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Clinical studies

Effects of trace element supplementation on the inflammatory response in a rabbit model of major trauma

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ABSTRACT

Patients with a severe trauma exhibit a strong oxidative stress, an intense inflammatory response, and long-lasting hypermetabolism, all of which are proportional to the severity of injury. In this study, we investigated the impact of trace element (TE) supplementation on the inflammatory response in an animal model of major trauma. New Zealand White rabbits were randomly assigned as a control group (n=5) and an experimental group (n=70) that, after receiving a major trauma, was subdivided into Trauma-Control (n=35) and Trauma-TE (n=35) groups. Systemic inflammatory response syndrome (SIRS) was observed in 40 out of 70 rabbits with a trauma, with a higher incidence in the Trauma-Control group (88.6%; 31/35) than the Trauma-TE group (28.6%; 10/35) ($p < 0.01$). The mortality rate was significantly different between the Trauma-Control and the Trauma-TE groups; (34% vs. 8%; $p < 0.01$).

There were significant post-trauma alterations in the levels of (1) serum and spleen zinc (Zn), copper (Cu), selenium (Se), and manganese (Mn), (2) serum AST and ALT, (3) serum interleukin-6/10, and (4) nuclear factor kappa binding (NF-κB) activity and the expression. TE supplementation: (1) improved blood urea nitrogen (BUN), and creatinine (Cr) levels, (2) stabilized IL-6/10 production, (3) decreased NF-κB p^{65} production. Appropriate TE supplementation can improve the TE status, mitigate SIRS, and reduce the mortality due to multiple organ dysfunction syndromes (MODS)/multiple organ failure (MOF) after major trauma.

Introduction

After major trauma, patients develop characteristic endocrine, immune, and metabolic alterations [1]. Blood trace element (TE) level is low during any critical illness due to loss in biological fluid, low intake, dilution by fluid resuscitation, and redistribution from plasma to tissues mediated by the inflammatory response [2–4]. TE and the related metalloenzymes play important roles in a number of essential metabolic functions [6]. For example, Cu is essential for collagen synthesis and wound healing [7]; Se participates in the activation of the nuclear transcription factor NF-κB, controlling the inflammatory response [8]; Zn is essential in protein synthesis, wound healing, gene replication, and is involved in antioxidant defense and NF-κB expression [9]; and Mn has an important antioxidant (AOX) function in wound healing (mucopolysacharide and glycoprotein synthesis plus mitochondrial AOX defense through Mn-SOD) [10]. Also, TE is directly involved in wound healing [11]. TE status and the related metalloenzyme activities are reportedly altered in a rat model of burn [5], but the exact roles of TE in acute phase response to trauma are still elusive.

Several studies have demonstrated that pro-inflammatory cytokines such as IL-6 and TNF-α are transiently up-regulated in the serum of injured patients [12,13]. Furthermore, the concentration of pro-inflammatory cytokines has been shown to be correlated with the severity and prognosis of systemic inflammatory response syndrome (SIRS) that comprises fever, leukocytosis, and tachypnea [14–16]. On the other hand, serum IL-10, an anti-inflammatory cytokine, has been shown up-regulated in patients suffering from severe SIRS with or without infection [17]. Therefore, the pro- and anti-inflammatory cytokine responses could reflect, to some degree, the pathophysiologic status in acute inflammatory response. These pro-inflammatory cytokines could activate neutrophils [18,19]. The excessive neutrophil response has been implicated in the pathogenesis of SIRS and could lead to multiple organ dysfunction syndromes (MODS), which is a progressive dysfunction of one or more organ systems resulting from an exaggerated and prolonged inflammatory response to severe illness and/or injury [20–24].

Clinical biochemistry values, such as serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT), and blood urea nitrogen (BUN) and serum creatinine (Cr), can reflect liver

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and renal functions, respectively. TE could regulate the gene expression of pro- and anti-inflammatory cytokines, such as IL-6 and IL-10, in monocyte/macrophage cells [25]. The transcription factor NF-κB plays a critical role in the development and maintenance of T cell-mediated immune and inflammatory response [26,27].

Therefore, the current study aimed to investigate (1) the status of TE (Zn, Cu, Se, and Mn) and the related metalloenzymes (ALT, AST), BUN and Cr, cytokine response, and NF-κB after a major trauma, and (2) the effect of TE supplementation, using a rabbit model of major trauma.

Materials and methods

Animal experimentation

Seventy-five healthy male New Zealand White rabbits weighing 2.0 ± 0.12 kg from Southern Medical University, China, were used in this study. All animal procedures were approved by the Animal Care and Use Committee of the Medical College of Shantou University. Prior to experiments, rabbits were quarantined for 2 weeks for adaptation to the local conditions. They were housed individually at 25 ± 0.5 °C with a relative humidity of 55% and on a 12 h light/dark cycle with food and water available ad libitum.

Rabbits were randomly assigned as control group (n=5) and experimental group (n=70). Under general anesthesia, multiple injuries were inflicted on limbs and pubic joints of the rabbits in the experimental group. The wounded limbs were wrapped in gauze and externally fixed with splints. Following the human Injury Severity Score (ISS) system, the ISS was calculated as the sum of the squared cores of the most severely injured body regions as described previously [28]. According to the Abbreviated Injury Scale (AIS-90) [29], we developed a rabbit model under general major trauma Injury model was 27. The experimental group was randomly divided into Trauma-Control group (n=35) and Trauma-TE group (n=35).

At 6 h post-trauma, according to the normal oral requirement for the various trace elements, each rabbit in the Trauma-Control group was given 100 ml of 10% glucose intravenously, whereas the Trauma-TE group received 100 ml of 10% glucose supplemented with 0.1 ml multi-TE compound, Addamel (Cr^{3+} 0.02 mmol, Cu^{2+} 2 μmol, Zn^{2+} 10 μmol, Fe^{3+} 2 μmol, Mn^{2+} 0.5 μmol, MoO_4^{2-} 0.02 μmol, SeO_3^{2-} 0.04 μmol, F^- 5 μmol, I^- 0.1 μmol/ml) (Fresenius Kabi AG, BadHomburg, Germany), to give a daily dose of 32.50 μg/kg of Zn, 6.35 μg/kg of Cu, 1.38 μg/kg of Mn, and 0.16 μg/kg of Se. During Days 1–5 (D1–D5) post-trauma, each rabbit in the Trauma-Control group received 20 ml of 10% glucose intravenously, while the Trauma-TE group received 20 ml of 10% glucose plus 0.1 ml TE intravenously. The amount of trace elements of Trauma-Control and Trauma-TE group were shown (Table 1).

Blood (1.5 ml) was collected from an ear-edge vein from all rabbits in the control group (D0) and the experimental groups at 6 h, D1, D3, D6, D9, and D14 post-trauma. Serum were separated within 2–3 h after collection and stored at −80 °C till used.

Maximum 5 rabbits each were sacrificed by carotid bloodletting at D0 (the control group) and D1, D3, D6, D9, and D14 (the experimental groups), and the spleens were collected and stored at −80 °C till used.

Defined SIRS

SIRS (systemic inflammatory response syndrome) was defined as the combination of an elevation of fever, leukocytosis, tachypnea in rabbit.

Biochemical and TE analyses

Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), and creatinine (Cr) were determined using a Hitachi Automatic Analyzer 7170 (Hitachi, Japan). Serum and spleen Se, Zn, Cu, and Mn concentrations were determined by flame atomic absorption spectrophotometry (AA-6800, Shimadzu corporation, Japan).

Measurement of cytokines in serum

The serum concentration of IL-6 and IL-10 was measured by a sandwich ELISA using a Rabbit IL-6/IL-10 ELISA kit (R&D Systems, Minneapolis, MN) following the manufacturer's instructions.

Measurement of NF-κB p65 activity and concentration

Spleen nuclear extracts were prepared according to the method previously described [30], and stored at −80 °C till used.

The activity of NF-κB p65 in the nuclear extracts was examined using a TransAM NF-κB p65 kit (Active Motif, Carlsbad, CA), in accordance with the protocols provided with the kit. The TransAM kit contains a 96-well plate coated with oligonucleotides containing either an NF-κB consensus binding site or a TPA-responsive element (TRE). The activated NF-κB dimers in nuclear extracts specifically bound to these oligonucleotides can be detected using specific antibodies. NF-κB p65 concentration in spleen was measured using a BCA protein assay kit (Pierce, Rockford, IL).

Statistical analysis

Data were analyzed using an SPSS software, version 13.0 (SPSS Inc., Chicago, Illinois, USA). Statistical analyses were performed by ANOVA and Student-Newman-Keuls tests. The P value of < 0.05 was considered statistically significant and $p < 0.01$ was considered statistically highly significant.

Table 1
The changes of trace elements in Trauma-Control and Trauma-TE groups.

Trace element	Normal oral requirement (μg/kg d)	Intravenous amount (μg/kg d)	Probable absorbed (%)	Absorbed amount (μg/kg d)	TE amount	
					Trauma-Control	Trauma-TE
Copper	4.6	6.35	40	1.84	↓	↑
Zinc	18.6	32.50	20	3.6	↓	↑
Manganese	31.4	1.38	3	0.94	↓	↑
Selenium	0.45	0.16	80	0.36	↓	↓