Available online at www.sciencedirect.com
ScienceDirect
Journal of Trace Elements in Medicine and Biology 23 (2009) 138–143
Journal of Trace Elements in Medicine and Biology
www.elsevier.de/jtemb

Physiology

Acute high-dose sodium selenite administration improves intestinal microcirculation without affecting cytokine release in experimental endotoxemia

Matthias Gründling[a,1], Sandra Rickert[a,1], Daniela Saeger[a], Dragan Pavlovic[a], Taras Usichenko[a], Konrad Meissner[c], Michael Wendt[a], Orlando Hung[b], Michael Murphy[b], Sara Whynot[b], Christian Lehmann[a,b,*]

[a]*Klinik und Poliklinik für Anästhesiologie und Intensivmedizin, Ernst-Moritz- Arndt-Universität, Friedrich-Loeffler-Str. 23, 17475 Greifswald, Germany*
[b]*Department of Anesthesia, Dalhousie University, 1278 Tower Road, 10 West, Victoria General Hospital, Halifax, Nova Scotia, Canada B3H 2Y9*
[c]*Department of Anesthesiology, Washington University in St. Louis, 660 S. Euclid Avenue, Box 8054, St. Louis, MO 63110, USA*

Received 17 July 2007; accepted 8 September 2008

Abstract

We evaluated the effects of acute high-dose sodium selenite (SEL) administration on the intestinal microcirculation and the release of the cytokines TNF-α, IL-1β, IL-6 and IL-10 in experimental endotoxemia (induced by lipopolysaccharide-LPS). Three groups of animals ($n = 30$) were studied: control group, endotoxemic group (15 mg kg^{-1} i.v. LPS from *E. coli*) and SEL-treated LPS group (100 μg kg^{-1} SEL i.v.). SEL treatment resulted in a significant reduced number of firmly adhering leukocytes in intestinal submucosal venules and reduced significantly the impairment of the intestinal functional capillary density. Despite of the improvement of microcirculatory parameters, we did not detect any changes in the pattern of cytokine release. In conclusion, administration of high-dose sodium SEL attenuates leukocyte adhesion and improves capillary perfusion within the intestinal microcirculation without affecting release of the cytokines TNF-α, IL-1β, IL-6 and IL-10 in experimental endotoxemia.

Keywords: Selenium; Endotoxemia; Microcirculation; Cytokines

Introduction

Decreased plasma selenium (Se) concentrations are common in critically ill patients [1,2]. In septic patients, it could be shown that survival and severity of the disease correlate significantly with the Se levels at admission of the patients [3]. A recently published study proved the concept that therapeutic Se administration may improve outcomes in patients with severe systemic inflammatory response syndrome, sepsis, and septic shock [4].

Acute high-dose Se administration has different effects compared to selenium's nutritional functions [5]. The effects of Se compounds on cells are strictly compositional and concentration-dependent. Sodium

*Corresponding author at: Department of Anesthesia, Dalhousie University, 1278 Tower Road, 10 West, Victoria General Hospital, Halifax, Nova Scotia, Canada B3H 2Y9. Tel.: +1 902 473 4344; fax: +1 902 423 9454.
E-mail address: chlehmann@dal.ca (C. Lehmann).
[1]First and second author equally contributed to the manuscript.

0946-672X/$ - see front matter © 2008 Elsevier GmbH. All rights reserved.
doi:10.1016/j.jtemb.2008.09.003

selenite (SEL) – an anorganic Se compound – can be utilized for GPX resynthesis and thus exert antioxidative action if administered in nutritional doses during several days. However, acute high-dose administration may result in production of reactive oxygen species [6]. Paradoxically, this acute pro-oxidant effect may also be beneficial in sepsis treatment by reversible inhibition of NF-KB to DNA binding through a peri-genomic action [7], by a transient pro-apoptotic action on pro-inflammatory circulating cells [8] and by direct pro-oxidative bactericidal or virucidal action [9].

The effect of pharmaceutical application of high-dose SEL upon the impaired microcirculation in sepsis is not known. Therefore, the aim of the present study was to evaluate the effects of acute high-dose SEL administration on the intestinal microcirculation during experimental endotoxemia. The intestine serves as a pathologically significant circulatory region in cases of clinical sepsis (“intestine as the motor of multiorgan failure” [10]). It was also attempted to determine whether SEL administration affected the cytokine release by evaluating the blood levels of the cytokines TNF-α, IL-1β, IL-6 and IL-10.

Materials and methods

Animals

Thirty male outbred Lewis rats (200–250 g, 6–8 weeks old) were obtained from Charles River Laboratories, Sulzfeld, Germany, housed in chip-bedded cages and, prior to experiments, acclimated for 1 week in the air-conditioned institutional animal care unit. The animals were kept on a 12 h light/dark circadian cycle with free access to water (drinking bottle) and standard rat chow (Altromin®, Lage, Germany). Animal experiments were approved by the State Animal Protection Commission and performed in accordance with federal legislation on protection of animals.

Anesthesia and monitoring

All animals were initially anesthetized with 60 mg kg^{-1} pentobarbital i.p. (Synopharm, Barsbüttel, Germany). Fixation of the animals was carried out in supine position on a heating pad maintaining a rectal body temperature of 38 °C. Tracheotomy was performed to maintain airway patency, while animals breathed room air spontaneously. The left jugular vein and right carotid artery were cannulated with polyethylene catheters (PE50; inner diameter, 0.58 mm; outer diameter, 0.96 mm; Portex, UK). Arterial pressure and heart rate were recorded continuously (Hewlett-Packard-Monitor Model 66S, Böblingen, Germany). Reactions to painful stimuli – i.e. muscular tonus, breathing pattern, depth and frequency, tonus of whiskers, piloerection, heart rate and blood pressure were continuously observed and registered during the experiments.

General protocol

Experiments started 15 min postcannulation. The rats were divided into three groups of 10 animals each. For the maintenance of anesthesia, all groups received pentobarbital intravenously as incremental injections of about 4.0 mg pentobarbital every 60 min. Group 1 served as control (CON). Groups 2 (lipopolysaccharide—LPS) and 3 (LPS + SEL) received LPS (15 mg kg^{-1} i.v.; 5 min short infusion; lipopolysaccharide from *E. coli*, serotype O26:B6; Sigma-Aldrich Chemie, Steinheim, Germany). LPS was diluted in 1.0 mL 0.9% saline. To study the microcirculatory effects of endotoxemia without influences of hypotension, the adequate endotoxin dosage was identified in previous pilot experiments. In group 3 (LPS + SEL) 100 μg kg^{-1} SEL i.v. (Selenase, Biosyn, Fellbach, Germany) was administered immediately after LPS administration. The dosage was selected according to recent clinical trials [4,11] and adapted to the higher metabolism of rodents. The controls received an equivalent volume of saline only. All fluids that were given, including anesthesia and intra-arterial flushing, were calculated in order to guarantee that all of the animals received an equal amount of intravenous fluids (total volume, 15 mL kg^{-1} h^{-1}).

At the end of the experiments (2.5 h following onset) heparinized arterial blood samples (total volume, 1.5 mL) were drawn to determine gas exchange, acid–base status and hematocrit (ABL 330, Radiometer, Copenhagen, Denmark) as well as plasma release of the cytokines TNF-α, IL-1β, IL-6 and IL-10 (Rat Quantikine ELISA, R&D Systems, Wiesbaden, Germany).

Laparotomy for intravital fluorescence microscopy was performed 30 min prior to initiation of the microscopy (1.5 h following onset of the experiment). The abdomen was opened through a midline incision and a section of the distal small intestine was placed carefully on a specially-designed stage attached to the microscope. During the entire in vivo microscopic procedure intestine was superfused with thermostat-controlled (37 °C/98.6 °F) normal saline to avoid drying. The duration of each experiment, including induction of anesthesia, did not exceed 240 min. Routinely performed blood gas analyses (data not shown) indicated, that the experimental animals did not develop acute lung injury following endotoxin administration during the observation time. At the end of the experiments, death of the

animals were induced by barbiturate overdose (i.v. 120 mg kg^{-1} body weight).

Intravital fluorescence microscopy

Intravital fluorescence microscopy (IVM) was performed only once (2 h following onset of the experiment) to prevent damage due to the phototoxic effects of repeated, long-lasting light exposure of the tissues. An epifluorescent microscope was used (Axiotech Vario, filter block No. 20, Zeiss, Jena, Germany) with a 50-W short arc mercury lamp and equipped with a 20 × water immersion (20/0.5; Achroplan, Zeiss) objective and a 10 × eyepiece. Images were taken by a video camera (BC-12, AVT-Horn, Aalen, Germany), transferred to a monitor (PM-159, Ikegami Electronics, Munich, Germany) and simultaneously recorded onto videotape using a video cassette recorder (Panasonic NV-SV120EG-S, Matsushita Audio Video, Tokyo, Japan) for off-line evaluation.

Leukocyte-endothelial interaction

Leukocytes were stained in vivo prior to initiation of the IVM examination by intravenous injection of 0.1 mL of 0.05% Rhodamine 6G (Sigma-Aldrich) for contrast enhancement, enabling visualization within the microvasculature. Microvessels in the intestinal submucosal layer were classified by their order of branching according to Gore and Bohlen [12]. Submucosal collecting venules (V1) as well as postcapillary venules (V3) were analysed. Firmly adherent leukocytes were defined within each vessel segment as cells that did not move or detach from the endothelial lining within an observation period of 30 s, and are given as number of cells per mm^2 of endothelial surface, calculated from diameter and length of the vessel segment studied, assuming cylindrical geometry. Five vessels of each population were evaluated in every animal. Evaluation of FCD and leukocyte adherence was performed in a blinded fashion.

Functional capillary density

Two hours from the onset of the experiment 50 mg kg^{-1} FITC-labeled bovine serum albumin (5% in saline i.v.; Sigma-Aldrich, Steinheim, Germany) was administered intravenously to distinguish plasma from the red blood cells (negative contrast). Assessment of functional capillary density in the intestinal mucosa and the circular as well as the longitudinal muscle layer was performed by morphometric determination for the length of the red-blood-cell-perfused capillaries per/area. Five separate fields were examined within each intestinal wall layer, and filmed for 30 s to be analyzed off-line later.

Statistical analysis

Data analysis was performed with a statistical software package (SigmaStat, Jandel Scientific, Erkrath, Germany). After it was demonstrated that data conformed to tests of normality of distribution and equality of variance, they were analysed using a one-way

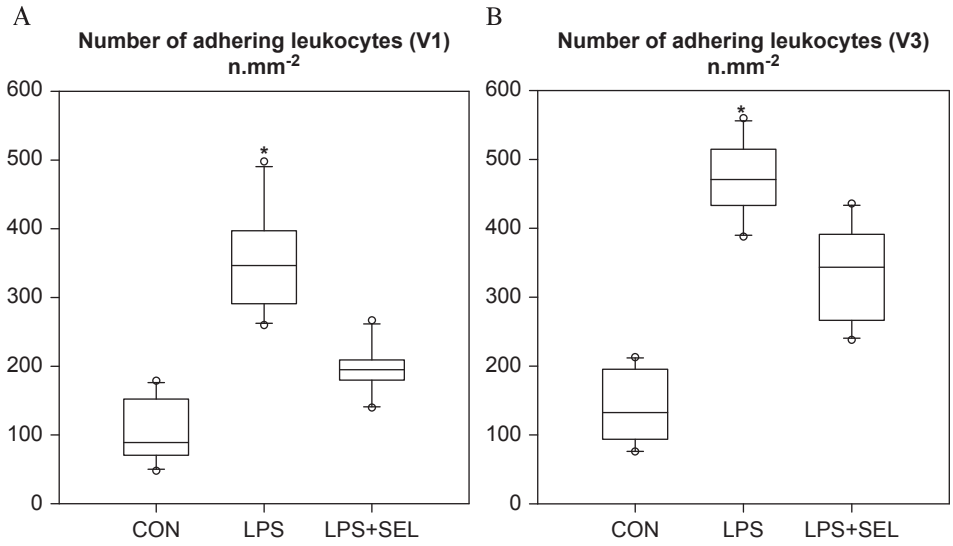

Fig. 1. Effects of sodium SEL administration on submucosal leukocyte-endothelial interaction 2 h after the beginning of the experiments; firmly adherent leukocytes in collecting venules (V1; Fig. 1A) and postcapillary venules (V3; Fig. 1B); n mm^{-2}; median, 25%, 75% confidence interval; CON = control, LPS = endotoxemia, LPS + SEL = sodium SEL treatment during endotoxemia. Significances: $*p < 0.05$ LPS vs. control group.

analysis of variance (ANOVA) followed by the Bonferroni corrected *t*-test for paired comparisons. Otherwise ANOVA on ranks was performed (post hoc Holm–Sidak test). A $p<0.05$ value was considered significant.

Results

The data of the adhesive behaviour of the leukocytes are shown in Fig. 1A and B. LPS induced leukocyte adhesion within the intestinal submucosa was significantly attenuated by SEL treatment ($p<0.05$). The number of adhering leukocytes to the endothelium was halved in V1 venules and reduced by one third in V3 venules.

Endotoxemia decreased drastically the mucosal FCD (Fig. 2A). Compared with the control group, nearly 50% of the mucosal capillaries were not perfused 2 h following endotoxin challenge. SEL administration appeared to attenuate the decrease of mucosal FCD in comparison to untreated animals ($p<0.05$). In addition, perfusion of the circular and longitudinal muscular layer of the intestinal wall was significantly diminished by endotoxemia and could be improved by SEL administration (Fig. 2B and C).

LPS administration resulted in an important cytokine release ($p<0.05$) which was unaffected by the SEL treatment (Fig. 3A–D). Arterial blood gas analyses along with hematocrit measurements revealed no pathological changes at the end of the experiments (2.5 h following onset; data not shown).

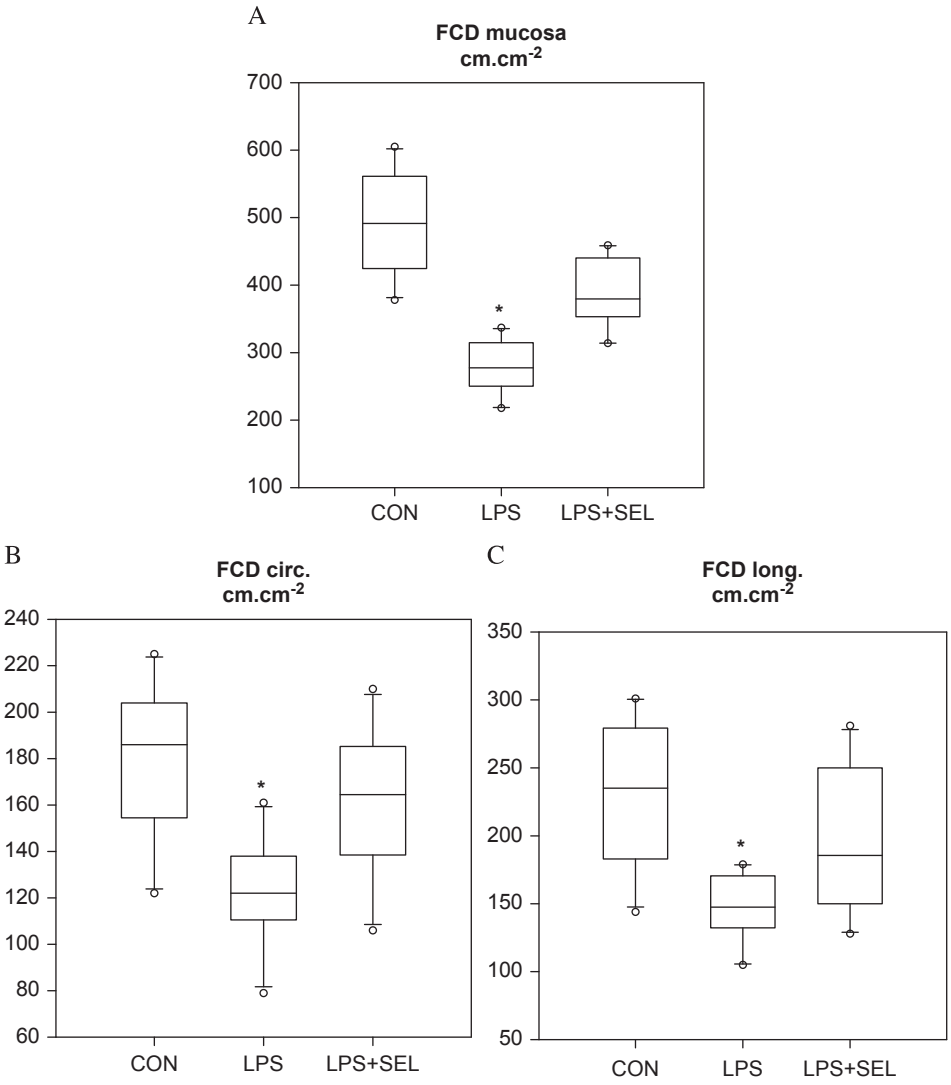

Fig. 2. Effects of sodium SEL administration on intestinal functional capillary density 2 h after the beginning of the experiments; intestinal functional capillary density (FCD) in the mucosa (FCD mucosa; Fig. 2A), the circular (FCD circ.; Fig. 2B) and longitudinal (FCD long.; Fig. 2C) muscle layer and ($cm\,cm^{-2}$); median, 25%, 75% confidence interval; CON = control, LPS = endotoxemia, LPS + SEL = sodium SEL treatment during endotoxemia. Significances: $*p<0.05$ LPS vs. control group.

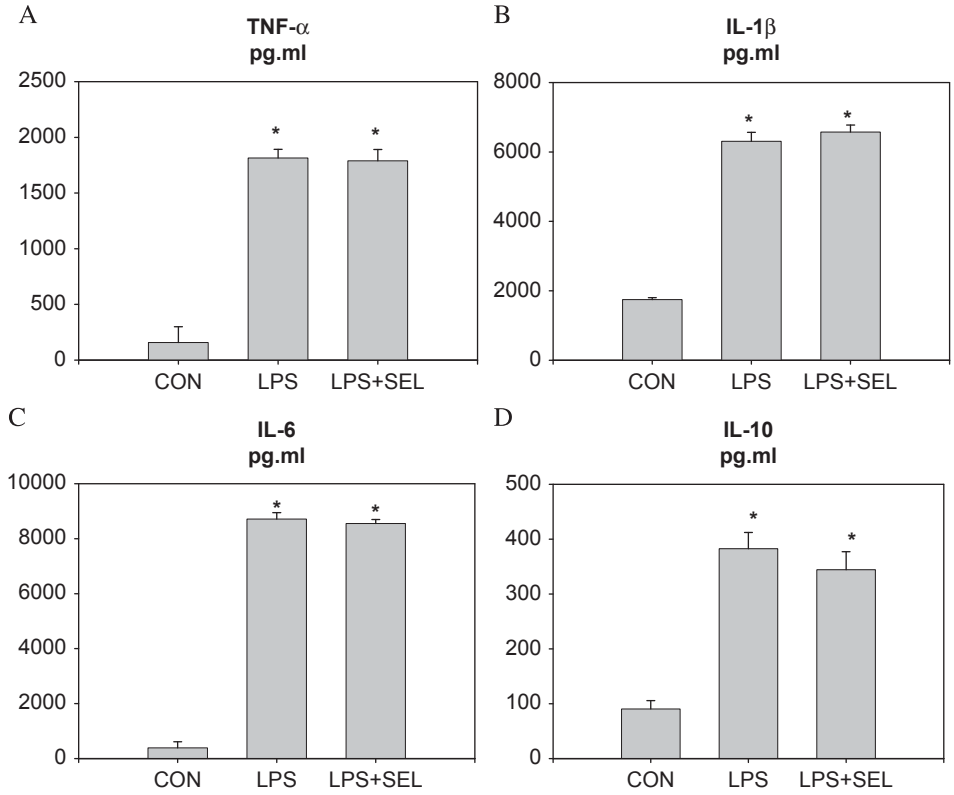

Fig. 3. Effects of sodium SEL administration on plasma cytokine release measured at the end of the experiments; pg ml; mean ± SD; CON = control, LPS = endotoxemia, LPS + SEL = sodium SEL treatment during endotoxemia. Significances: $*p < 0.05$ LPS vs. control group, LPS + SEL vs. control group.

Discussion

In this study, SEL treatment immediately following endotoxin challenge attenuated leukocyte adhesion and improved capillary perfusion within the intestinal microcirculation without affecting release of the cytokines TNF-α, IL-1β, IL-6 and IL-10.

One of the methodological strengths of intravital microscopy in microvascular research is the ability to visualize interactions between the blood cells and endothelium. During endotoxemia and sepsis a sequential activation of leukocytes can be observed: selectin-mediated temporary adhesion to the endothelium (rolling), integrin-mediated firm endothelial adhesion (sticking) and the transmigration process [13]. The firm adhesion of inflammatory cells to the endothelium is the pivotal step in this process. It is well-known, that reactive oxygen metabolites promote leukocyte-endothelial interactions in postcapillary venules [14,15]. Acute high-dose sodium SEL administration may result in production of reactive oxygen species [6]. However, this acute pro-oxidant effect may also cause reversible inhibition of NF-KB to DNA binding through a perigenomic action [7] and a transient pro-apoptotic action on pro-inflammatory circulating cells [8]. This acute effect of sodium SEL could be responsible for the significant decrease in leukocyte sticking in these experiments.

Zapletal et al. [16] found a reduction in the number of adherent leukocytes in liver sinusoids and venules by high-dose SEL administration in warm ischemia/reperfusion. In addition, Lindenblatt et al. [17] reported, that the oxidant stress-induced upregulation of P-selectin could be inhibited by ebselen, a seleno-organic compound. In pilot experiments we verified whether SEL by itself could affect normal microcirculation of the rat intestine and did not observe any changes.

The reduction in functional capillary density observed following endotoxin challenge is caused by different mechanisms, e.g. vasoconstriction, edema of the endothelial cells, intravascular coagulation or adhesion of blood cells [18]. FCD reduction in the intestinal mucosa is especially detrimental due to the impairment of the mucosal barrier function. If the mucosal barrier is deteriorated, bacteria and toxins may be translocated from the intestinal lumen to the systemic circulation [19]. Consequently, the protection of the capillary perfusion by SEL administration is an important finding. Also in the study of Zapletal et al. [16], the percentage of regular perfused sinusoids was increased

in high-dose SEL-treated animals during warm liver ischemia/reperfusion. The improvement of the functional capillary density appears to be closely related to the anti-adhesive effect on the leukocytes.

With regard to the effect of SEL administration on the cytokine release two different effects are possible: reduced cytokine production by inhibition of NF-KB to DNA binding [7] and/or increased cytokine production by stimulation of the oxidant-dependent NF-kB pathway [17]. The net effect on the inflammation remains questionable. We could not find an impact of SEL administration on the release of the cytokines TNF-α, IL-1β, IL-6 and IL-10 during endotoxemia. This may be also related to the short observation time or the SEL dosage.

Conclusions

Selenium is a trace element essential to human health. Acute high-dose anorganic selenium (SEL) administration has different effects compared to selenium's nutritional functions. Using leukocyte adhesion and capillary perfusion within the intestinal microcirculation as model, our experiments showed that high-dose SEL administration is able to reduce pathologic changes of microcirculation in animals with sepsis without affecting cytokine release.

Acknowledgements

The technical assistance of Barbara Egerer, Department of Anesthesiology and Intensive Care Medicine (Head: Prof. Dr. Claudia Spies), University Medicine Berlin, Germany, is gratefully acknowledged.

References

[1] Berger MM, Cavadini C, Chioléro R, Dirren H. Copper, selenium, and zinc status and balances after major trauma. J Trauma 1996;40:103–9.
[2] Lehmann C, Weber M, Krausch D, Wauer H, Newie T, Rohr U, et al. Parenteral selenium supplementation in critically ill patients – effects on antioxidant metabolism. Z Ernährungswiss 1998;37(Suppl. 1):106–9.
[3] Vitoux DD. Low plasma selenium in patients admitted in an intensive care unit is related to systemic inflammatory response syndrome and sepsis. In: Nève, et al. editors. Therapeutic use of trace elements. New York: Plenum Press; 1996. p. 127–31.
[4] Angstwurm MW, Engelmann L, Zimmermann T, Lehmann C, Spes CH, Abel P, et al. Selenium in intensive care (SIC): results of a prospective randomized, placebo-controlled, multiple-center study in patients with severe systemic inflammatory response syndrome, sepsis, and septic shock. Crit Care Med 2007;35:118–26.
[5] Heyland DK, Dhaliwal R, Suchner U. Berger MM: antioxidant nutrients: a systematic review of trade elements and vitamins in the critically ill patient. Intensive Care Med 2005;31:327–37.
[6] Seko Y, Saito Y, Kitahara J, Imura N. Active oxygen generation by the reaction of selenite with reduced glutathione *in vitro*. In: Wendel A, editor. Selenium in biology and medicine. Berlin: Springer; 1989. p. 70–3.
[7] Jeong DW, Yoo MH, Kim TS, Kim JH, Kim IY. Protection of mice from allergen-induced asthma by selenite: prevention of eosinophil infiltration by inhibition of NF-kappa B activation. J Biol Chem 2002;277:17871–6.
[8] Nilsonne G, Sun X, Nyström C, Rundlöf AK, Potamitou Fernandes A, Björnstedt M, et al. Selenite induces apoptosis in sarcomatoid malignant mesothelioma cells through oxidative stress. Free Radic Biol Med 2006;41:874–85.
[9] Stewart MS, Spallholz JE, Neldner KH, Pence BC. Selenium compounds have disparate abilities to impose oxidative stress and induce apoptosis. Free Radic Biol Med 1999;26:42–8.
[10] Marshall JC, Christou NV, Meakins JL. The gastrointestinal tract. The "undrained abscess" of multiple organ failure. Ann Surg 1993;218:111–9.
[11] Forceville X. Effects of high doses of selenium, as sodium selenite, in septic shock patients a placebo-controlled, randomized, double-blind, multi-center phase II study-Selenium and sepsis. J Trace Elem Med Biol 2007;21(Suppl. 1):62–5.
[12] Bohlen HG, Gore RW. Preparation of rat intestinal muscle and mucosa for quantitative microcirculatory studies. Microvasc Res 1976;11:103–10.
[13] Ley K. Leukocyte adhesion to vascular endothelium. J Reconstr Microsurg 1992;8:495–503.
[14] Suzuki M, Asako H, Kubes P, Jennings S, Grisham MB, Granger DN. Neutrophil-derived oxidants promote leukocyte adherence in postcapillary venules. Microvasc Res 1991;42:125–38.
[15] Haddad JJ. Oxygen sensing and oxidant/redox-related pathways. Biochem Biophys Res Commun 2004;316:969–77.
[16] Zapletal C, Heyne S, Golling M, Kraus T, Gebhard MM, Herfarth C, et al. Influence of selenium therapy on liver microcirculation after warm ischemia/reperfusion: an intravital microscopy study. Transplant Proc 2001;33:974–5.
[17] Lindenblatt N, Schareck W, Belusa L, Nickels RM, Menger MD, Vollmar B. Anti-oxidant ebselen delays microvascular thrombus formation in the rat cremaster muscle by inhibiting platelet P-selectin expression. Thromb Haemost 2003;90:882–92.
[18] Nolte D, Zeintl H, Steinbauer M, Pickelmann S, Messmer K. Functional capillary density: an indicator of tissue perfusion? Int J Microcirc Clin Exp 1995;15:244–9.
[19] Wiest R, Rath HC. Gastrointestinal disorders of the critically ill. Bacterial translocation in the gut. Best Pract Res Clin Gastroenterol 2003;17:397–425.