Supplemental Data

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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1. **Design ThAlb infusion**

The treating clinicians determined the amount and rate of 20%-ThAlb to increase as quickly as possible the circulating concentration of HSA in the range 25 to 30 g/L by infusing ThAlb every 8 h. The 4%-ThAlb was infused continuously at a rate of 12.5 mL/kg/24 h until norepinephrine infusion was stopped. All other treatments were prescribed at the discretion of the treating clinicians, as were all other aspects of care.

1. **Clinical data and Outcome Measures**

Data collected at admission included age, sex, Body Mass Index (BMI), SAPS II, SOFA score, comorbidities, motive for referral, HSA, and lactate. At inclusion, HSA concentration, length of time from norepinephrine infusion commencement to first ThAlb infusion and SOFA score were additionally recorded. From inclusion, we recorded on a daily basis: HSA over 5 days, Chromogranin A and VS-I over 48 h, time from first ThAlb infusion to peak norepinephrine infusion, time from first ThAlb infusion to peak minus 30% hourly output and full time from first ThAlb infusion to norepinephrine stop. We also measured time from first to last ThAlb infusion and the total amount of ThAlb/patient. Blood samples for the assessment of HSA, Plasma Chromogranin A and VS-I concentrations were measured by ELISA(10, 8 in the main text). We calculated the ratio of VS-I/Alb adjusting for a possible hemodilution related to the high volume of saline infusion at the acute phase of the disease (severe systemic inflammation).

Death from any cause within randomization was recorded. Every 3 days from inclusion and at each occurrence of fever >38°C until hospital discharge, we collected blood samples, lower respiratory tract, urines and skin samples to check the presence of microbes (bacteria, fungi) and to detect care-related infection. Each time an in dwelling catheter was removed, it was sent for microbial culture (whether fever was present or not). 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 (1, in the main text). Colonization was defined as the presence of bacteria or fungus in an expectedly sterile focus, in the absence of a simultaneous increase in C-reactive protein and fever.

1. **Analysis of the interaction between HSA/BSA and VS-I**

In a first series of experiments the analysis of the interaction between HSA/BSA and several VS-I-derived peptides employing surface SPR was performed using a SPR Navi 200 (Bionavis Ltd, Finland) at a fixed wavelength of 670 nm. After mounting the gold sensor in the SPR cell, phosphate buffer (PBS; pH 7.4) was injected continuously at a rate of 0.5 mL/min at a temperature of 22°C. After equilibrium a uniform layer of polyethylenimine (PEI) was first deposited to make the surface positively charged. Then, a solution of HSA or BSA, was injected at a concentration of 1 mg/mL (1.5 µM) to form a protein monolayer on the biosensor. After rinsing with buffer and reaching equilibrium, 20 µM of different VS-I-derived fragments were injected and their specific interactions with HSA/BSA were studied.

A second series of experiments was performed on a Biacore 2000 instrument (Biacore Inc.) at 20 °C and HSA was immobilized at high surface densities (∼15000 response units) on an activated CM5 chip using standard amine-coupling procedures as described by the manufacturer. To perform binding assays, rVS-I, VS-I13-40 and VS-I61-76 at different concentrations (from 5 to 20 µg in 200 µl) were injected in 10 mM sodium acetate, pH 7.4 at a flow rate of 10 μL/min. Blank surfaces were used for background corrections. To regenerate surfaces between two binding experiments 15 µL of 10 mM glycine, and pH 2.0 were used. We used steady state analysis to estimate the affinity of peptide to HSA. Association constant (*K*a) was estimated using 1:1 Langmuir association model as indicated by the manufacturer.

1. **Antioxidant properties of ThAlb**

Analysis by reverse phase HPLC is obtained with a Dionex Dual Gradient System (Dionex, Sunnyvale USA) and a Vydac 208 TP C8 column (5 µm particle size, 300 Å pore size, 150 mm x 2.1 mm, Grace Davison Discovery Science, Columbia, USA). Solvent system includes 0.1% (v/v) trifluoroacetic acid in water (solvent A) and 0.09% (v/v) trifluoroacetic acid in 70% (v/v) acetonitrile-water (solvent B). Gradient of elution (%B) was reported on the chromatograms. Elution was obtained with a flow rate of 200 µL/min and monitored at 214 nm. After elution, the peaks were manually collected and analyzed by mass spectrometry.

Mass spectrometry measurements were performed in the positive ion mode on an electrospray time-of-flight mass spectrometer (Synapt G2 HDMS mass spectrometer, Waters, Manchester, UK) equipped with an automated chip-based nanoESI source (Triversa Nanomate, Advion). Calibration of the instrument was performed using multiply charged ions of a 2 μM horse heart myoglobin solution. For analysis in denaturing conditions, samples were diluted to 20 μM for infusion in a 1/1 water/acetonitrile mixture (v/v) acidified with 0.5-1% formic acid and interface parameters were fixed to 45 V for accelerating voltage (Vc) and to 2 mbar for backing pressure (bP) in order to obtain the best mass accuracy.