Current Evidence from Phase III Clinical Trials of Selenium Supplementation in Critically Ill Patients: Why Should We Bother?

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Abstract: The importance of the trace element selenium for human health is well established. Selenium plays a central role in the formation of selenocysteine, a modified amino acid located in the catalytic center of selenoenzymes. The crucial role of selenium in these enzymes revolves around the maintenance of many redox systems in cellular and extracellular compartments. In addition, selenium plays an important role in thyroid hormone metabolism. Several clinical trials of selenium supplementation in critically ill patients have been conducted to date, providing an interesting and provoking mix of findings. Despite some promising results, no definitive answers regarding the effects of selenium supplementation on critically ill patient mortality or morbidity exist. Further research in the setting of well-designed, prospective, randomized trials is necessary to better define the role of selenium supplementation in critically ill patients.

Key Words: Selenium supplementation, intensive care unit, critically ill patients, review of clinical trials.

INTRODUCTION

The essential trace element selenium was first discovered by Swedish physician Jons Jakob Berzelius in 1817, who named the element after the Greek moon goddess Selene [1]. Early on, selenium was thought to be very toxic, with no known health benefits [1, 2]. It was not until Schwarz and Foltz published evidence of the beneficial and essential role of selenium in 1957 that our perception of the 'moon' element began to change [3]. This was followed by the demonstration that selenium is an integral part of glutathione peroxidase by Flohe in 1973 [4]. Selenium is now known to be incorporated into at least 25 specific human selenoproteins [1].

Trace element metabolism is significantly affected by major physiologic stress [5]. Plasma levels of selenium are decreased in severe illness, injury and sepsis [6, 7]. It has been postulated that low serum selenium concentrations are associated with low glutathione peroxidase activity in critically ill patients [8]. This, in turn has been hypothesized to lead to decreased cleavage of free radicals and worsened clinical outcomes secondary to impaired regulation of inflammatory processes [8, 9]. Focusing on glutathione peroxidase, this paper reviews the available literature on selenium supplementation in critically ill patients, its side effects, as well as potential benefits of its administration.

SELENOCYSTEINE AND GLUTATHIONE PEROXIDASE

Selenium is nearly three times less abundant than its close relative sulfur. These elements are similar in their general chemical properties, with the redox potentials of selenium compounds being lower than those of their sulfur analogues [1, 10]. Generally more reactive than sulfur, selenium plays a crucial role in the formation of selenocysteine, also known as the 21st amino acid [1] (Fig. **1**). Selenocysteine is located in the catalytic center of selenoenzymes [6].

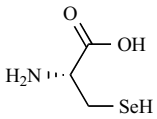

Fig. (1). Chemical structure of selenocysteine.

Perhaps the most important role of the selenoenzymes is the maintenance of nearly all redox systems in cellular and extracellular compartments. Thus, selenoenzymes are thought to play a major role in protecting cells against peroxidation, especially lipid peroxidation [9]. One of the best-known and well-characterized redox systems is the glutathione complex consisting of a selenium-dependent peroxidase (Fig. **2**). The activity of glutathione peroxidase has been linked to the amount of available selenium [6].

There are currently three known mechanisms for selenoprotein formation – posttranslational selenium binding as a cofactor, non-specific selenium incorporation, and specific incorporation during translation [11-13]. Selenoprotein synthesis is a multistep process, and our understanding of the eukaryotic process is based largely on studies of bacterial systems. Detailed overview of selenoprotein synthetic steps, as well as description of other selenoprotein-dependent metabolic processes (including thyroid hormone metabolism) has been described elsewhere [1, 6].

OUR CURRENT UNDERSTANDING OF GLUTATHIONE PEROXIDASES

The many selenoproteins identified thus far in humans share little sequence homology, and while many selenoproteins have no specific function ascribed to them, they seem to serve a number of diverse functions [1]. One of the best

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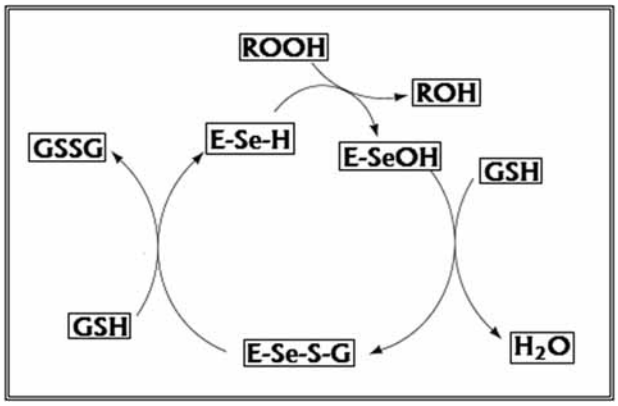

Fig. (2). Proposed catalytic mechanism of glutathione peroxidase. Glutathione peroxidase catalyses the reduction of harmful peroxides by glutathione and protects the cell membrane from oxidative damage. The enzyme's catalytic site includes a selenocysteine residue in which the selenium undergoes a redox cycle involving the selenol (E-Se-H) as the active form that reduces hydrogen peroxides and organic peroxides. The selenol is oxidized to selenenic acid (E-SeOH), which reacts with reduced glutathione (GSH) to form selenenyl sulfide adduct (E-Se-S-G). A second glutathione then regenerates the active form of the enzyme by attacking the ESeSG to form the oxidized glutathione (GSSG). As a result, 2 equivalents of GSH are oxidized to the disulfide and water, while the hydroperoxide (ROOH) is reduced to the corresponding alcohol (ROH). Modified from Gromer, S.; Eubel, J.K.; Lee B.L.; *et al. Cell Mol Life Sci*. 2005, 62, 2414.

characterized selenoprotein families, and a focus of this review, is the glutathione peroxidase family. Glutathione peroxidase was one of the first mammalian selenoproteins identified, and currently seven isoenzymes of this protein are known in humans [14].

The importance of glutathione peroxidases and their potentially beneficial role in critically ill patients revolves around the mechanism of detoxification of peroxides to their respective alcohols at the expense of glutathione (Fig. **2** and Fig. **3**). It seems that all of the glutathione peroxidase isoforms share the same catalytic mechanism, with a highly conserved sequence of selenocysteine, tryptophan and glutamine [15, 16]. A discussion of the known isoforms of glutathione peroxidase will now follow.

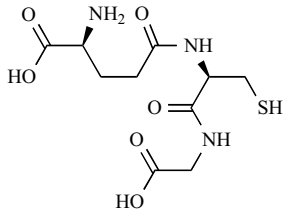

Fig. (3). Chemical structure of glutathione.

Glutathione peroxidase 1 (GP-1) is abundant in the liver and erythrocytes, and its concentration is dependent on the nutritional selenium status [17]. Of interest, GP-1 deficient mice show no obvious phenotypic changes under normal conditions, but are severely affected by oxidative stress [18, 19]. Furthermore, it seems that GP-1 may play a presently undefined role in several infectious processes, with evidence showing that the genomes of HIV-1, HIV-2, hepatitis C virus, coxsackievirus B3 and measles virus encode for glutathione peroxidase homologues [20]. Even more intriguing, GP-1 polymorphisms are reported to be associated with an increased risk of bladder cancer and vascular diseases [21, 22].

Glutathione peroxidase 2 (GP-2) is found in the liver and the gastrointestinal tract [1, 23]. It appears to be absent from the heart and kidney. Due to that fact, some refer to it as gastrointestinal glutathione peroxidase-1 [1]. The distribution of GP-2 in the intestine appears to decline as one proceeds from the crypts towards the luminal surface [24]. GP-2 is a homotetrameric, cytoplasmic enzyme. Its substrates include hydroperoxides such as t-butyl hydroperoxide, cumene hydroperoxides, and linolic acid hydroperoxide [1]. Of interest, GP-2 appears to be conserved under conditions of inadequate selenium supply, and has been considered as the first line of defense against ingested organic hydroperoxides [25-27]. Other evidence points to possible involvement of GP-2 in the processes of apoptosis and cellular proliferation [24]. Of further interest, GP-1 and GP-2 double knockout mice demonstrate inflammatory bowel disease and bacteria-induced tumors [28].

Glutathione peroxidase 3 (GP-3) is a homotetrameric glycoprotein found in the plasma, the intestine, the adrenal gland, pulmonary lavage fluid, breast milk, as well as in renal proximal tubules [23, 29, 30]. Although its physiologic function has not been definitively resolved, it is speculated that GP-3 may be involved in regulatory functions associated with oxidative stress and malignancy [1]. Hypoxia appears to induce GP-3 expression [30]. Decreased levels of GP-3 have been associated with familial childhood stroke and human renal cell carcinoma [30-31]. In addition, GP-3 is expressed in the renal proximal tubules and can be used as a plasma marker of tubular integrity [32].

Glutathione peroxidase 4 (GP-4) is a monomeric enzyme found in testes, lung, heart, and cerebellum, with several interesting characteristics [23]. Utilization of alternative initiation sites allows GP-4 to assume either mitochondrial or cytoplasmic isoforms [1]. In addition, GP-4 is capable of transforming into an essential structural component of the sperm's midpiece *via* alternative splicing, and is required for sperm fertilization [33-35]. GP-4 is thought to have the broadest substrate specificity of all glutathione peroxidases, being capable of reducing phospholipid hydroperoxides as well as hydroperoxides still integrated in cellular membranes [36, 37]. Therefore, GP-4 is a good candidate for being a universal antioxidant that serves in the protection of biomembranes [36, 37]. GP-4 is also involved in redox signaling and regulatory processes, including apoptosis and inhibition of lipoxygenases [16, 38]. Studies of GP-4 knockout mice show that complete lack of GP-4 is lethal at an early embryonic stage, and the conceptus exhibits abnormal structural compartmentalization [1]. Heterozygous cells are significantly more susceptible to induced oxidative stress, corroborating the important role of GP-4 in oxidative stress states [39].

Glutathione peroxidase 5 (GP-5) is found exclusively in the epididymis [40]. Although its function has not been well-characterized, this non-selenocysteine containing isoform can be secreted or membrane-bound, and it has been suggested to function as a backup for the selenocysteine-containing isoforms in sperm [41]. Given the extremely low level of GP-5 expression in humans, its importance as a potential radical scavenger is unlikely [42].

Glutathione peroxidase 6 (GP-6) appears to be involved in olfaction [1]. It has been demonstrated only in olfactory epithelium and embryonic tissues [43]. GP-6 is known to be expressed in or near the Bowman's glands, which is a site of several olfactory-specific biotransforming enzymes. Further studies have shown GP-6 to be a putative odorant metabolizing enzyme [44].

Table 1. Glutathione Peroxidase Isoenzymes

Glutathione Peroxidase Isoenzyme	Tissue and cellular localization	Function	Additional comments
GP-1 [a]	Liver, Erythrocytes	Important in severe oxidative stress. Role in viral infectious processes.	Concentration dependent on nutritional selenium supplementation. Polymorphisms associated with increased risk of bladder cancer and vascular diseases. Genomes of HIV-1, HIV-2, Measles virus, Hepatitis C virus, and Coxsackievirus B3 encode for GP-1 homologs.
GP-2 [b]	Liver, Gastrointestinal tract (stomach and intestines)	First line of defense against ingested organic hydroperoxides. Involvement in apoptosis, cellular proliferation.	Homotetrameric, cytoplasmic enzyme. Conserved with inadequate selenium supply. Demonstrated inflammatory bowel disease and bacteria-induced tumors.
GP-3 [c]	Plasma, Intestine, Breast milk, Adrenal gland, Pulmonary lavage fluid, Renal proximal tubules	Thought to have largely regulatory functions associated with oxidative stress and malignancy. Expression induced by hypoxia.	Homotetrameric glycoprotein. Deficiency associated with familial childhood stroke and renal cell carcinoma. Expressed in renal proximal tubules, can be used as plasma marker of tubular integrity.
GP-4 [d]	Distributed in cytoplasm, nucleus and mitochondria Expressed in testis, lung, heart, and cerebellum Essential structural component of the sperm's midpiece	Universal antioxidant involved in protection of biomembranes. Contains broadest substrate specificity (reduces phospolipid hydroperoxides in cellular membranes). Involved in redox signaling and regulatory processes (apoptosis, inhibition of lipoxygenases). Required for sperm fertilization. Lack of GP-4 is lethal at an early embryonic stage.	Monomeric enzyme. Utilization of alternative initiation sites allows GP-4 to assume either mitochondrial or cytoplasmic isoforms.
GP-5 [e]	Epididymis	Potentially serves a 'backup' function for selenocysteine containing GP isoforms in sperm. Can be secreted or membrane-bound.	Non-selenocysteine containing isoform. Low expression in humans (unlikely to be of importance as a free radical scavenger).
GP-6 [f]	Bowman's gland, olfactory epithelium, embryonic tissues	Olfaction. Putative odorant metabolizing enzyme.	A close homolog of plasma GP-3. A selenoprotein in humans, this isoform has been found to be a non-selenium containing enzyme in rodents.
GP-7 [g]	Breast tissue	Breast cancer cell defense against oxidative stress.	Non-selenocysteine isoform. Possible involvement in BRCA-1 related breast cancer.

Legend:
[a] = References 17-20
[b] = References 1, 23-28
[c] = References 1, 23, 29-32
[d] = References 1, 23, 33-39
[e] = References 40-42
[f] = References 1, 43-44
[g] = Reference 45

Glutathione peroxidase 7 (GP-7) is another non-selenocysteine isoform of glutathione peroxidase. This cytoplasmic protein has been shown to have only miniscule detectable glutathione peroxidase activity *in vitro* [45]. It may be involved in breast cancer cell defense against oxidative stress [45].

A table summarizing all of the major isoforms of glutathione peroxidase has been constructed for the reader's convenience (Table **1**).

DOSAGE AND SIDE EFFECT PROFILE OF EXOGENOUSLY SUPPLEMENTED SELENIUM

Selenium is usually supplemented as sodium selenite or ebselen, an organic selenium-containing compound, which appears to mimic glutathione peroxidase [6, 46]. Selenium is very tempting as a therapeutic candidate in management of critically ill patients partly because of its relatively harmless nature at the doses used in published intensive care unit (ICU) clinical trials [46]. These dosages vary from 35 micrograms to 1000 micrograms daily, in various combinations (including tapered and non-tapered regimens) [6, 46-48]. The most frequently reported initial dosage was 500 micrograms daily [6, 46, 48]. The reported duration of selenium administration in most studies was between 5 and 28 days [6, 46, 48]. Of note, the recommended dosage of selenium for patients who are receiving parenteral nutrition is between 70 and 200 micrograms per day [6]. In one clinical trial, the dose of sodium selenite was reduced when administered to patients with renal failure because selenium is excreted in the urine [6]. More detailed discussion of selenium dosage and toxicity will now follow. A summary of selenium deficiency states and toxicities is presented in Table **2**.

The reported toxic dose of selenium in humans is 3000 micrograms per day taken over a period of weeks [6]. This represents a threefold increase over the highest reported administration dosage regimen in the ICU trials [46]. Based on previous studies, intakes of 400 micrograms per day and plasma selenium levels of 1000 nanograms per milliliter were established as having no observed adverse effects [49]. Serum half-life of 17.5 hours was observed in one case of massive selenic acid ingestion in a patient with normal renal function [50].

Table 2. Clinical Characteristics of Selenium Deficiency and Toxicity States

Parameter	Selenium Deficiency	Selenium Toxicity
Blood selenium level [a]	**Keshan Disease**: 0.021 ± 0.010 μg/mL	**Chronic toxicity (selenosis)**: 3.2 μg/mL
Clinical signs and symptoms of deficiency [b], chronic [c] and acute toxicity [d]	**Keshan Disease** – a syndrome consisting of dilated cardiomyopathy seen in children and women of child-bearing age, endemic to regions of China. Cardiomyopathy, similar to Keshan disease, associated with total parenteral nutrition (TPN). Skeletal myopathy associated with TPN. Reduction in CD4 counts in HIV-infected patients.	**Chronic toxicity (selenosis)** – a syndrome consisting of: • Loss of hair, regrowth of unpigmented hair, scalp rash with intolerable itching. • Nail changes consisting of brittleness, white spots and longitudinal streaks, nail regrowth thickened with rough, striped surface. Thumbs typically affected first. • Skin lesions on extremities (back of hands and feet, outer side of legs, thighs and forearms, and back of neck) initially red and swollen, progress to blisters and eruptions. • Garlic odor of breath and increased risk of dental caries. • Nonspecific gastrointestinal symptoms. • Polyneuritis characterized by peripheral neuropathy, pain in extremities, tendon hyperreflexia, numbness, convulsions, paralysis, motor disturbances, hemiplegia in severe cases. **Acute toxicity** – a syndrome consisting of: • Severe gastrointestinal symptoms (nausea and vomiting). • Sinus tachycardia with ST wave alteration. • Fatal intoxication associated with nausea, vomiting, pulmonary edema, and cardiovascular collapse
Populations at highest risk for selenium deficiency [e] and toxicity [f]	Geographic regions with low soil selenium concentrations: some regions of China, New Zealand, Central Africa, Finland, Poland, and Yugoslavia. Patients requiring parenteral nutrition. Patients with malabsorptive conditions.	Geographic regions with high soil selenium concentrations, including some regions of China.

Legend: [a] = Reference 52
[b] = References 61-65
[c] = Reference 52
[d] = References 50, 54
[e] = References 62-64
[f] = Reference 52

In one study of high-dose selenium administration as a chemopreventive agent for prostate cancer progression, doses of up to 3200 micrograms per day were utilized with no significant selenium-related adverse events [49]. Of interest, patients were on selenium doses ranging from 1600 micrograms to 3200 micrograms per day for an average of approximately 12 months [49]. While the 3200 microgram per day group reported more selenium-related side effects, blood chemistry and hematology results were all within normal limits for both treatment groups, and symptoms of selenium toxicity did not correspond to peaks in plasma selenium levels [49]. This may be due to the presence of intervening metabolic steps, resulting in delayed physiologic response to the supplemented selenium before toxicity becomes apparent.

The classic condition associated with chronic selenium toxicity in humans is called selenosis [51]. The characteristic features of this toxicity pattern include hair and nail loss and brittleness, gastrointestinal problems, skin rash, garlic breath odor, and nervous system abnormalities [52]. In China, selenosis was reported in patients who consumed 1000 micrograms of selenium daily as sodium selenite for more than 2 years [52]. In the United States, selenosis was reported in cases of dietary supplement ingestion containing over 27 milligrams of selenium per tablet [53].

Acute selenium toxicity is associated with ingestion of large amounts of selenium (grams), with associated severe gastrointestinal symptoms, transient electrocardiographic changes, and slight elevation of serum bilirubin [50]. Classic electrocardiographic changes, which have been reported to occur in acute selenium intoxication, include sinus tachycardia with ST wave alteration [54]. Fatal cases of selenium intoxication were also reported, usually involving ingestion of massive amounts of selenium-containing substances [54-55]. These cases manifested as nausea and vomiting, followed by pulmonary edema and rapid cardiovascular collapse approximately 3 to 4 hours after ingestion [54].

STUDIES OF SELENIUM SUPPLEMENTATION IN THE INTENSIVE CARE SETTING

Most clinical studies of selenium supplementation are phenomenological in nature, examining the responses to the presence or absence of selenium. Since many of these studies do not consider the precise mode of action, the recorded parameters and results are difficult to interpret. With numerous potential confounding variables, the general applicability of these results is limited. These confounding variables include, but are not limited to, the variability in renal function between individual patients, varied methods of nutritional supplementation, concurrent administration of other pharmaceutical agents that may have a physiologic interaction with selenium-dependent physiologic pathways, and the effects of varied degrees of oxidative stress associated with any given clinical diagnostic entity (i.e., mild, moderate, or severe sepsis).

Low plasma selenium levels in the setting of severe illness and sepsis have been linked to decreased glutathione peroxidase activity [6]. In a recent prospective, randomized, controlled study of patients with severe sepsis, selenium supplementation was shown to result in a significant increase in serum selenium levels as well as increased plasma glutathione peroxidase activity [6]. In addition, selenium supplementation has been associated with improved clinical outcomes among patients with systemic inflammatory response syndrome (SIRS), major traumatic injuries, burns, and necrotizing pancreatitis [8, 9, 56-60].

In terms of patient mortality, a recent review of selenium supplementation for critically ill adults demonstrated mixed clinical results [46]. In that review, four major studies of selenium supplementation were analyzed. Pooling of the mortality data from these studies showed a statistically significant difference in mortality in favor of selenium treatment with a fixed effects model [46]. However, when a random effects model was used, no statistically significant mortality differences were observed [46]. Looking at individual studies, an analysis of a subset of patients with APACHE-II score greater than 20 in one trial demonstrated significant reduction in mortality between the high-dose selenium supplementation group (30% mortality) and the control group (70% mortality, $P = 0.013$) [8]. In another study, while no mortality was noted among patients with necrotizing pancreatitis who received supplemental selenium, 89% of the control group patients died [58]. Although certainly impressive, these results should be considered carefully until reproduced by other investigators in well-controlled, randomized trials. Zimmermann, *et al.* reported significant reduction in mortality among 20 patients with SIRS who were treated with 1000 micrograms of sodium selenite daily. In that trial, 15% of patients in the treatment group died compared to 40% in the control group [59]. Of note, all of the above selenium studies suffer from significant methodologic deficiencies and inadequate sample sizes [46].

In terms of the effect of selenium supplementation on infectious complications in critically ill patients, no statistically significant differences have been reported [56]. Two trials reported on the number of ventilator days, with no significant differences between the selenium-supplemented and non-supplemented groups [47, 56]. Likewise, no significant differences were seen in terms of the length of stay in the intensive care unit [20] or length of hospital stay [47, 56].

Of additional interest, one of the trials of selenium supplementation showed that while only 3/21 patients in the selenium replacement group required continuous veno-venous hemodialysis, 9/21 control group patients required this intervention in the face of renal failure [47]. Others also report decreased incidence of renal failure in selenium-supplemented critically ill patients [8, 57].

None of the trials of selenium supplementation reported on quality of life data, details of costs, or economic impact of selenium administration [46]. Important studies of selenium-containing supplementation in critically ill patients have been summarized in Table **3**.

CONCLUSIONS

The beneficial effects of selenium supplementation on outcomes in critically ill patients remain to be fully elucidated. Although some promising observations have been made in a limited number of relatively poor quality trials,

Table 3. Summary of Important Studies of Selenium Supplementation in Critically Ill Patients

Study	Number of patients	Mortality	Complications and interventions	Additional comments
Kuklinski B, *et al*., 1991 Reference 58 Patients with acute necrotizing pancreatitis	8 patients in sodium selenite-supplemented group 9 patients in placebo group	No mortality in treatment group 89% mortality in control group	6/8 control group patients underwent surgical exploration and 4/8 treatment group patients underwent surgery	Treatment period of 6 days
Zimmermann T, *et al*., 1997 Reference 59 Patients with SIRS	20 patients received 1000 micrograms of sodium selenite daily 20 control patients	15% mortality in treatment group 40% mortality in control group	No complication-related information reported	Treatment period of 28 days
Gartner R, *et al*., 1997 Reference 8 Patients with sepsis	21 patients received sodium selenite taper (500, 250, and 125 micrograms for 3 days each) 21 patients received 35 micrograms of sodium selenite daily	33.5% in high-dose selenium supplemented group 55% in control group	Acute renal failure necessitating hemodialysis present in 9/21 control patients *vs* 3/21 high-dose selenium group ($P < 0.04$)	Treatment period of 9 days Analysis of patient subset with APACHE-II score >20 demonstrated significant reduction in mortality (70% *vs* 30%) in the selenium-supplemented group ($P = 0.013$)
Berger MM, *et al*., 1998 Reference 60 Burn patients	10 patients received trace elements (including high-dose selenium) 10 patients received standard trace elements (controls)	10% mortality in high-dose selenium group No mortality in control group	1.9 infections per patient reported in treatment group, 3.1 infections per patient reported in control group ARDS in 4/10 treatment group patients and 8/10 in control group patients	Treatment period of 8 days ICU length of stay was 30 days for treatment group and 39 days for controls Hospital length of stay was 54 days for treatment group and 66 days for controls
Porter JM, *et al*., 1999 Reference 57 Trauma patients	9 patients in combined selenium and antioxidant treatment group 9 patients in control group	No mortality reported in either treatment or control groups	Treatment group had 8 incidences of infectious complications, control group had 18 incidences of infectious complications Nine incidences of organ dysfunction in control group versus none in treatment group	Treatment period of 7 days
Berger MM, *et al*., 2001 Reference 48 Trauma patients	20 patients received selenium-containing regimen 11 patients received placebo	2/20 in selenium group 1/11 in placebo group	Respiratory failure 6/20 in selenium group, 4/11 in placebo group Hemorrhage or persistent bleeding 9/20 in selenium group and 6/11 in placebo group	Treatment period of 5 days No significant differences in ventilator days (5.1 *vs* 4.2), ICU length of stay (6.1 *vs* 6.1 days), or hospital length of stay (68 *vs* 59 days)

there are no definitive answers regarding the beneficial effects of selenium supplementation in this population of patients. In addition, due to many potentially confounding variables, the general applicability of these results is limited. Well-designed, adequately powered randomized controlled trials, accounting for cellular mechanisms of selenium activity, are needed to determine whether there is true clinical benefit to selenium supplementation in the critically ill.

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