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TITLE PAGE

DELAYED INCREASE OF PLASMA SELENOPROTEINS AND ABSENCE OF SIDE EFFECT INDUCED BY INFUSION OF PHARMACOLOGICAL DOSE OF SODIUM SELENITE IN SEPTIC SHOCK: SECONDARY ANALYSIS OF A MULTICENTER, RANDOMIZED CONTROLLED TRIAL

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Statements

The authors state that informed consent was obtained for this experimentation in septic shock patients. The privacy rights of human subjects have been always observed.

The work described has not been published previously and is not under consideration for publication elsewhere. Its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright holder.

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Health's future policy in this area.

Competing interests

Dr Xavier Forceville created a very small start-up in 2005 (Sérénité-Forceville). In 2015, Pharm. V Cotereau volunteer manager of Sérénité-Forceville, former vice president of the French pharmacist association, filed a new patent for the treatment of sepsis entitled: "Kit for treating sepsis and/or any systemic (SIRS) or damaging cellular hyper-inflammation". Its reference numbers are: PCT number PCT/FR2016/051569, European demand 16742342.5, US demand Attorney Docket Number: 0727-1267.

I am the main shareholder of this company. One of my brothers is also a shareholder.

The other authors (BL, JG, AB, PVA, EP, DA, and EB) declare that they have no competing interests.

ABSTRACT

Background

In sepsis, neutrophil respiratory bursts participate in endothelium damage, the first step to multiple organ failure. In plasma two antioxidant selenoenzymes, which protect the endothelium, decrease: selenoprotein-P, and to a lesser extent glutathione peroxidase (GPX3). Sodium selenite (Na_2SeO_3) is a Se donor, but also an oxidant chemotherapy drug depending on its concentration. In a previous published study, Na_2SeO_3 continuous infusion in septic shock patients at a pharmacological dose of 4 mg Se/day on day-1, followed by a high nutritional dose of 1 mg Se/day during 9 days, showed no beneficial effect on weaning of catecholamine nor on survival. In this ancillary study, we report clinical and biological effects of such continuous infusion of Na_2SeO_3 .

Methods: This was a multicenter, placebo-controlled, double-blind study on 60 patients. Na₂SeO₃ or placebo in continuous infusion as described above. Evolution with time of plasma Se, selenoprotein-P, GPX3, Organ dysfunction (sequential organ failure assessment SOFA scores, including PaO₂/FiO₂, for respiratory failure, and plasma lactate) and quality of life at 6 months (by SF36 scores) were analyzed using two-way (time, treatment) non-parametric repeated-measures analysis of variance (Friedman test).

Main Results: At baseline, plasma Se was about a quarter of reference values. From baseline to day-4 plasma Se, selenoprotein-P and GPX3 significantly increased by 3.9, 2.7 and 1.8 respectively in the Na₂SeO₃ group as compared with placebo and remained elevated by 2.3, 2.7 and 2.1 at day-14 respectively (p<0.001). Na₂SeO₃ did not affect global and organ by organ SOFA Scores and plasma lactate concentration at day-1 and later up to day-14. The evolution of PaO₂/FiO₂ until day-14 was similar in the two groups. Quality of life in the surviving patients at 6 months was similar between the two groups.

Conclusion: Continuous infusion of Na₂SeO₃ at 4 mg Se at day-1 seems to have neither beneficial nor toxic effect at day-1 or later and induces a late increase of selenoprotein-P at day-4. Preclinical studies are required to confirm the use of Na₂SeO₃ as a cytotoxic drug against neutrophils and protection of the endothelium by selenoprotein-P.

Name of the registry: Centre Hospitalier de Meaux

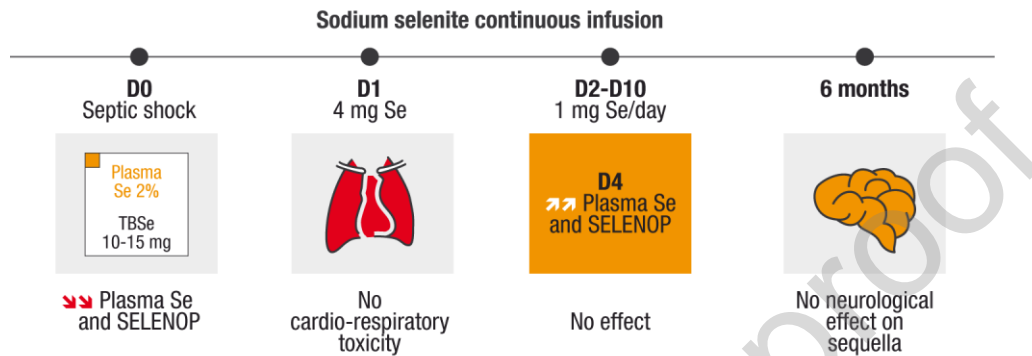
Trial registration: NCT00207844.

Date of registration: 13 September 2005, retrospectively registered

URL of the trial registry record: www.ch-meaux.fr

KEYWORDS (6)

Sepsis; Sodium Selenite; Selenoprotein P; Glutathione peroxidase; Organ failure

GRAPHICAL ABSTRACT**Highlights**

- Continuous infusion of 4 mg selenium (Se) as selenite (Na_2SeO_3) corresponds to about a third of total body Se content (10 to 15 mg) in Se repleted people
- Plasma selenium content represents less than 2% (0.3 mg) of the 10 -15 mg Se body content in Se repleted people.
- No side effect was observed with continuous infusion of sodium selenite (Na_2SeO_3) - at a dose corresponding to 4 mg Se- in septic shock patients during the first day.
- Increase of selenoprotein P was observed at day4, long after endothelium damage.

INTRODUCTION

Septic shock is a major public health issue characterized by multiple organ failure (MOF), high mortality rate in intensive care [1-3], and frequent reduced long-term quality of life [2, 4]. It is the first cause of mortality in hospitals, and one of the main causes of death worldwide, even outside of a pandemic, as with COVID 19, with which severe forms correspond to a sepsis state [5-8]. It remains to date without specific treatment, at the exception of low dose steroids [9, 10]. At the early phase of sepsis, endothelial dysfunction is a major event [2, 11, 12]. This dysfunction is notably related to the neutrophil respiratory bursts [12-16]. The selenium (Se) is required for the vital antioxidant selenoenzymes under

the form of selenocystein aminoacid [17, 18]. With about 0.3 mg Se, plasma Se represents only 2% of body Se content, which is 10-15 mg Se in a Se-repleted person [19, 20]. The antioxidant selenoprotein-P accounts for about 60% of plasma Se, and glutathione peroxidase (GPX3) for about 30%. The rest of the plasma Se is incorporated into proteins under the non-biologically active form selenomethionine [19, 21-24]. The plasma selenoproteins are a critical point in sepsis between activated leukocytes, including neutrophils and endothelium [19, 23]. Like many small selenocompounds, sodium selenite (Na_2SeO_3) - despite being a good Se provider at low concentration - is oxidant and cytotoxic above a micromolar threshold [25]. Na_2SeO_3 has been studied for a long time as a chemotherapeutic agent as the more the cells are activated the lower the cytotoxicity threshold [26-30].

At the acute phase of sepsis, there is an early and major decrease in plasma Se and selenoprotein-P concentrations in mammal models and in patients [24, 31-36]. This has been the basis the hypotheses of: (i) a selenium deficiency at the acute phase of sepsis and (ii) a need to increase selenium supply in sepsis patients. However such hypotheses were not based on preclinical data [35-40]. On the contrary several elements or experiments are against such hypotheses: (i) there is no increased losses of selenium atom during sepsis [41]; (ii) there is an increased protein catabolism at the acute phase of sepsis that should release selenium incorporated into selenomethionine and selenocystein [36]; and (iii) recent experimental data have confirmed a major multi-step down-regulation of selenoprotein-P synthesis and hepatic release at the acute phase of sepsis [36]. This is likely to be the major factor for the decrease of selenoprotein-P plasma concentration together with an increased binding to endothelium at the acute phase of sepsis [36]. In our previous SÉRÉNITÉ study the first day Na_2SeO_3 infusion, corresponding to 4 mg Se, was performed with the goal of reaching the cytotoxicity threshold for hyperactivated neutrophils [41], contrary to most sepsis studies performed on the hypothesis of a Se deficiency, requiring Se supplementation for selenoenzyme synthesis

[37-40]. Our Na₂SeO₃ first day dose was based on preclinical data, reporting this dose as the maximum safe single oral dose for cancer research [42] and administrated by continuous infusion in the absence of preclinical studies allowing a bolus injection. Performed in patients with a 70% expected mortality rate at 6 months, our study should be considered as a fast-track study similar to drug studies performed in advanced-stage cancer patients with unmet medical needs. This first day infusion was followed by 1 mg Se/day as Na₂SeO₃ during 9 days. We observed no effect on the weaning of catecholamine and on survival [43]. Some authors suggested that this first day dose toxicity might have overwhelmed the beneficial effect of Se supplementation by increasing the incidence of MOF and especially respiratory failure due to Na₂SeO₃ toxicity [44, 45].

To further investigate the clinical and biological effects of this Na₂SeO₃ infusion on oxidation and plasma selenoproteins, we performed a secondary analysis of the SÉRÉNITÉ study. Our aim was to assess if this Na₂SeO₃ infusion had an impact on plasma selenoproteins, and especially on selenoprotein-P, and if it had any clinical side effect during the early 24 hours of pharmacological continuous dose infusion [43].

METHODS

The SÉRÉNITÉ trial (high doses of selenium, as sodium selenite, in septic shock, NCT00207844) was a multicenter, placebo-controlled, randomized, double-blind study performed in 60 patients with documented septic shock. Main inclusion criteria were: severe community or nosocomial infection, need for mechanical ventilation, septic shock with at least one hour of 0.2 µg/kg/min norepinephrine after fluid loading of at least 1.000 ml, Simplified Acute Physiologic Score II (SAPS II score) of 25 or more and written informed consent. Patients with limitation of care, previous circulatory failure, or with urinary infection or peritonitis related to dialysis or trauma, and pregnant women were excluded. Modalities of

selenite infusion as well as main efficacy and safety endpoints have been described elsewhere [43].

The sample size was based on the assumption of 60% of patients would be free of catecholamine at day 10 (end of the study treatment) the sample size would have allowed the detection of an absolute increase of 25 % of this percentage of patients with a type I error of 5% and a power of 80%. Analyses were performed according to the intention to treat principle. Time to vasopressor therapy withdrawal was analyzed using cumulative event curves constructed by the Kaplan–Meier method and the effect of treatment using the log-rank test and similarly for mortality. Otherwise, comparisons between groups were performed using the Student t test or Wilcoxon rank sum test as appropriate for continuous variables, and using the chi-square test, the Fisher exact test or the Cochran–Mantel–Haenzel test as appropriate for categorical variables. All reported p are two sided and $p < 0.05$ was considered significant [43].

Patients were randomly assigned in a 1:1 manner to receive either sodium selenite or matching placebo for 10 days. Treatments (Laboratoires Aguettant, Lyon, France) were conditioned in ampoules containing 1 mg selenium as sodium selenite diluted in 48 ml saline and were administered intravenously by continuous infusion (2 ml/hour) at the following doses, expressed in selenium content: 4,000 μg on the first day and 1,000 $\mu\text{g}/\text{day}$ on the nine following days. Randomization was stratified on each centre by blocks of four. All patients, medical and nursing staff, and pharmacists remained blinded throughout the study period [43].

Organ dysfunctions

Organ dysfunctions were assessed through the computation of global and of specific organ Sequential Organ Failure Assessment (SOFA) scores [46, 47]. Lactate concentrations were

also measured. Quality of life at 6 months was evaluated using the French Short Form (36) Health Survey (SF-36) [48].

Supportive care

Cardio-vascular: cardiac pressures and indexes (measured using a Swan-Ganz catheter), fluid loading requirements and occurrence of arrhythmias were recorded.

Pulmonary: partial pressure arterial oxygen and fraction of inspired oxygen ratio ($\text{PaO}_2/\text{FiO}_2$), partial pressure of carbon dioxide (PCO_2), level of positive end-expiratory pressure (PEEP), use of nitric oxide (NO) or of prone position, and duration of mechanical ventilation (MV) were recorded.

Renal: number of patients requiring renal replacement therapy (RRT), duration of RRT and number of RRT free days were recorded.

Nosocomial infections

The occurrence of the following nosocomial infections were recorded: nosocomial pneumonia during mechanical ventilation by using broncho-alveolar lavage and protected specimen brush [49, 50], post-operative wound infection [51], catheter infection using Brun-Buisson technique [52], and bacteremia using blood cultures. The number of infections per patient was also recorded.

Plasma Se and plasma selenoproteins

Plasma Se concentrations were determined using graphite furnace atomic absorption spectrometry (GFAAS) using Unicam 989 QZ Solaar apparatus and the values were expressed as nanogram per milliliter [53]. The method was validated using Seronorm™ lyophilized human reference serum samples (Nycomed Pharma AS, Oslo, Norway, batch No. 605113) and through participation in an inter-laboratory quality assurance program. The mean serum Se level of reference was 78.0 $\mu\text{g/L}$ while that obtained in our laboratory was 77.4 \pm 5.0 $\mu\text{g/L}$.

Plasma selenoprotein-P concentration was determined by sandwich Elisa procedure. Ninety six well microplates Maxisorp (Nunc) were coated overnight with anti-human selenoprotein-P monoclonal antibody (Anti-selenoprotein P 37A1; AbFRONTIER (Korea), cat. no. LF-MA0141) at room temperature, and were further incubated at 37°C with plasma samples and with biotinylated anti-human selenoprotein-P antibody. Color reaction development was achieved with alkaline phosphatase conjugated with streptavidin and p-nitrophenylphosphate (pNPP) as the substrate. The reaction was stopped with 0.1 M Ethylenediaminetetraacetic acid (EDTA) and absorbance measured on a micro-plate reader at wave length $\lambda=405$ nm.

Plasma samples were diluted 1:1000 to 1:2000 with PBS-Tween buffer before analysis. Pooled human plasma was used as standard. Selenoprotein-P concentrations were expressed as arbitrary unit/ml. Plasma selenoprotein-P concentration was calculated using exponential standard curve.

GPX3 activities were assayed by the coupled method of Paglia and Valentine with t-butyl hydroperoxide as the substrate [54]. The reaction was carried out at 25°C in a spectrophotometer fitted with constant-temperature cell housing. The method based on a nicotinamide adenine dinucleotide phosphate (NADPH)-coupled reaction whereby oxidized glutathione produced by GPX3 and exogenous t-butyl hydroperoxide was reduced by exogenous glutathione reductase and NADPH. Enzymatic activities were expressed as units per ml of plasma. One unit of enzyme was defined as 1 μ mol NADPH oxidized per minute per ml of plasma. The intra-assay coefficient of variation for both materials (six to eight analyses) was below 3%.

Biological variables

Plasma lactate and C-reactive protein concentration, aspartate aminotransferase (AST), alanine aminotransferase (ALT) activities were measured using standard biochemical methods.

Inflammation

Circulating plasma interleukin-6 (IL-6) and vascular-endothelial-growth-factor (VEGF) levels were measured by enzyme-linked immunosorbent assays (ELISA) according to manufacturer's recommendations (IL-6 and VEGF DuoSet kit R&D systems, Abingdon, UK). Detection thresholds were 3 pg/ml and 5 pg/ml for IL-6 and VEGF, respectively.

Circulating plasma procalcitonin (PCT) levels were measured using time-resolved amplified cryptate-emission technology (Kryptor® procalcitonin; Brahms AG, Hennigsdorf, Germany) with an assay sensitivity of 0.06 ng/ml.

Lipid peroxidation

Lipid peroxidation was assessed according to the method proposed by Yagi [55], by adding 4.0 mL of sulfuric acid (N/12) and 500 µL of phosphotungstic acid (10%) to 100 µL of plasma. After 5 min, the tubes were centrifuged at 1,600 g for 10 min, and the supernatant discarded. The residue was suspended in sulfuric acid (2.0 mL) and phosphotungstic acid (300 µL) for 10 min. The tubes were centrifuged at 1,600 g for 10 min, and the supernatant discarded again. The residue was weighed and dissolved in 4.0 mL of water and 1.0 mL of a thiobarbituric acid reagent in acetic acid (335 mg in 50.0 mL of water diluted 50:50 in acetic acid 99%). The tubes were incubated for 1 h at 95°C. The solutions were cooled and the product of the reaction was extracted by 5.0 mL of n-butanol. The fluorescence of the organic layer was measured at 553 nm with an excitation at 515 nm. The concentration of the total lipid peroxide (expressed in nanomoles per milligram of residue) was calculated with the slope obtained from a gradient concentration of tetramethoxypropane pure standard diluted in 4.0 mL of water and 1.0 mL of the thiobarbituric acid reagent.

Statistical analysis

The statistical analysis was performed using Statistical Analysis System (SAS) statistical software V9.3 (SAS Institute, Cary, NC). Variables are expressed as mean ± standard

deviation unless otherwise noted. All available data were included in the statistical analysis. Qualitative variables were compared between the two groups using the Chi square or Fisher Exact test if necessary and quantitative variables were compared using the Student t test or Wilcoxon rank sum test if necessary. The evolution with time of quantitative variables was analyzed using two-way (time, treatment) non-parametric repeated-measures analysis of variance (Friedman test). In case of significant group effect, time-by-time comparisons were performed by use of the least-squares means procedure with a Bonferroni correction to adjust for multiple comparisons. In time by time comparisons, reported effects are calculated from median values in placebo and Na₂SeO₃ groups. For all analyses, a p value <0.05 was considered significant. All reported p values are two-sided.

RESULTS

Organ dysfunctions and supportive care

Global SOFA score (Fig. 3a) and plasma lactate concentration (Fig. 3b) significantly decreased with time ($p<0.001$ and $p=0.026$, respectively) but without significant difference between the two groups ($p=0.625$ and $p=0.485$, respectively). The evolution with time of PaO₂/FiO₂, AST and ALT until day-14 was similar in the two groups ($p=0.845$, $p=0.831$, and $p=0.961$, respectively see electronic supplementary material, Fig. S2a, S2b, and S2c).

There was no difference between the Na₂SeO₃ and placebo groups in terms of number of patients requiring renal replacement therapy (13/31 vs. 16/29, respectively, $p=0.545$), number of days free of renal replacement therapy (37 ± 55 vs. 26 ± 49 , respectively, $p=0.303$), and duration of ventilation (34 ± 54 vs. 25 ± 43 days, respectively, $p=0.762$).

The quality of life at 6 months was evaluated in 6/10 and 8/12 of the surviving patients in the two groups. Global, physical and behavioral SF-36 scores were similar between the two groups ($p = 0.846$, 0.953 and 0.905 , respectively, data not shown).

Acute tolerance of Na₂SeO₃ on specific organ dysfunctions

There was no deleterious effect of Na₂SeO₃ on cardiac and respiratory functions as assessed by the SOFA cardiologic and respiratory scores especially at day-1 (see table S1). In the same way, there was no hepatotoxicity in the Na₂SeO₃ group (as assessed by AST and ALT, see electronic supplementary material S2b and S2c).

Nosocomial infections

The occurrence of the first nosocomial infection was delayed in the Na₂SeO₃ group as compared to the placebo group (34 ± 28 vs. 18 ± 24 days, respectively, $p<0.001$). Mean time before bacteremia, first catheter and surgical wound infection was delayed in the Na₂SeO₃

treated group ($p<0.05$), but not the first VAP ($p=0.27$). However, the number of infections was similar in the two groups (1.8 ± 3.9 vs. 2.1 ± 3.7 infections per patient ($p=0.924$)).

Plasma selenium and selenoproteins

There were significant treatment effects on plasma Se concentrations (Fig. 1a, $p<0.001$), plasma selenoprotein-P concentrations (Fig. 1b, $p<0.001$), and GPX3 activity (Fig. 1c, $p<0.001$). Na_2SeO_3 infusion significantly increased plasma Se and selenoprotein-P concentrations, and GPX3 activity from day-4 to day-14 as compared to placebo ($\times 3.9$ on day-4 and $\times 2.3$ on day-14 for Se, $\times 2.7$ day-4 and $\times 2.6$ day-14 for selenoprotein-P, and $\times 1.8$ on day-4 and $\times 2.1$ on day-14 for GPX3 activity). Mean plasma selenium concentrations were above the reference value from day-4 to day-14 (i.e. 4 days after the end of Na_2SeO_3 administration).

Inflammation and lipid peroxidation

Plasma procalcitonin concentration significantly decreased with time ($p<0.001$) but without significant difference between the two groups (Fig. 2a, $p=0.869$). The evolution with time of IL6, and VEGF until day-14 was similar in the two groups ($p=0.769$ and $p=0.398$, respectively, see electronic supplementary material, Fig. S1a and S1b). Similarly, there was no difference on lipid peroxidation at any time point (Fig. 2b, $p=0.110$).

DISCUSSION

The additional clinical and biological data of the SERENITE study showed no evidence for clinical or biological effects in severe septic shock patients of continuous infusion of Na_2SeO_3 at a dose corresponding to 4 mg Se the first day followed by 1 mg/day during 9 days. There was in particular no argument in favor of pulmonary toxicity of Na_2SeO_3 as shown by the

similar evolution of PaO₂/FiO₂ ratio, especially from day1 to day3, and duration of ventilation. SOFA score, cardiac and respiratory SOFA scores, and plasma lactate also have a similar evolution. Cardio-respiratory distress is a critical point of acute Na₂SeO₃ toxicity, which is concentration and dose dependent [30, 56]. There is therefore no argument in favor of a toxic effect of continuous infusion of Na₂SeO₃ at a dose corresponding to 4 mg Se the first day.

As constantly seen in septic shock patients, we observed a profound decrease of plasma Se concentration at inclusion, which is between half to three-quarter of the reference value [12, 24, 32, 33, 36, 39]. In contrast with the absence of clinical effect, we observed a marked increase of plasma Se selenoprotein-P concentrations as well as GPX3 activity with Na₂SeO₃ administration until day-4 that seemed to have no impact on plasma inflammatory markers and lipid peroxidation. This increase at day4 of plasma Se and selenoenzymes is in accordance with the reported increase in GPX3 activity at day3 after Na₂SeO₃ administration at the dose of 2 mg Se in 1-hour bolus followed by 1.5 mg Se/day continuously during 14 days [57]. No clinical effect (neither positive, nor toxic) was reported in this study, which was based on the hypothesis of Se deficiency [57]. Similarly, in the phase III study of Bloss et al, based on a similar hypothesis, a marked increase of plasma Se concentration was observed in the treated group at Day4 and after [39].

Plasma procalcitonin (PCT) concentration - a precursor hormone of calcitonin - decreases according to recovery of bacterial sepsis [58]. We recorded increased interleukin 6 (IL6) concentrations at D0, - an important marker of cytokine driven inflammation but with huge individual variations as observed in our study. Mean IL6 concentration is known to be associated with the severity and progression of sepsis, leading to COVID-19 treatment proposal [2, 59-61]. The analogous decrease of PCT and IL-6 between groups observed in our study is in accordance with an absence of effect of the selenite infusion despite septic shock

being characterized by an acute oxidative stress [12], plasma lipid peroxidation has seldom been studied in sepsis patients [62-64]. An increased plasma lipid peroxidation was not observed in either group. A marked increase in thiobarbituric acid reactive substance (TBARS) has been previously described, especially measured in erythrocytes, which was not examined in our study [64]. In order to understand the impact of our continuous infusion of Na_2SeO_3 the first day in 2009 we performed a similar continuous infusion in a resuscitated peritonitis sheep model (4 $\mu\text{g}/\text{kg}\cdot\text{h}$ Se as Na_2SeO_3 ; e.g. Se 2.4 mg/24h in these 25 kg body weight animals) with sequential measurements of plasma Se concentration [31]. This continuous infusion of Na_2SeO_3 was performed 9 hours after the induction of the peritonitis by injection of autologous feces. It was performed one hour before the onset of septic shock in this resuscitated animal model. These intubated and ventilated sheep received fluid loading based on the data of arterial pressure and swan ganz catheter (including central venous pressure) with an algorithm similar to that of ICU patients [31]. At baseline, sheep have a slightly lower plasma Se concentrations than humans. Plasma Se decreased rapidly after the onset of peritonitis and at H9 was at about a quarter of its baseline value [31]. Despite the Na_2SeO_3 dose above our given dose in septic shock patients, continuous infusion of Na_2SeO_3 was unable to increase the plasma Se concentration above 1 $\mu\text{mol}/\text{L}$ [31]. The obtained plasma Se concentration by continuous infusion of Na_2SeO_3 remained thus below the oxidant cytotoxic concentration threshold, contrary to our expectation. The Na_2SeO_3 cytotoxicity threshold is above 1 to 5 $\mu\text{mol}/\text{L}$ depending on cell activity. This cytotoxicity threshold is all the more lower when the cells are activated and detached [25-29]. The continuous infusion peritonitis sheep group had a similar evolution to that of the control group in terms of microcirculatory perfusion, cardiac function, blood lactate concentration and survival time. This is in accordance with the lack of any benefits or side effect of our therapeutic intervention.

The brain seems to have a specific sensitivity to oxidative stress as shown by the susceptibility to damage of the glycocalyx of the hippocampal region in sepsis, alteration of the blood-brain barrier, and the neuronal dysfunction due to excessive microglial activation leading to long term neurological sequelae [2, 65, 66]. It is also one of the privileged organs for intracellular antioxidant selenoenzymes, with a specific cycle of selenoprotein-P from the astrocytes to the neurons especially in cortical, striatum as well as hippocampal regions [18, 19, 21, 67]. Nevertheless, in our study no beneficial effect was observed in long-term brain sequelae in surviving patients. This result represents an absence of early selenoenzyme induction in brain, especially brain selenoprotein-P, during the acute phase of sepsis despite a massive Se administration. Such induction might have protected the neurons before their damage.

Decrease of plasma Se has been proposed as a protective mechanism against pathogens, as most of them require Se for growth and antioxidant defense [36, 68-70]. In the treated patient group, nosocomial infections did not increase; on the contrary, the first nosocomial infection was delayed. This could be related to a slight improvement of immunodepression following septic shock, or a cytotoxic effect of Na_2SeO_3 on pathogens.

The absence of beneficial effect of the selenite infusion on any clinical and biological parameter despite the 4 mg Se intake in the first day goes against the hypothesis of a relative Se deficiency. This hypothesis is debated at the early phase of sepsis due to the low plasma Se and selenoprotein-P concentration observed at this phase, such as observed in our study at baseline and other studies [32, 33, 37]. According to such hypothesis, a large intake of Se is required at the early phase of sepsis for restoring antioxidant defense especially selenoprotein-P concentration [37, 40]. As indicated in the introduction, numerous elements oppose this hypothesis [71]. One can cite: (i) the absence of extra Se losses in sepsis [32]; (ii) the increased protein catabolism at acute phase of sepsis increasing the Se availability [19, 36, 71-

73]; (iii) the content of only 2% of body Se within the plasmatic compartment [19, 20, 71]; (iv) the rapid decrease of plasma Se in multiple animal models after lipopolysaccharide injection [19, 31, 34, 71], associated with a major and multistep downregulation of hepatic selenoprotein-P synthesis and excretion [34, 71, 74]. In the peritonitis sheep model previously cited numerous blood sampling could be performed during the first hours after the peritonitis induction and the initiation of selenite infusion or injection [31]. In the continuous infusion peritonitis sheep group with no beneficial effect, the plasma Se concentration remained below the baseline value despite a similar Se administration of the one provided the first day in our treated group, which is against an early induction of selenoprotein-P synthesis [31]. Together with the negative phase III study of Bloos [39], there seems to be no interest in using Na_2SeO_3 in septic shock patients as the Se donor for selenoenzyme induction, including selenoprotein-P. But this did not rule out the interest of Na_2SeO_3 as a cytotoxic drug against hyperactivated leukocytes that may need rapid IV injection, nor the interest of selenoprotein-P administration. Selenoprotein-P is known to protect the endothelium against oxidative stress through peroxynitrite (ONOO^-) detoxification [75, 76]. It might also protect endothelium in sepsis (submitted data) and may thus increase the Na_2SeO_3 margin of safety. However, in order to protect the endothelium, an early administration of a recombinant selenoprotein-P seems to be required before endothelium damage occurs [77, 78].

In order to achieve blood cytotoxic concentration, from 4 to 14 $\mu\text{M/L}$ Se under the form of Na_2SeO_3 a bolus injection of Na_2SeO_3 at a dose corresponding to 2 mg Se was required in peritonitis sheep weighing between 23 to 28 kg. This pharmacologic administration of Na_2SeO_3 leads to transient beneficial effects from lactate to micro-, macro-circulation and survival time [31]. Such 4 to 14 $\mu\text{M/L}$ concentrations are above the cytotoxic threshold of Na_2SeO_3 in in-vitro cancer studies [26-29, 31, 36]. However, its effect is limited in time (few

hours) [31]. The general toxicity is a threat. The toxicity is characterized by cardio-respiratory distress symptoms similar to those of sepsis [30, 56]. In addition Na_2SeO_3 cytotoxicity seems increased in sepsis as observed in a rat lipopolysaccharide (LPS) model. The minimum lethal doses were between 0.3 to 0.6 Se mg/kg as Na_2SeO_3 in LPS rats, compared to 3 mg/kg Se as Na_2SeO_3 in healthy rats [30, 56]. Our present study illustrates that pharmacological use of Na_2SeO_3 cytotoxicity against hyperactivated neutrophils requires preclinical drug development to determine the adapted dose and delivery (e.g. rapid intravenous injection vs. continuous infusion) for an efficient and safe treatment.

On one hand, selenoprotein metabolisms in sepsis may be further explored by studies focusing on genomic and proteomic expression in circulating cells of broad panel proteins including selenoproteins - under selenocompound administration at nutritional or pharmacological ranges - such has been done in sepsis patients for other purpose [79, 80].

Several studies in LPS mice on selenoprotein expression have already been performed in hepatic and in a lesser extent lung, kidney and spleen tissues [71, 74]. But further studies might be of great interest on modification of genomic expression of selenoproteins in sepsis using knock-out models focusing on endothelium, brain -especially hypothalamus-, thyroid, renal and muscular tissues similar to studies in mice or rats investigating metabolic syndrome [81, 82], thyroid disease [83, 84] or pregnancy - especially in preeclampsia - [85].

On the other hand, for oxidant direct cytotoxic effects of high blood concentrations of selenite studying its effect on circulating cells, especially their binding to endothelium, might be a key point. Studies on selenoprotein-P direct protective effects on endothelium against sepsis-induced hyper hyperoxidation might be also of great interest.

CONCLUSIONS

In conclusion, the absence of beneficial effects observed in the SERENITE study using high doses of Na_2SeO_3 in septic shock may be explained by (i) a low plasma Na_2SeO_3 concentration below the cytotoxic concentration of hyperactivated leukocytes and (ii) a delayed increase of plasma selenoprotein-P occurring after initiation of endothelium damage. Further preclinical studies need to be conducted to explore antioxidant protection of selenoprotein-P on septic endothelium, and the effects of rapid injection of Na_2SeO_3 for cytotoxic purposes on hyperactivated leukocytes. Furthermore, it might be useful to study if a combination of selenoprotein-P infusion and rapid intravenous injection of Na_2SeO_3 leads to a cytotoxic effect on leukocytes while protecting endothelium. The selenoprotein-P infusion might in addition reduce the pulmonary side effects and increase the Na_2SeO_3 margin of safety by limiting endothelium activation.

Additional files

Evolution of cardiac and respiratory SOFA scores, Table S1.

Evolution of IL6, and VEGF until day-14 in the two groups, see electronic supplementary material, Fig. S1a and S1b. Evolution of $\text{PaO}_2/\text{FiO}_2$, AST and ALT until day-14 in the two groups, see electronic supplementary material, Fig S2a, S2b, and S2c.

DECLARATIONS

Ethics approval and consent to participate

The study has been approved by the ethics committee (*Comité consultatif de protection des personnes dans la recherche biomédicale*, CCPPRB) of Saint Germain en Laye on the 10th of May, 2001 ; A statement of intention for the study of a test drug has been filed with the French Office for the safety of health products (*Agence Française de Sécurité Sanitaire des Produits de Santé* AFSSAPS), in May 2001.

The study has received the labels of the French Speaking Society of Intensive Care (*Société de Réanimation de Langue Française*, SRLF) in Nov 1999, and the French Speaking Society of Study and Research on Essential and Toxic Elements (*Société Francophone d'Etude et de Recherche sur les Eléments Toxiques et Essentiels*, SFERETE) in May 2001.

Consent for publication

Acceptation

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

XF obtained the financing, developed the link with the administrative staff of Meaux Hospital – especially for pharmaceutical aspects – and the coordination between centers, participated in the study design and execution, in interpretation of the data, and in the writing of the manuscript. BL coordinated the monitoring of the study, performed the medical analysis of adverse events, and participated in the statistical analysis, in the interpretation of the data, and in the writing of the manuscript. BL, JG, AB, EP, PVA, and DA, participated in the execution of the study and in the interpretation of the data. EB realized the methodology of the study, coordinated the monitoring, data management, statistical analysis, interpretation of the data, and analysis of adverse events, and participated in the writing of the manuscript. All authors read and approved the final manuscript.

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FIGURE LEGENDS

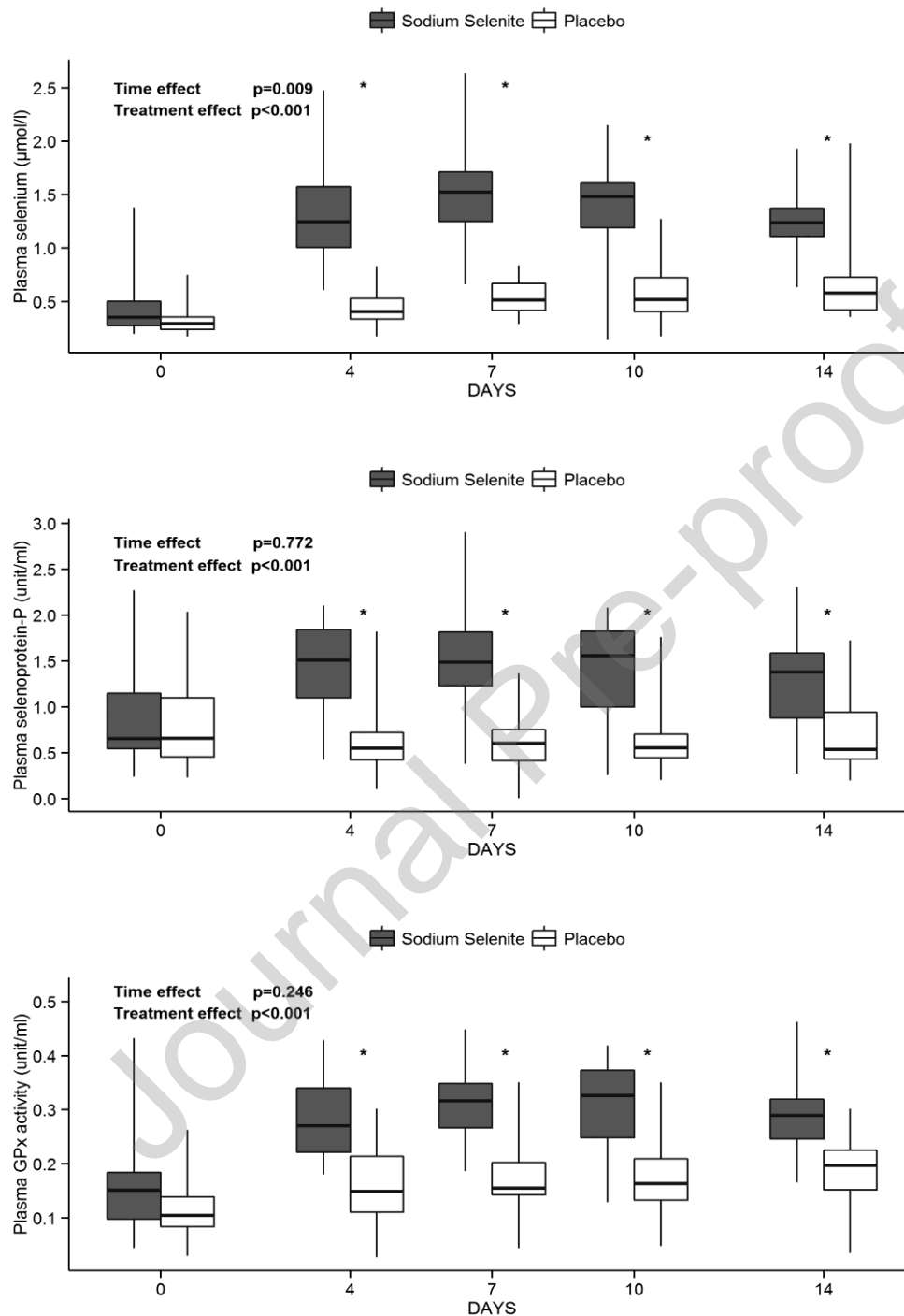


Figure 1. Evolution of plasma selenium (1a), selenoprotein-P (1b), and Glutathione Peroxidase activity (1c)

Plasma GPx: Plasma Glutathione Peroxidase (or GPX3).

Data are presented as box plots. The lower and higher boundaries of the box indicate the 25th and 75th percentile. The line within the box marks the median, and error bars below and above the box respectively indicate $Q1-1.5 \times \text{Interquartile}$ and $Q1+1.5 \times \text{Interquartile}$.

Reference values for plasma selenium concentration in the French area are 1.00 ± 0.15 $\mu\text{mol/L}$.

*, $p < 0.05$ vs placebo

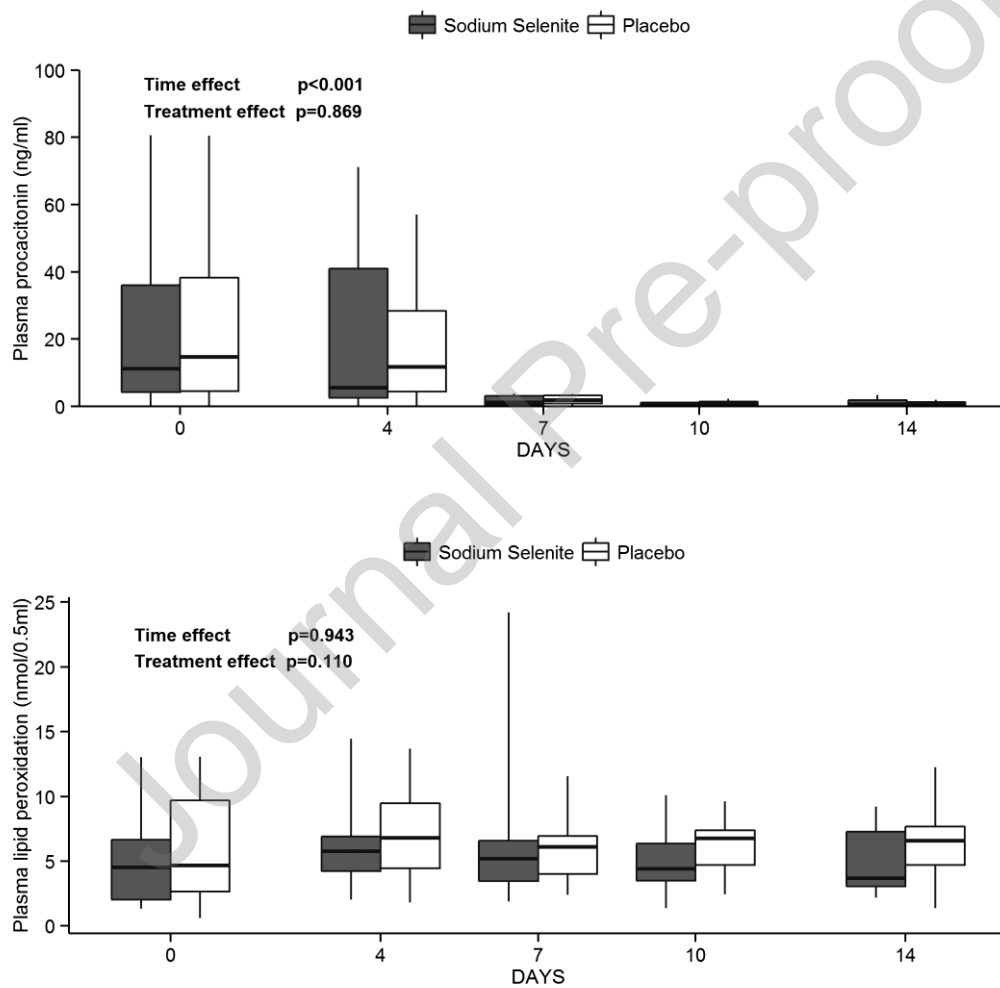


Figure 2. Evolution of plasma procalcitonin (2a) and lipid peroxidation (2b)

Data are presented as box plots. The lower and higher boundaries of the box indicate the 25th and 75th percentile. The line within the box marks the median, and error bars below and above the box respectively indicate $Q1-1.5 \times \text{Interquartile}$ and $Q1+1.5 \times \text{Interquartile}$.

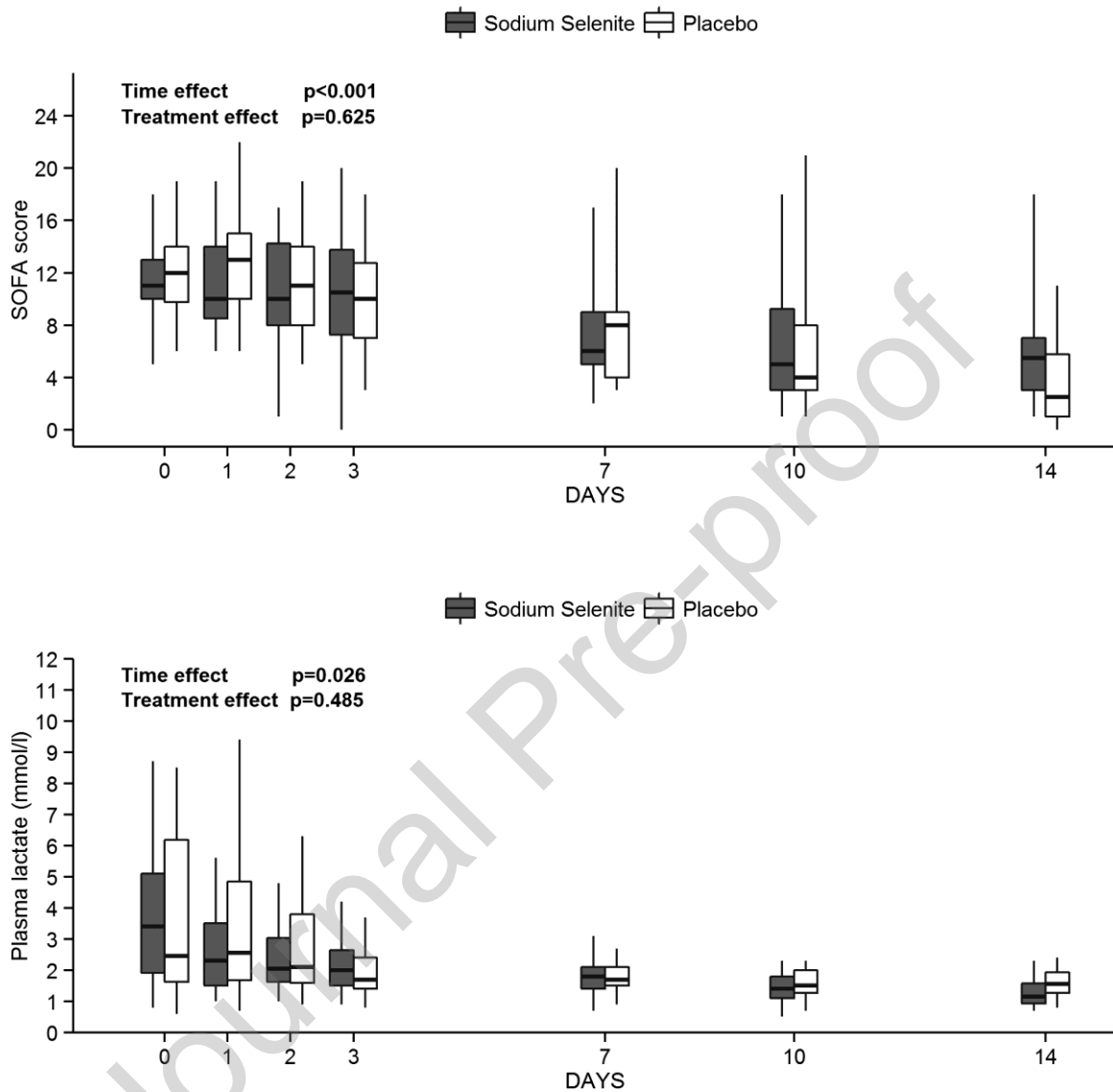


Figure 3. Evolution of SOFA score (3a) and plasma lactate (3b)

SOFA: Sequential Organ Failure Assessment

Data are presented as box plots. The lower and higher boundaries of the box indicate the 25th and 75th percentile. The line within the box marks the median, and error bars below and above the box respectively indicate $Q1 - 1.5 \times \text{Interquartile}$ and $Q1 + 1.5 \times \text{Interquartile}$.

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Biographies

Dr Xavier Forceville created a very small start-up in 2005 (Sérénité-Forceville). In 2015, Pharm. V Cotereau volunteer manager of Sérénité-Forceville, former vice president of the French pharmacist association, filed a new patent for the treatment of sepsis entitled: "Kit for treating sepsis and/or any systemic (SIRS) or damaging cellular hyper-inflammation". Its reference numbers are: PCT number PCT/FR2016/051569, European demand 16742342.5, US demand Attorney Docket Number: 0727-1267.

I am the main shareholder of this company. One of my brothers is also a shareholder.

The other authors (BL, JG, AB, PVA, EP, DA, and EB) declare that they have no competing interests.

Author statement

I have the written consent from all authors to submit this manuscript and all authors accept complete responsibility for the contents. This manuscript is not currently under consideration elsewhere and the reported work will not be submitted for publication elsewhere until a final decision has been made as to its acceptability. The manuscript is a truthful, original work.