per year.

Patients: Fifty shock patients with serum albumin less than 20 g/L.

Interventions: In vivo colonization and nosocomial infections were recorded and time-dependent changes in serum albumin, chromogranin A, and vasostatin-I concentrations as well. In vitro, we studied biochemical albumin-vasostatin-I relationship using biochemical methods.

Measurements and Main Results: Over 18 days, we recorded a decrease in colonization (four vs 12 episodes; $p = 0.035$) and nosocomial infection frequency (two vs 13 episodes; $p = 0.002$) in patients infused continuously 4% albumin versus controls. In vitro, albumin interacts with the disulfide loop vasostatin-I (residues 17–40) and continuous 4% albumin infusion restores its oxidative status required for antimicrobial activity.

Conclusions: Continuous 4% albumin is effective in reducing care-related infections in shock patients by increasing the availability of antimicrobial vasostatin-I. This might guide future care of shock patients.

Key Words: antimicrobial peptides; chromogranin A; critical care; nosocomial infections; protein-protein interaction; therapeutic albumin

Care-related infections still affect near 5% of hospitalized people and up to 11% in ICUs (1). They are responsible for increased morbidity, costs, and also mortality. For this reason, the World Health Organization expressed recently the wish that new strategies of treatment emerge to lessen the millions of deaths (2). At the acute phase of a life-threatening disease, as a consequence of the stress, molecular and cellular constituents undergo oxidative stress (3) that induces oxidative damage both in tissues and in the circulation where proteins become prone to aggregation in relation with the opening of disulfide bridges by oxidation. Thus, oxidation contributes to severity of the initial injury and increases the risk of care-related infections by modifying the functional status of endogenous proteins belonging to defense (4).

Human serum albumin (HSA) carries many biologic properties, including oncotic power, scavenging, anti-inflammatory, and also antioxidant activities (5). Critically ill patients with shock frequently demonstrate a rapid decrease of the concentration of HSA that is negatively related to outcome (6). To correct hypoalbuminemia, therapeutic albumin (ThAlb) is infused, but clinical studies differ from each other as far as the protocol of ThAlb infusion is concerned. In addition, it has been demonstrated that infusion of 4%-ThAlb, but not 20%-ThAlb, provided a protective effect both on endotoxemic mice survival and on endothelial dysfunction during septic challenges (7).

The use of the potential antioxidant properties of albumin in trials involving critically ill patients has never been addressed to restore the native function of circulatory proteins and peptides. We set the hypothesis that infusion of nonoxidized ThAlb would prevent nosocomial infections in ICU patients and improve in vitro its antioxidative activity on antimicrobial peptides. In the present pilot study, we, therefore, tested in a random open-labeled study two protocols of ThAlb infusion (4%-ThAlb continuously vs the standard method with 20%-ThAlb intermittently) to check whether one of these two treatments would be better at limiting the occurrence of colonization and nosocomial infections in ICU patients with shock. We have completed the in vivo study with a biochemical analysis of the interaction of active albumin with vasostatin-I (VS-I), a chromogranin A–derived peptide released in circulation of critically patients during shock (8) and for which the antimicrobial activity is related to its nonoxidative state (9).

MATERIALS AND METHODS

Setting

This study was performed in a tertiary 30-bed ICU caring for an average of 1,000 patients per year.

Subjects Setting

This study was performed in a tertiary 30-bed ICU caring for an average of 1,000 patients per year.

Subjects

The study design is available at Clinical trials NCT02755155. The protocol was approved by our institutional ethics committee and by our National Drug Safety Agency (no: 160451A-61). Informed written consent was obtained from all patients. Fifty consecutive patients 18 years old or older, who had been admitted between January and June 2017, were assessed for eligibility for this prospective study. Eligible patients were those whom the treating physician judged to require norepinephrine infusion together with saline administration to maintain a target mean arterial pressure of 65 mm Hg (or 75 mm Hg if previous hypertension was part of the medical history) and who were displaying HSA concentration less than or equal to 20 g/L.

Design

As indicated in **Figure 1**, eligible patients were randomly assigned (n = 24 and n = 26) to receive 4%-ThAlb continuously and 20%-ThAlb (Vialebex; LFB BIOMEDICAMENTS, Les Ulis, France) intermittently, respectively. The design of the study corresponds to: 1) the collection of data at inclusion and the outcome assessment; 2) the characteristics of treatments by ThAlb; 3) the evaluation of HSA, chromogranin A, and VS-I concentration; 4) the analysis of episodes of colonization and nosocomial infections; 5) the identification of the VS-I–derived fragment interacting with HSA/bovine serum albumin (BSA)/ThAlb in vitro; and 6) the characterization in vitro of the antioxidative properties of continuous 4%-ThAlb infusion to reverse the antimicrobial form of VS-I.

Clinical Data and Outcome Measures

Methods used for the analyses of comorbidities/outcome and the data collected at admission/inclusion are provided in the **supplemental data** (Supplemental Digital Content 1, http://links.lww.com/CCX/A84). Blood samples for ex vivo and in vitro tests were collected, and plasma was separated as previously reported (10).

Colonization was defined as the presence of bacteria or fungi in an expectedly sterile focus, in the absence of a simultaneous increase in *C*-reactive protein and fever. Care-related infections were defined as episodes with clinical signs of focal infection, with fever or hypothermia, increased *C*-reactive protein, and the presence of a microbe at the focus of infection.

Statistical Analysis

Clinical data are presented as median (quartile 1, quartile 3). Differences between medians of groups were examined by either the Mann-Whitney *U* test or the nonparametric analysis of variance (also called "Kruskal-Wallis test"). Differences between proportions were tested using either Fisher exact probability test or the chi-square test. In all cases, p values of less than 0.05 were considered significant.

Analysis of the Interaction Between HSA/BSA and VS-I

BSA and HSA were purchased from Euromedex (Souffelweyersheim, France) and ThAlb as Vialebex from the LFB BIOMEDICAMENTS.

Natural VS-I and recombinant human VS-I (rVS-I) were prepared as previously reported (9, 11). Several synthetic peptides (VS-$I_{4–16}$, VS-$I_{47–66}$, VS-$I_{7–57}$, and VS-$I_{65–76}$) were prepared on an Applied Biosystems 433A peptide synthesizer (Illkirch, France), using the stepwise solid-phase approach with 9-fluorenylmethoxycarbonyl chemistry (12). Two other peptides VS-$I_{13–40}$ and VS-$I_{61–76}$ were prepared from Proteogenix (Schiltigheim, France).

The methods used for the analysis of the interaction between HSA/BSA and several VS-I–derived peptides employing surface

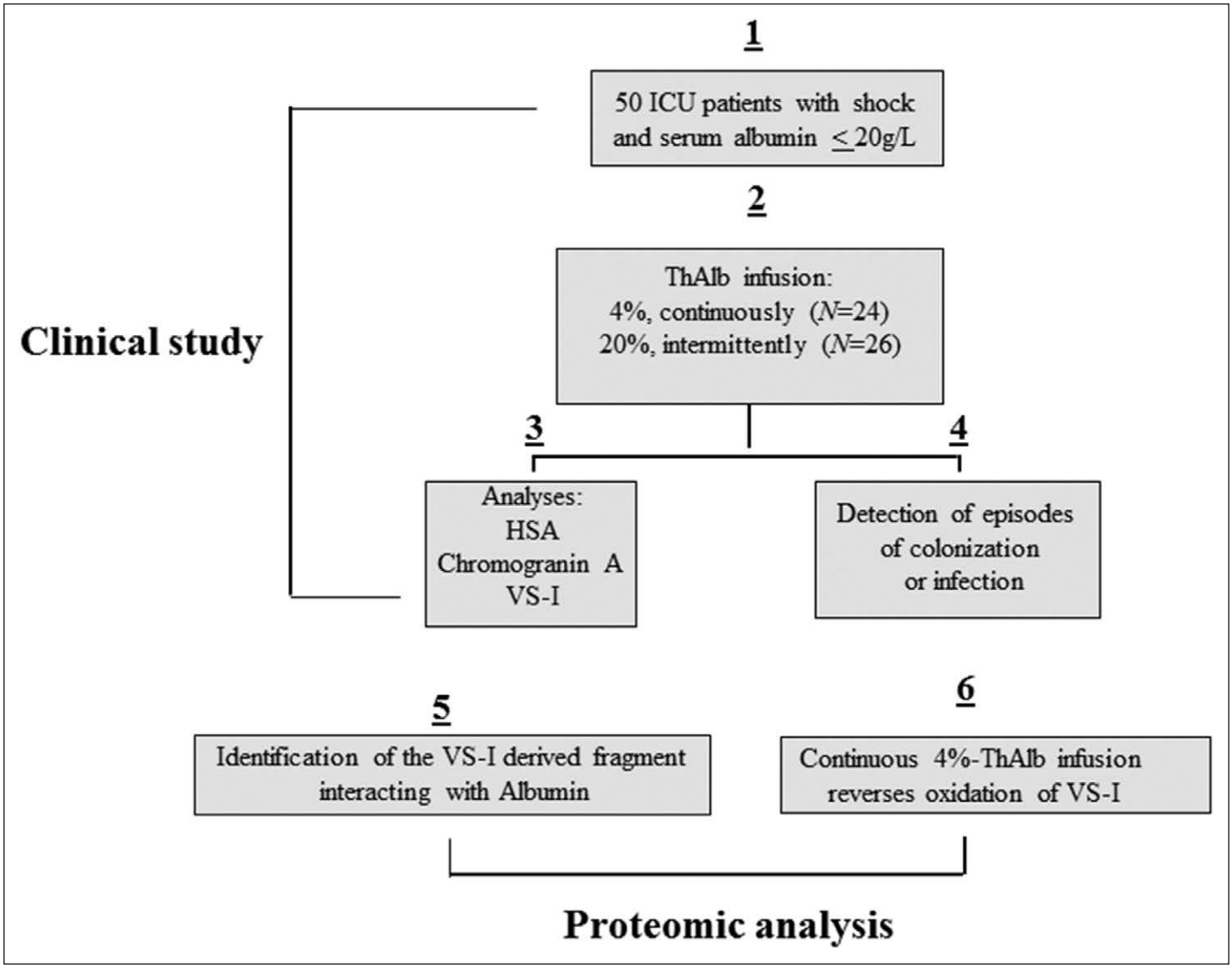

Figure 1. Design of the experimental strategy. HSA = human serum albumin, ThAlb = therapeutic albumin, VS-I = vasostatin-I.

pasmon resonance (SPR) are presented in the supplemental data (Supplemental Digital Content 1, http://links.lww.com/CCX/A84).

Antioxidant Properties of ThAlb

rVS-I (0.8 mg/mL) was oxidized as previously reported (9). Oxidized rVS-I (10 µL) was treated with 2.5 µL of different concentrations of ThAlb (0.02 mg/mL, 0.0004%; 0.2 mg/mL, 0.004%; 2 mg/mL, 0.04%; 20 mg/mL, 0.4%; 200 mg/mL, 4%) during 30 minutes at room temperature. Then, in order to show that ThAlb displays antioxidant property, the mixtures were analyzed by reverse-phase HPLC (RP-HPLC) and mass spectrometry as indicated in the supplemental data (Supplemental Digital Content 1, http://links.lww.com/CCX/A84).

RESULTS

The design of this pilot study is shown in Figure 1. Critically ill patients (n = 50) demonstrating acute circulatory failure requiring norepinephrine infusion to maintain circulation and with HSA concentration less than or equal to 20 g/L were enrolled.

In Vivo ThAlb Impacts on Both Colonization and Care-Related Infection Occurrences

Random assignments for a study treatment were made with n equals to 24 to the 4%-ThAlb treatment and n equals to 26 to the 20%-ThAlb treatment (Fig. 1). On ICU admission, study inclusion and at H48, critically ill patients can be considered as displaying similar characteristics ($p > 0.05$) with the exception of lactate at inclusion ($p = 0.04$) (**Table 1**). Most patients were admitted for septic shock (for 4%-ThAlb group, n = 20 [83%]; and for 20%-ThAlb group, n = 17 [65%]) (**Table 2**). Among non–septic shock patients, the origin of shock was as follows: acute pancreatitis (n = 3), acute-on-chronic liver failure (n = 3), and miscellaneous (n = 7). At the completion of the study, information on vital status 28 days after randomization was available for all patients (Table 2). Mortality was similar within groups ($p > 0.05$): within 28 days after

TABLE 1. Biologic Data of Interest at Inclusion and at 48 Hours After Inclusion

Biologic Data	4%-ThAlb ($n = 24$)	20%-ThAlb ($n = 26$)	p
Status at study inclusion, medians (quartile 1, quartile 3)			
Sequential Organ Failure Assessment	12 (8, 12.75)	10 (8.75, 13)	0.82
Albumin (g/L)	17 (14.25, 18)	19.5 (16.25, 19.75)	0.99
Chromogranin A (ng/mL)	167.9 (70.9, 235.1)	151.8 (83.6, 373.2)	0.34
Chromogranin A/albumin ratio	10.9 (3.78, 14.5)	9.17 (5.47, 20.6)	0.37
Vasostatin-I (ng/mL)	3.03 (1.73, 6.04)	2.52 (1.17, 6.54)	1.00
Vasostatin-I/albumin ratio (10^{-6})	0.16 (0.1, 0.41)	0.19 (0.08, 0.44)	0.94
Vasostatin-I/chromogranin A ratio	0.024 (0.01, 0.04)	0.016 (0.01, 0.04)	0.41
Lactate (mmol/L)	2.95 (1.97, 5.89)	1.85 (1.67, 2.7)	0.04
Status at 48 hr after inclusion, medians (quartile 1, quartile 3)			
Albumin (g/L)	23 (19, 25.25)[a]	25 (20.75, 28)[a]	0.001
Chromogranin A (ng/mL)	123.1 (65.6, 252.2)[b]	208.5 (90, 412.3)[b]	0.22
Chromogranin A/albumin ratio (10^{-6})	6.21 (3.44, 10.8)[b]	6.95 (3.42, 16.7)[b]	0.46
Vasostatin-I (ng/mL)	1.71 (1.01, 4.23)[b]	2.34 (1.44, 3.43)[b]	0.81
Vasostatin-I/albumin ratio (10^{-6})	0.09 (0.05, 0.19)[c]	0.09 (0.05, 0.12)[c]	0.66
Vasostatin-I/chromogranin A ratio	0.018 (0.01, 0.05)[b]	0.01 (0.01, 0.02)[b]	0.18

ThAlb = therapeutic albumin.

[a]$p < 0.001$.

[b]Nonsignificant.

[c]$p = 0.01$.

n: number of patients; data are medians (quartile 1, quartile 3); *p* value is calculated between median values with the Mann-Whitney *U* test according to the two arms of ThAlb (4% vs 20%). Statistical comparisons within arms of treatment (between inclusion and 48 hr after inclusion) are performed with analysis of variance for nonparametric distribution and in a second time with the Mann-Whitney *U* test.

randomization, nine patients (38%) in the 4%-ThAlb group and six patients (23%) in the 20%-ThAlb group had died ($p = 0.36$). There was no difference in either ICU length of stay (13.5 d [7.45–26 d] vs 17.4 d [13.9–45.2 d]) or hospital length of stay (28.8 d [13.1–41.8 d] vs 39.1 d [16.3–46.8 d]) between 4%-ThAlb group and 20%-ThAlb group, respectively (Table 2). ThAlb administration was well tolerated without detectable side effects in either group of treatment. The median HSA concentration was similar in both groups on 96 hr after inclusion (H96) (**Fig. 2**); from baseline to 48 hr after inclusion (H48) of ThAlb infusion, HSA concentrations were significantly lower in the 4%-ThAlb treatment group ($p < 0.001$) (Table 1 and Fig. 2). On inclusion, patients displayed plasma concentrations of both chromogranin A and VS-I in the range of those previously reported at admission in an ICU among nonselected patients (8, 10). Over H48, the impact of ThAlb concentration (4% or 20%) on the plasma concentrations of VS-I was not different in either group (**Supplemental Fig. 1**, Supplemental Digital Content 2, http://links.lww.com/CCX/A85; **legend**, Supplemental Digital Content 9, http://links.lww.com/CCX/A92), but at H48, the Mann-Whitney *U* test applied to the decrease in the medians of the ratio VS-I/HSA (10^{-6}) indicated a significant decrease in both groups when compared with inclusion values ($p < 0.001$) (Table 1).

Over H96 (Fig. 2), equivalent HSA concentrations were achieved in both arms of treatment, although this corresponded to significantly higher volume of ThAlb infusion in the 4%-ThAlb group ($p < 0.001$; **Supplemental Table 1**, Supplemental Digital Content 3, http://links.lww.com/CCX/A86), but to similar amounts of ThAlb. The only difference between the groups was the rate of ThAlb infusion (continuous in the 4%-ThAlb group and intermittent in the 20%-ThAlb group). Of note is also the fact that the infusion of ThAlb was without significant effect on norepinephrine treatment in both groups (Supplemental Table 1, Supplemental Digital Content 3, http://links.lww.com/CCX/A86).

Finally, over hospital stay, we noticed a significant decrease in colonization (four vs 12 episodes; $p = 0.035$) (**Fig. 3**) and nosocomial infection frequency (two vs 13 episodes; $p = 0.002$) in patients infused with continuous 4%-ThAlb when compared with those receiving intermittent 20%-ThAlb (Fig. 3). The foci of infections, the time dependence from inclusion, and the species of microbes involved are depicted in **Supplemental Table 2** (Supplemental Digital Content 4, http://links.lww.com/CCX/A87). We noted that there was a trend toward a longer length of time from first ThAlb infusion to colonization outbreak in the 4%-ThAlb arm of treatment compared with 20%-ThAlb. The median values are 9.25 versus 5.83 days ($p = 0.11$; Supplemental Table 2, Supplemental Digital Content 4, http://links.lww.com/CCX/A87).

In order to understand the role of ThAlb infusion in the protection against infections, we decided to investigate if albumin (BSA, HSA, or ThAlb) is able to interact with VS-I to protect its antimicrobial activity.

TABLE 2. Characteristics of the Patients at ICU Admission, Comorbidities, and Outcome

Characteristics of the Patients	4%-ThAlb (*n* = 24)	20%-ThAlb (*n* = 26)	*p*
Age, yr, medians (quartile 1, quartile 3)	69.5 (58.25, 78.25)	68 (57, 74.5)	0.23
Male sex, *n* (%)	15 (63)	18 (69)	0.77
Body mass index, medians (quartile 1, quartile 3)	25.4 (24.1, 31.2)	28 (25.97, 29.95)	0.39
Admission types, *n* (%)			
Medical	16 (67)	19 (73)	0.76
Surgical	8 (33)	7 (27)	0.76
Simplified Acute Physiology Score II, medians (quartile 1, quartile 3)	68 (57, 83)	64 (51.25, 75.5)	0.18
Sequential Organ Failure Assessment, medians (quartile 1, quartile 3)	12 (9, 15)	9 (7, 13)	0.06
Lactate	3.5 (1.9, 5.3)	2.1 (1.8, 3.9)	0.19
Albumin (g/L)	19 (16.75, 24)	20 (17.75, 23)	0.47
Causes of shock, *n* (%)			
Septic shock	20 (83)	17 (65)	0.20
Non–septic shock	4 (17)	9 (35)	0.20
Comorbidities, *n* (%)			
Chronic obstructive lung disease	5 (21)	7 (27)	0.74
Chronic heart disease	10 (42)	12 (46)	0.78
Hypertension	12 (50)	18 (69)	0.25
Diabetes	5 (21)	7 (27)	0.74
Chronic renal failure (clearance < 30 mL/min)	3 (13)	8 (31)	0.18
Hematologic disease (within 1 yr)	4 (17)	6 (23)	0.73
Solid cancer (within 1 yr)	11 (46)	9 (35)	0.57
Ongoing aplasia	3 (13)	2 (8)	0.66
Therapeutic immune suppression	5 (21)	6 (23)	1.00
Ongoing cirrhosis	3 (13)	3 (12)	1.00
Therapeutic immune suppression	5 (21)	6 (23)	1.00
Outcome			
Status at 28 d, *n* (%)			
Dead	9 (38)	6 (23)	0.36
Alive in ICU	12 (50)	18 (69)	0.25
Length of stay in ICU (d), medians (quartile 1, quartile 3)	13.5 (7.45, 26)	17.4 (13.9, 45.2)	0.06
Length of stay in hospital (d), medians (quartile 1, quartile 3)	28.8 (13.1, 41.8)	39.1 (16.3, 46.8)	0.07

ThAlb = therapeutic albumin.
n: number of patients; when data are medians (quartile 1, quartile 3), the *p* value is calculated with the Mann-Whitney *U* test according to the two arms of ThAlb (4% vs 20%).

In Vitro Natural VS-I and rVS-I Develop Biochemical Relationship With HSA, BSA, and ThAlb

Because BSA and HSA have 76% amino acid sequence homology, we investigated the possible interaction of VS-I with these two proteins. SPR binding experiments showed that BSA and HSA interact with bovine VS-I (Fraction E; **Supplemental Fig. 2, *A* and *B***, Supplemental Digital Content 5, http://links.lww.com/CCX/A88; legend, Supplemental Digital Content 9, http://links.lww.com/CCX/A92) and rVS-I (**Supplemental Fig. 2*C***, Supplemental Digital Content 5, http://links.lww.com/CCX/A88; legend, Supplemental Digital Content 9, http://links.lww.com/CCX/A92). To specify the domain of VS-I able to interact with BSA/HSA, we tested by SPR the interaction of several peptides covering all the VS-I sequence (**Supplemental Fig. 3*A***, Supplemental Digital Content 6, http://links.lww.com/CCX/A89; legend, Supplemental Digital Content 9, http://links.lww.com/CCX/A92). No binding was observed with

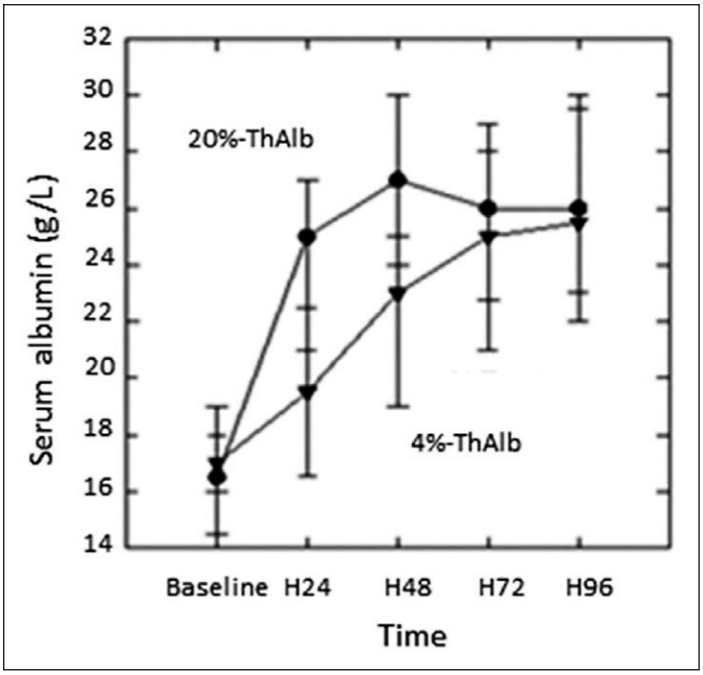

Figure 2. Influence of ThAlb on HSA concentrations in critically ill patients. *Circles* and *triangles* represent medians in patients infused with 20%-ThAlb and 4%-ThAlb, respectively. The lower bounds of the *error bars* equal the first quartiles and the upper bounds the third quartiles. Sample sizes are $n = 26$ (20%-ThAlb) and $n = 22$ (4%-ThAlb; provided that two patients died after inclusion). At 48 hr after inclusion (H48), the Mann-Whitney *U* test shows a significant difference ($p < 0.001$) between the HSA concentrations in both arms. H24 = 24 hr after inclusion, H72 = 72 hr after inclusion, H96 = 96 hr after inclusion, HSA = human serum albumin, ThAlb = therapeutic albumin.

$VS\text{-}I_{4–16}$, $VS\text{-}I_{7–57}$, $VS\text{-}I_{47–66}$, and $VS\text{-}I_{65–76}$ (**Supplemental Fig. 3*B***, Supplemental Digital Content 6, http://links.lww.com/CCX/A89; legend, Supplemental Digital Content 9, http://links.lww.com/CCX/A92). In contrast, binding was observed with disulfide-bridged $VS\text{-}I_{1–64}$ or $VS\text{-}I_{17–40}$ (**Supplemental Fig. 3*C***, Supplemental Digital Content 6, http://links.lww.com/CCX/A89; legend, Supplemental Digital Content 9, http://links.lww.com/CCX/A92) suggesting that the region of VS-I capable of interacting with BSA or HSA is located within residues $VS\text{-}I_{17–40}$ including the disulfide bridge and that this interaction is stable at pH 6 (**Supplemental Fig. 3*D***, Supplemental Digital Content 6, http://links.lww.com/CCX/A89; legend, Supplemental Digital Content 9, http://links.lww.com/CCX/A92). We confirm these data by using Biacore technique, and binding was also observed with rVS-I and $VS\text{-}I_{13–40}$, but not with $VS\text{-}I_{61–76)}$, with an affinity constant (K_a) = 5.8×10^5 M^{-1} (**Supplemental Fig. 3*E***, Supplemental Digital Content 6, http://links.lww.com/CCX/A89; legend, Supplemental Digital Content 9, http://links.lww.com/CCX/A92).

These results point to the fact that the sequence of $VS\text{-}I_{17–40}$ (highly conserved during evolution) is more hydrophobic than the regions $VS\text{-}I_{1–16}$ and $VS\text{-}I_{41–76}$, with 33.3% of hydrophobic residues versus 25.0% and 26.3%, respectively (Supplemental Fig. 3*A*, Supplemental Digital Content 6, http://links.lww.com/CCX/A89; legend, Supplemental Digital Content 9, http://links.lww.com/CCX/A92).

Antioxidative Properties of ThAlb Impact on the Oxidation of VS-I

Under oxidative stress conditions occurring during the acute phase of shock, endogenous HSA and VS-I might undergo structural modifications (9, 14). Thus, we previously demonstrated that oxidation of VS-I prevents its antimicrobial activity (9), suggesting that such structural modifications were an obstacle to microbe lysis. We have, therefore, hypothesized that ThAlb with its antioxidative properties may restore the antimicrobial activity of VS-I.

To address this hypothesis, we oxidized rVS-I according to the procedure previously reported (9). As a control, rVS-I, its oxidized form ([O]rVS-I), and ThAlb were purified by RP-HPLC (Supplemental Fig. 4*A*, Supplemental Digital Content

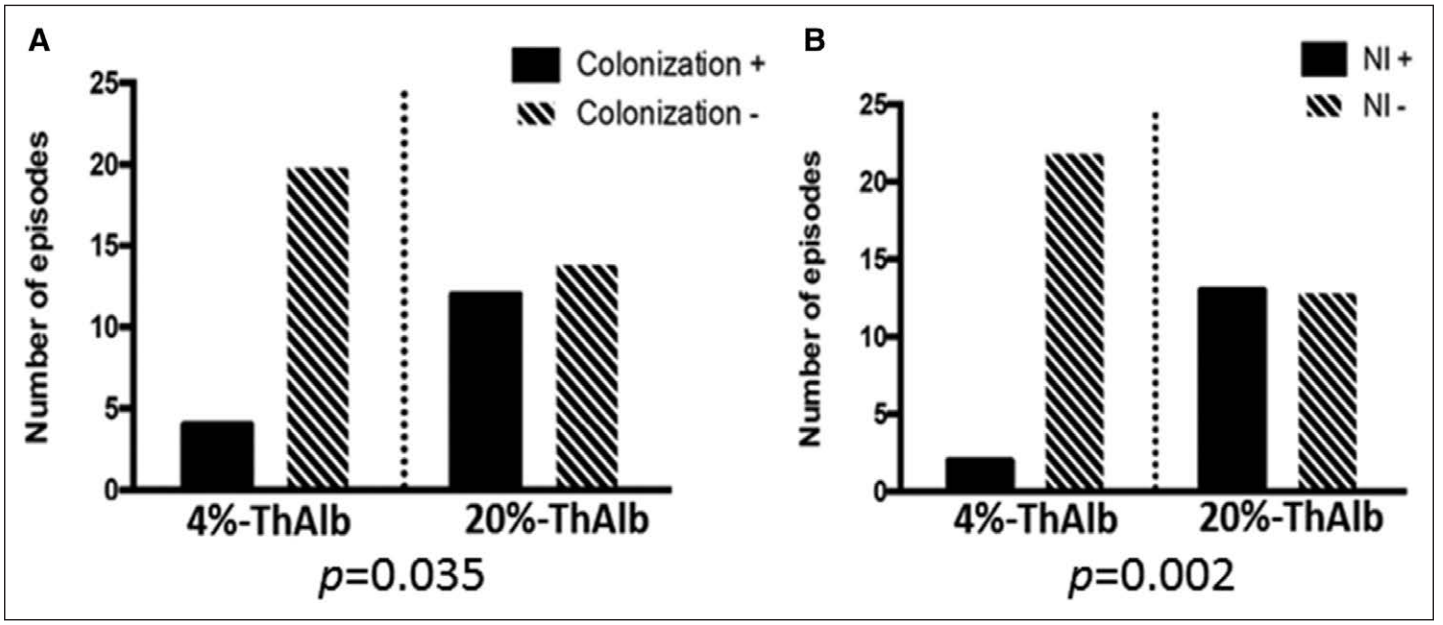

Figure 3. Colonization and nosocomial infection in critically ill patients according to the arm of ThAlb treatment (4%-ThAlb and 20%-ThAlb). Frequency of ThAlb treatment on (**A**) colonization and (**B**) nosocomial infection (NI+) or (NI–). ThAlb = therapeutic albumin.

7, http://links.lww.com/CCX/A90; legend, Supplemental Digital Content 9, http://links.lww.com/CCX/A92). As shown on chromatogram control, the oxidized form [O]rVS-I is eluted faster (36.1 min) than the nonoxidized form (rVS-I) (37.6 min) and ThAlb is eluted at 38.6 minutes (**Supplemental Fig. 4**, Supplemental Digital Content 7, http://links.lww.com/CCX/A90; legend, Supplemental Digital Content 9, http://links.lww.com/CCX/A92). The expected molecular mass of rVS-I (with the disulfide bridge) and [O]rVS-I (oxidation of C_{17}, C_{38}, and three methionine sulfone M_7, M_{15}, and M_{32}) was evaluated at 9,069.5 Da and 9,263.5 Da (Supplemental Fig. 4*C*, Supplemental Digital Content 7, http://links.lww.com/CCX/A90; legend, Supplemental Digital Content 9, http://links.lww.com/CCX/A92). However, after oxidation, we obtain a value of 9,365.3 Da corresponding to the presence of 18 oxidations (Supplemental Fig. 4*C*, Supplemental Digital Content 7, http://links.lww.com/CCX/A90; legend, Supplemental Digital Content 9, http://links.lww.com/CCX/A92). Then, we treated [O]rVS-I (0.8 mg/mL) with different concentrations of ThAlb (0.0004–4%) for 30 minutes at room temperature and analyzed the mixture by RP-HPLC (Supplemental Fig. 4*B*, Supplemental Digital Content 7, http://links.lww.com/CCX/A90; legend, Supplemental Digital Content 9, http://links.lww.com/CCX/A92). Only for the 4%-ThAlb solution treatment, the profile of chromatography indicates a decrease by 53.8% of the complete [O]rVS-I form (fraction 1), showing that ThAlb partially reverses the oxidation of rVS-I to produce forms included in fractions 2 and 3 (Supplemental Fig. 4, B and C, Supplemental Digital Content 7, http://links.lww.com/CCX/A90; and **Supplemental Fig. 5**, Supplemental Digital Content 8, http://links.lww.com/CCX/A91; legend, Supplemental Digital Content 9, http://links.lww.com/CCX/A92). Interestingly, a form with only two oxidations was detected (Supplemental Fig. 4*C*, Supplemental Digital Content 7, http://links.lww.com/CCX/A90; and Supplemental Fig. 5, Supplemental Digital Content 8, http://links.lww.com/CCX/A91; legend, Supplemental Digital Content 9, http://links.lww.com/CCX/A92).

DISCUSSION

Conflicting results on outcome have been observed in meta-analyses of ThAlb use (15), and to our knowledge, no studies report for critical ill patients the impact of ThAlb infusion rate on the activity of antimicrobial peptides, an efficient system used to fight microbes (16).

In critically patients with shock, different types of oxidative processes occur and may hamper the activity of antimicrobial peptides (17). The antioxidant activity of different ThAlb preparations was reported, and Vialebex turned out to possess the highest property (18).

In the present study, we report that the use of continuous infusion of 4%-ThAlb in nonselected critically ill patients with shock and acute hypoalbuminemia results in a significant decrease of the frequency of both colonization and care-related infections, when compared with the standard intermittent 20%-ThAlb treatment (even though circulating concentrations of serum albumin reach similar levels). We also found that in vitro BSA, HSA, and ThAlb are able to interact with the hydrophobic domain VS-$I_{17–40}$ that includes the disulfide bridge C_{17}–C_{38} (Fig. 3; Supplemental Fig. 4, Supplemental Digital Content 7, http://links.lww.com/CCX/A90; legend, Supplemental Digital Content 9, http://links.lww.com/CCX/A92). We also show that in vitro the interaction ThAlb-[O]rVS-I reverses oxidation of rVS-I (**Supplemental Fig. 4,** Supplemental Digital Content 7, http://links.lww.com/CCX/A90; and Supplemental Fig. 5, Supplemental Digital Content 8, http://links.lww.com/CCX/A91; legend, Supplemental Digital Content 9, http://links.lww.com/CCX/A92), which is mandatory for its optimal antimicrobial activity (9).

ThAlb may rapidly be oxidized by the shock-responsive chemical processes, rendering it unable to interplay properly during the early stage of the stressing disease. Thus, when injecting ThAlb intermittently, after a time exceeding the time-scale required for oxidizing ThAlb following each injection, only the remaining nonoxidized HSA is left active to restore antimicrobial VS-I after oxidation. On the other hand, when a low concentration of fresh ThAlb is infused continuously, there always remains nonoxidized ThAlb able to protect VS-I for oxidation and keeping the antimicrobial activity. Our data on care-related infection are also in agreement with the fact that less colonization in our patients may be related with lesser pneumonia frequency in experimental models (19, 20) with colonization by yeasts, which are sensitive to VS-I (9).

Finally, our data are in line with a higher protection by continuous 4%-ThAlb infusion—than by intermittent 20%-ThAlb infusion—which had been suggested in mice (21) and more recently in chronically decompensated cirrhosis (15).

In vitro arguments proving that VS-I interacts with ThAlb are a prerequisite to ascribe a role for ThAlb in somehow enhancing defence in vivo. We prove that BSA and HSA interact with VS-I and rVS-I (Supplemental Fig. 2, Supplemental Digital Content 5, http://links.lww.com/CCX/A88; legend, Supplemental Digital Content 9, http://links.lww.com/CCX/A92) through the fragments including the disulfide bridge (Supplemental Fig. 3, Supplemental Digital Content 6, http://links.lww.com/CCX/A91; legend, Supplemental Digital Content 9, http://links.lww.com/CCX/A92). In addition, SPR experiments show that interaction is maintained when pH decreases at 6 (Fig. 3), which is a possible condition in microcirculation during shock. Furthermore, it is important to point out that during oxidative stress, VS-I without its disulfide bridge is not able to display antimicrobial activity (9). Cationic antimicrobial peptides bind with their hydrophobic parts to drug site II of HSA (22). The domain VS-$I_{17–40}$ possesses a yield of hydrophobic residues 33.3% higher than that of domain VS-$I_{1–16}$ and VS-$I_{41–76}$ with 25.0 and 26.3, respectively. It is also important to point out that the domain VS-$I_{17–40}$ includes the disulfide bridge (C_{17}–C_{38}) and three main residues sensitive to oxidation (C_{17}, M_{32}, and C_{38}) (Supplemental Fig. 3, Supplemental Digital Content 6, http://links.lww.com/CCX/A89; legend, Supplemental Digital Content 9, http://links.lww.com/CCX/A92). This point suggests the importance of the antioxidative property of ThAlb to maintain the structure of the domain VS-$I_{17–40}$ and the antimicrobial activity of VS-I.

The main limitation of our study is the small number of subjects (which is nevertheless taken into account by statistical analyses), and our data need replicating in future clinical multicenter studies.

In conclusion, these data provide support for pharmacologic interventions to restore physiologic activities using a continuous infusion of 4%-ThAlb and suggest that during systemic inflammatory response syndrome, other physiologic peptides may undergo similar functioning inhibition. This could be relevant to limit morbidity in many critically ill patients with similar clinical characteristics.

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