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Research Article
Investigation of Simvastatin on Micro and Macro Element Levels in Intestinal Tissue for During Early Phase of Sepsis

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Abstract

Background: There are limited studies in the literature micro and macro elements of level during early phase of sepsis. The changes of micro and macro element levels plays important roles normal physiologic states and pathological states especially inflammation. Intestine is main organ for absorption of element. There are a lot of studies shown that simvastatin possess potent anti-inflammation and antioxidation capacity. But it is not clear simvastatin on element levels and its mechanism. **Aim:** This study aimed to observe the effect of prior treatment with simvastatin on elements in intestinal tissue of rats during the early-phase of sepsis. **Methodology:** Rats were divided into four groups: control, lipopolysaccharide (LPS) (20 mg kg^{-1}, i.p.), simvastatin (20 mg kg^{-1}, p.o.) and the simvastatin+LPS group. Selenium, zinc, iron, manganese, magnesium, calcium, sodium, copper and potassium element levels in intestinal tissue were analyzed by inductively coupled plasma-optical emission spectroscopy (icp-oes). This study measured serum and tissue levels of Tumor Necrosis Factor-α (TNF-α), interleukin-10 (IL-10) using ELISA. Serum and tissue TNF-α and IL-10 levels were higher in the simvastatin+LPS and LPS groups ($p<0.05$). The IL-10 levels was found higher in simvastatin+LPS group compared to LPS group ($p<0.01$). In the simvastatin+LPS group, levels of calcium and sodium were lower than those of LPS and controls ($p<0.05$ and $p<0.01$, respectively). **Results:** The levels of zinc, iron, potassium and copper were found increased in the simvastatin+LPS group compared with LPS group ($p<0.05$). The present study simvastatin have important effect on element metobolism especially zinc, copper, iron, potassium during sepsis. **Conclusion:** This study suggests that prior treatment with simvastatin may be effective in reducing tissue damage by increasing levels of zinc, copper, iron, potassium and IL-10 and decreasing levels of sodium and calcium in septic rats.

Key words: Elements, intestine, sepsis, simvastatin

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Sepsis is a complex syndrome that results from a systemic inflammatory response to infection and is the leading cause of death in critically ill patients[1]. Sepsis triggers an excessive inflammatory response with release of secondary mediators, including cytokines and the generation of Reactive Oxygen Species (ROS)[2]. Systemic injection of lipopolysaccharide (LPS) to experimental animals is a widely-used *in vivo* model for the study of endotoxic shock and acute systemic inflammation. The LPS activates the immune system leading to the release of endogenous proinflammatory cytokines such as Tumor Necrosis Factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6)[3].

Intestinal tissue contains endogenous and exogenous microorganisms that can sometimes become potential pathogens of sepsis. Intestinal barrier integrity is an important defense against microbial pathogens. Its damage may lead to the escape of microbial pathogens from the gut into the body, which induces an exaggerated inflammatory response and consequently promotes multiple organ dysfunction and failure[4].

It is known that ROS increase in organisms when deprived of micronutrients during sepsis[5]. Systemic inflammation causes a redistrubution of micronutrients from the circulating compartment to tissues and organs that are involved in immune defense. It has been reported that the circulation total iron, zinc, copper and selenium changed during inflammation[6].

Trace minerals include macrominerals such as calcium, magnesium, phosphorus and sulfur and trace elements, which are defined as those existing in less than one part per million body weight, e.g., zinc, copper, manganese, selenium, iron, iodine, chromium, molybdenum[7]. Trace elements are essential for direct antioxidant activity as well as functioning as cofactors for a variety of antioxidant enzymes[8].

Selenium, zinc, iron, manganese and copper have an antioxidant capacity and the elements magnesium, calcium, sodium and potassium are essential trace minerals for cellular function. Trace minerals and trace elements status could influence oxidative stress and inflammatory responses in patients with sepsis.

Experimental and observational studies have shown that statin therapy reduces inflammatory cytokines[9]. Statins are 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors[10]. In the literature, there have been shown antioxidant and anti-inflammatory properties of statins to preventing in the generation of oxidative stress species by affecting on trace element status such as iron, zinc, selenium, copper in microvascular function, angiogenesis, atherosclerosis, cardiovascular diseases. However, there are a few studies about the effects of statins on the level of micro and macroelements during sepsis[11,12].

This study aimed to investigate the effects of prior treatment with simvastatin on the levels of selenium, zinc, iron, manganese, magnesium, calcium, sodium, copper and potassium and on intestinal tissue during the early phase of sepsis.

MATERIALS AND METHODS

Experimental groups: This study was conducted at the Experimental Research Center, University of Istanbul (Resolution No. 2012/138). Male adult Wistar albino rats that weighed 200-250 g were used in the experiments. The animals were fed a commercial diet and provided tap water *ad libitum.* The rats were housed in cages and kept at a controlled temperature (22±2°C) and humidity (55-60%) with a 12 h light/dark cycle.

A thirty-two rats were divided into four groups, each composed of eight rats, (1) control group, (2) LPS-treated group, (3) simvastatin-treated group and (4) simvastatin plus LPS-treated group.

Experimental procedures: Lipopolysaccharide from *Escherichia coli* O127:B8 (Sigma, St. Louis, MO) was dissolved in 1 mL of sterile saline solution and a single dose of 20 mg kg^{-1} was injected intraperitoneally and simvastatin (20 mg kg^{-1}) (Sigma) was given p.o. via oral gavage for 5 days. In the simvastatin plus LPS-treated group, LPS was given 1.5 h after the fifth dose of simvastatin. All animals that were treated with LPS (LPS and simvastatin+LPS groups), simvastatin and control groups were sacrified after 4 h.

Cytokine levels: Blood samples were centrifuged at 2500 rpm for 20 min and the serum was stored in eppendorf tubes at -20°C until analysis. The cytokine levels were measured using enzyme-linked immune assay (ELISA) in serum and tissue for TNF-α (BioSource, Invitrogen, USA, Cat No KRC3012) and IL-10 (BioSource, Invitrogen, USA, Cat No, KRC0101). The absorbance values were measured at 450 nm using a micro-ELISA automatic analyzer.

Analyze of elements levels: The levels of selenium, zinc, iron, manganese, magnesium, calcium, sodium, copper and potassium in intestinal tissue were analyzed by inductively

coupled plasma-optical emission spectroscopy (icp-oes) (PerkinElmer-Optima 7000 DV). The tissues about 0.200/0.250 g were mesured and were taken in falcon tubes and incubated in a microwave with 10 mL 20% nitric acid (HNO_3) at 145°C for 5 min and 165°C for 5 min, 175°C for 20 min. After cooling, the samples were filtered using Whatman filters and made up to 50 mL with ultrapure water in volumetric flasks and then stored in falcon tubes. The samples were then read using icp-oes with a 50 ppb^{-1} ppm standard. Results were calculated based on the unit mg kg^{-1} and dilution.

Histologic procedures: The intestinal tissue samples were fixed in 10% buffered formalin and embedded in parafin wax. Five-micrometer-thick sections were placed on polylysine-coated slides and stained with Hematoxylin and Eosin (H and E). The slides were evaluated under light microscopy (Olympus BX51; Olympus Corp, Tokyo, Japan) at 40× magnification.

Statistical analysis: The data were expressed as Mean±Standard deviation. Overall statistical significance between the groups was tested with a oneway ANOVA and Turkey as a *post hoc* test. In all cases p<0.05 was set as limit of significance.

RESULTS

Findings of cytokine levels: Serum TNF-α and IL-10 levels were found higher in simvastatin+LPS and LPS groups compared the other groups (p<0.05) (Fig. 1). Intestinal tissue TNF-α and IL-10 were determined by the increase in simvastatin+LPS and LPS groups (p<0.01). Moreover, IL-10 levels was found higher in simvastatin+LPS group compared to LPS group (p<0.01) (Fig. 1a-d).

Histology findings: This study examined the intestinal tissue sections, the structural morphology of the simvastatin group was the same as the controls. In the LPS group, deterioration of the structural villi was observed together with villi damage in apical area especially and determined injury to epithelial cells. In simvastatin+LPS group, improvements of damaged tissue which were occurred after LPS treatment (Fig. 2a-d).

Findings of element levels: There were decrements in the tissue levels of selenium in both the LPS and simvastatin+LPS groups compared with those of controls (p<0.01, for both).

There were decrements in the tissue levels of iron in both the LPS and simvastatin+LPS groups compared with

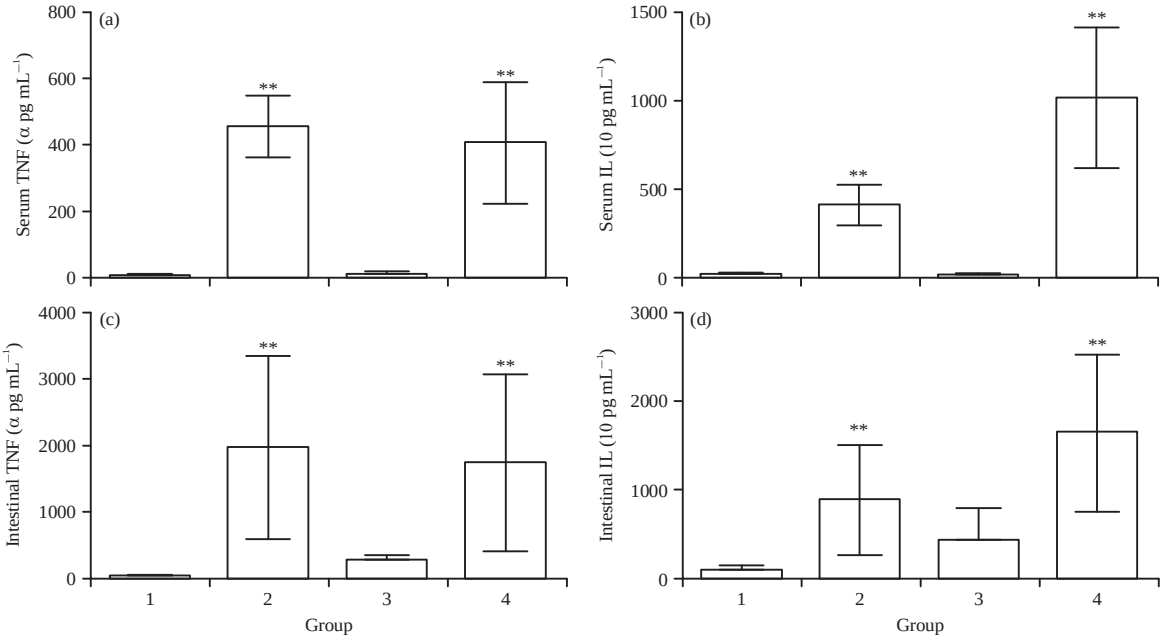

Fig. 1(a-d): Cytokine values in groups 1-4 of experimental rats and within groups (control group 1, LPS group 2, simvastatin group 3, simvastatin+LPS group 4), (a) Serum TNF-α values in experimental groups, (b) Serum IL-10 values in experimental groups, (c) Intestinal TNF-α values in experimental groups. **Significant differences at p<0.01, LPS and simvastatin+LPS vs. other groups and (d) Intestinal IL-10 values in experimental groups. **Significant differences at p<0.01 simvastatin+LPS and LPS groups vs control group and simvastatin+LPS group vs LPS group

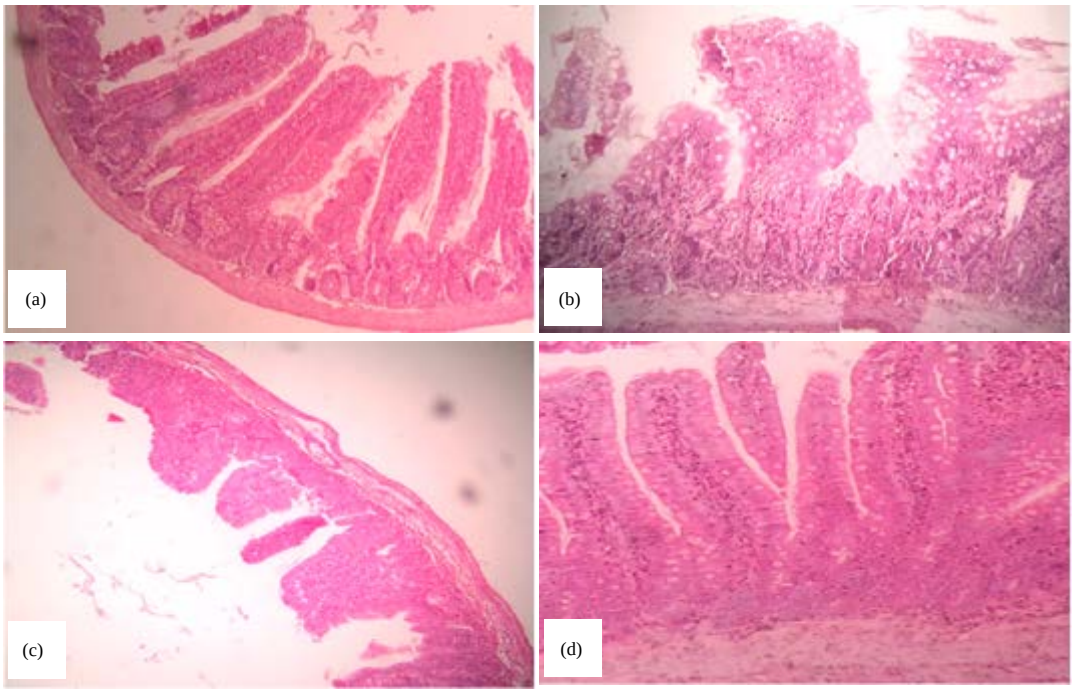

Fig. 2(a-d): Section of intestinal tissue stained with H and E. Section of liver tissue from (a) Control group stained with H and E, x40 magnification, (b) Section of intestinal tissue from control group, x40 magnification, (c) LPS group, x40 magnification and (d) Simvastatin group, x40 magnification. Simvastatin+LPS group, x40 magnification

those of controls ($p<0.01$ and $p<0.05$, respectively). In the simvastatin+LPS group, iron levels were increased compared the LPS ($p<0.05$).

In the simvastatin and LPS group, zinc levels were decreased compared the controls ($p<0.05$, for both). In the simvastatin+LPS group, zinc levels were increased compared with those of LPS and simvastatin groups (both $p<0.05$).

There were significant decrements in the tissue levels of manganese in both the LPS and simvastatin+LPS groups compared with those of controls ($p<0.01$, for both).

In the LPS groups, copper levels were lower than in the controls ($p<0.01$).

In the LPS group, tissue sodium levels were higher than in any of the other experimental groups and control group ($p<0.05$). In the simvastatin+LPS groups tissue sodium levels were lower than in the controls ($p<0.01$).

There were decrements in the tissue levels of potassium in both the LPS and simvastatin+LPS groups compared with those of controls ($p<0.01$, for both). In the simvastatin+LPS group, potassium levels were increased compared the LPS ($p<0.05$).

There were decrements in the tissue levels of magnesium in both the LPS and simvastatin+LPS groups compared with those of controls ($p<0.01$, for both).

When it is compared the ratio of copper zinc (copper/zinc ratio), there were decreased of ratio in LPS groups than that of controls ($p<0.01$). There was increased of ratio in simvastatin+LPS groups than that of LPS ($p<0.01$) (Fig. 3a-j).

DISCUSSION

The LPS initiates a cascade of signaling reactions to the expression of inflammatory cytokines chemokines and other inflammatory markers[13]. This study determined significantly increased levels of serum and tissue TNF-α and IL-10 in in the simvastatin+LPS and LPS groups compared with controls. Also tissue IL-10 level was higher in simvastatin+LPS group than LPS group. The LPS-induced endotoxemia in pigs elicited a pro- and anti-inflammatory cytokine response detectable in peripheral blood and at the organ level[14]. Experimental data suggest that Tumor Necrosis Factor (TNF) is a pivotal endogenous mediator of septic and endotoxic shock[15].

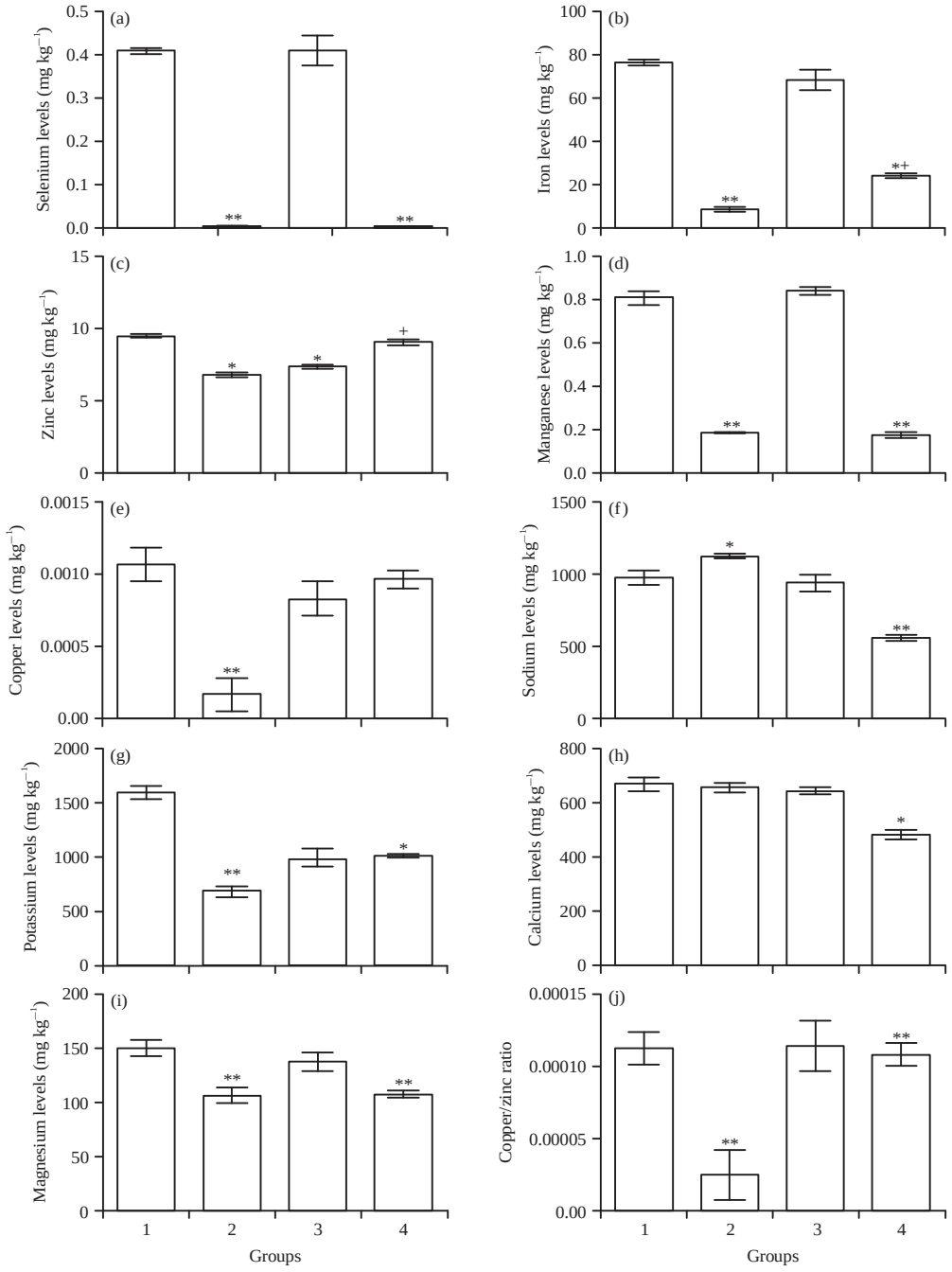

Fig. 3(a-j): Amounts of elements (mg kg^{-1} tissue) in groups 1-4 of experimental rats and within groups (control group 1, LPS group 2, simvastatin group 3, simvastatin+LPS group 4). Intragroup Mean±SD are shown. *: Cases of significant differences ($p<0.05$), **: Cases of significant differences ($p<0.01$), (a) Amounts of selenium in groups; **Control vs LPS and simvastatin+LPS groups, (b) Amounts of iron in groups; **+Control vs LPS and simvastatin+LPS groups; *LPS vs simvastatin+LPS group, (c) Amounts of zinc in groups; *Control vs LPS and Simvastatin groups, *LPS and simvastatin vs simvastatin+LPS group, (d) Amounts of manganese in groups; **Control vs LPS and simvastatin+LPS groups, (e) Amounts of copper in groups; **Control vs LPS group, (f) Amounts of sodium in groups; **Control vs simvastatin+LPS groups; *Control vs LPS, (g) Amounts of potassium in groups; **Control vs LPS and simvastatin+LPS group; *LPS vs simvastatin+LPS group, (h) Amounts of calcium in groups; *simvastatin+LPS group vs Control and LPS groups, (i) Amounts of magnesium in groups; **Control vs LPS and simvastatin+LPS groups and (j) Ratio of copper/zinc; **LPS vs control and simvastatin+LPS vs control

The IL-10 plays a beneficial role in gut-derived sepsis model, the cecal ligation and puncture model and various other models of infection[16].

The LPS causes also alterations in plasma levels of trace elements. The most common responses include a decrease in the plasma concentrations of iron and zinc and an increase in plasma copper. This triad of trace element changes has been reported in bacterial, viral, rickettsial and parasitic infections[17]. This study investigate the effects of simvastatin at a dosage of 20 mg kg^{-1} for 5 days on element status during the early phase of sepsis.

For decades LPS has been known to have influences on zinc and iron metabolism, particularly for producing hypozincemia and hypoferremia[18]. Zinc is an essential micronutrient that has numerous biologic roles and its deficiency increases susceptibility to infection. Zinc and copper are essential trace elements and function as co-factors of antioxidant enzymes[19]. Low zinc concentrations would promote oxidative stress and result in more pronounced inflammatory responses[20]. Hypozincemia has been suggested to support production of acute phase proteins, restriction of zinc from acquisition systems of pathogens, immune cell function and as shown in various systems, regulation of signaling pathways including inhibition of phosphatases[21]. In the present study, it is found that levels of zinc were decreased in both the LPS and simvastatin groups compared with the controls, but its level was higher in the simvastatin+LPS group than in the LPS and simvastatin groups. There were similar zinc level results in the simvastatin+LPS and control groups. In parallel to this, a previous study reported that stating treatment was associated with a significant reduction in mean serum zinc and these effects may be due in part to the anti-inflammatory properties of statins[22]. Hypoferremia is also associated with inflammation and infection. Mechanisms that result in reduced serum iron in response to both acute and chronic stimuli focus on the regulatory peptide hepcidin[23]. Hypoferremia has been suggested as a host defense process to restrict iron from pathogens. Redistribution of iron to maintain energy metabolism in specific tissues is another likely reason for regulated tissue iron accumulation during inflammation and infection[24]. It was observed the decrements of iron levels in both the LPS and simvastatin+LPS groups compared to controls, also its level was increased in the simvastatin+LPS group compared with the LPS and simvastatin groups. Shanbhogue and Paterson[25] found that serum iron levels were significantly decreased in adults with sepsis compared with controls. In recent study, acute inflammation resulted in hypoferremia, which was associated with an increased expression of hepcidin in the liver and ferritin in the duodenum[26].

Oxidative stress is a major contributing factor to the high mortality rates associated with several diseases, such as septic shock. Like sepsis, in critically ill patients, there are reduced stores of antioxidants, which have been associated with an increase in free radical generation, an augmentation of the systemic inflammatory response, subsequent cell injury, increased organ failure and even higher mortality[27]. The body has extensive defense mechanisms against the ROS produced during sepsis and other critical illnesses. One of these mechanisms involves a group of proteins that utilize selenium, copper and manganese[28]. Copper is known to play an important role in the immune system. Copper deficiency could reduce neutrophil function and down regulate inflammatory cytokine expression[29]. This study found a greater reduction of copper levels in the LPS group than in the controls; the results of simvastatin+LPS group were similar to the controls. In bacterial infections (septicemia, pneumonia and meningitis), the plasma concentrations of selenium, iron and zinc were decreased[30]. Manganese serves as a component of superoxide dismutase and is also important for arginine and pyruvate metabolism[31]. Copper and manganese constitute the active centres of CuZn-SOD and MnSOD, respectively. Hence, a deficiency of these metal ions would be expected to lead to a decrease in the activity of these enzymes[32]. Substantial decrements of manganese levels were determined in both the LPS and simvastatin+LPS groups.

Serum copper/zinc ratio has been used as a prognostic parameter in some disease processes by several investigators[33]. When the ratio of copper and zinc evaluated, the decrements in LPS group compared to that of control. Also, this study found the levels of copper and zinc ratio in simvastatin+LPS group as similar as controls. Serum zinc and copper levels and copper/zinc ratio were lower in infants with sepsis compared to healthy infants and infants with mild infection[34].

Selenium is an important component of a subset of enzymes called selenoenzymes. The most important member of this group of enzymes is glutathione peroxidase. There are multiple subtypes of glutathione peroxidase that play an important role in reducing hydrogen peroxide within the cytosol of cells and offer protection against oxygen free radical damage[35]. Significant reductions in tissue levels of selenium were found in both the LPS and simvastatin+LPS groups compared with the controls. Decreased plasma selenium concentrations are common in critically ill patients[36]. Ghayour-Mobarhan *et al.*[22] reported that no significant effects were seen in serum selenium in either of their statin or control groups. Selenium has been identified as the corner stone of the endogenous defence against oxidative stress and serum selenium levels correlate inversely with the severity of sepsis[37].

Magnesium plays an essential role in controlling immune phenomena. Previous data have demonstrated that magnesium sulfate has strong anti-inflammatory properties[38]. Magnesium sulfate could inhibit LPS-induced release of TNF, IL-1, IL-6 and IL-10 both *in vivo* and *in vitro*[39]. In this study, it is found significantly lower magnesium levels in the LPS and simvastatin+LPS groups than in the controls.

The calcium ion plays an important part in many cellular functions. Several studies over the past few years have implicated endotoxin-mediated alterations in transmembrane Ca^{2+} movements and cellular Ca^{2+} homeostasis[40].

The levels of calcium were determined to decrease in simvastatin+LPS group compared with both of in LPS and controls. There was also decrement in the LPS group but this difference did not reach significancy. Endotoxin acts through inducing calcium influx from extracellular space to produce a transient increase in intracellular calcium level. The endotoxin-induced transient calcium increase is crucial in regulating signaling pathways expression and subsequent inflammatory molecule production during endotoxemia[41]. Sakaguchi and Furusawa[28] demonstrated that oxidative stress caused by endotoxin can decrease the levels of scavengers or quenchers of free radicals and that calcium may participate in free radical formation during endotoxemia.

Natrium and potassium homeostasis is vitally important for organ function, high energy phosphate metabolism, cellular volume and osmolarity, cardiac muscle activity and piruvate kinase activity[42]. A few studies have shown that sodium and potassium levels change in sepsis[43,44]. In the present study, lower sodium levels were seen in the simvastatin+LPS group compared with controls; however, its level was higher than any other group in the LPS group. The levels of potassium were decreased in the LPS and simvastatin+LPS groups compared with the controls and it was also higher in the simvastatin+LPS group than in the LPS group.

Several studies have demonstrated that statins have anti-inflammatory properties; they reduce the inflammatory cell content and cytokine expression by plaque cells[45]. Other investigators have reported that statins have antioxidant properties[46,47].

In this study, there was significant damage to the intestinal villi, epithelial and goblet cells in the intestinal sections of LPS group. Cell injury was reduced in the histologic sections of the simvastatin+LPS group. This study determined prior treatment with simvastatin decreased intestinal damage caused by cytokines during the early phase of sepsis.

CONCLUSION

In conclusion, the prior use of simvastatin functions as a protective agent against intestinal tissue damage during sepsis by increasing zinc, copper, iron and potassium and IL-10 levels and decreasing sodium and calcium levels.

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REFERENCES

1. Hotchkiss, R.S. and I.E. Karl, 2003. The pathophysiology and treatment of sepsis. N. England J. Med., 348: 138-150.
2. Zarjou, A. and A. Agarwal, 2011. Sepsis and acute kidney injury. J. Am. Soc. Nephrol., 22: 999-1006.
3. Ando, H., T. Takamura, T. Ota, Y. Nagai and K.I. Kobayashi, 2000. Cerivastatin improves survival of mice with lipopolysaccharide-induced sepsis. J. Pharmacol. Exp. Ther., 294: 1043-1046.
4. Suliburk, J., K. Helmer, F. Moore and D. Mercer, 2008. The gut in systemic inflammatory response syndrome and sepsis: Enzyme systems fighting multiple organ failure. Eur. Surg. Res., 40: 184-189.
5. Dupertuis, Y.M., M.M. Meguid and C. Pichard, 2009. Advancing from immunonutrition to a pharmaconutrition: A gigantic challenge. Curr. Opin. Clin. Nutr. Metab. Care, 12: 398-403.
6. Han, M., Z. Lin and Y. Zhang, 2013. The alteration of copper homeostasis in inflammation induced by lipopolysaccharides. Biol. Trace Elem. Res., 154: 268-274.
7. Agarwal, A., P. Khanna, D.K. Baidya and M.K. Arora, 2011. Trace elements in critical illness. J. Endocrinol. Metab., 1: 57-63.
8. Demling, R.H. and M.A. DeBiasse, 1995. Micronutrients in critical illness. Crit. Care Clin., 11: 651-673.
9. Viasus, D., C. Garcia-Vidal, F. Gudiol and J. Carratala, 2010. Statins for community-acquired pneumonia: Current state of the science. Eur. J. Clin. Microbiol. Infect. Dis., 29: 143-152.
10. Cordle, A., J. Koenigsknecht-Talboo, B. Wilkinson, A. Limpert and G. Landreth, 2005. Mechanisms of statin-mediated inhibition of small G-protein function. J. Biol. Chem., 280: 34202-34209.
11. Ky, B., A. Burke, S. Tsimikas, M.L. Wolfe and M.G. Tadesse *et al*., 2008. The influence of pravastatin and atorvastatin on markers of oxidative stress in hypercholesterolemic humans. J. Am. Coll. Cardiol., 51: 1653-1662.

12. Zacharski, L.R., R.G. DePalma, G. Shamayeva and B.K. Chow, 2013. The statin-iron nexus: Anti-inflammatory intervention for arterial disease prevention. Am. J. Public Health, 103: e105-e112.
13. Yadav, U.C., S.K. Srivastava and K.V. Ramana, 2007. Aldose reductase inhibition prevents endotoxin-induced uveitis in rats. Invest. Ophthalmol. Vis. Sci., 48: 4634-4642.
14. Brix-Christensen, V., C. Vestergaard, S.K. Andersen, J. Krog and N.T. Andersen *et al*., 2005. Evidence that acute hyperinsulinaemia increases the cytokine content in essential organs after an endotoxin challenge in a porcine model. Acta Anaesthesiologica Scandinavica, 49: 1429-1435.
15. Beutler, B., I.W. Milsark and A.C. Cerami, 1985. Passive immunization against cachectin/tumor necrosis factor protects mice from lethal effect of endotoxin. Science, 229: 869-871.
16. Mosmann, T.R., 1994. Properties and functions of interleukin-10. Adv. Immunol., 56: 1-26.
17. Beisel, W.R., 1998. Metabolic Response of the Host to Infections. In: Textbook of Pediatric Infectious Diseases, Feigen, R.D. and J.D. Cherry (Eds.). Saunders, Philadelphia, pp: 54-69.
18. Cousins, R.J., 1985. Absorption, transport and hepatic metabolism of copper and zinc: Special reference to metallothionein and ceruloplasmin. Physiol. Rev., 65: 238-309.
19. Rink, L. and P. Gabriel, 2000. Zinc and the immune system. Proc. Nutr. Soc., 59: 541-552.
20. Prasad, A.S., B. Bao, F.W.J. Beck and F.H. Sarkar, 2011. Zinc-suppressed inflammatory cytokines by induction of A20-mediated inhibition of nuclear factor-κB. Nutrition, 27: 816-823.
21. Haase, H. and L. Rink, 2009. Functional significance of zinc-related signaling pathways in immune cells. Annu. Rev. Nutr., 29: 133-152.
22. Ghayour-Mobarhan, M., D.J. Lamb, A. Taylor, N. Vaidya, C. Livingstone, T. Wang and G.A.A. Ferns, 2005. Effect of statin therapy on serum trace element status in dyslipidaemic subjects. J. Trace Elem. Med. Biol., 19: 61-67.
23. Means, R.T., 2004. Hepcidin and cytokines in anaemia. Hematology, 9: 357-362.
24. Armitage, A.E., L.A. Eddowes, U. Gileadi, S. Cole and N. Spottiswoode *et al*., 2011. Hepcidin regulation by innate immune and infectious stimuli. Blood, 118: 4129-4139.
25. Shanbhogue, L.K.R. and N. Paterson, 1990. Effect of sepsis and surgery on trace minerals. J. Parent. Enteral Nutr., 14: 287-289.
26. Sukumaran, A., J. Varghese, J. Tamilselvan, V. Jeyaseelan and T. Mani *et al*., 2012. Effects of acute and chronic inflammation on proteins involved in duodenal iron absorption in mice: A time-course study. Br. J. Nutr., 108: 1994-2001.
27. Quasim, T., D.C. McMillan, D. Talwar, N. Sattar, D.S.J. O'Reilly and J. Kinsella, 2003. Lower concentrations of carotenoids in the critically ill patient are related to a systemic inflammatory response and increased lipid peroxidation. Clin. Nutr., 22: 459-462.
28. Sakaguchi, S. and S. Furusawa, 2006. Oxidative stress and septic shock: Metabolic aspects of oxygen-derived free radicals generated in the liver during endotoxemia. FEMS Immunol. Med. Microbiol., 47: 167-177.
29. Pan, Q., C.G. Kleer, K.L. van Golen, J. Irani and K.M. Bottema *et al*., 2002. Copper deficiency induced by tetrathiomolybdate suppresses tumor growth and angiogenesis. Cancer Res., 62: 4854-4859.
30. Srinivas, U., M. Abdulla, B. Akesson, P.A. Ockerman, J.H. Braconier and B. Jeppsson, 1988. Trace element alterations in infectious diseases. Scand. J. Clin. Lab. Invest., 48: 495-500.
31. Dickerson, R.N., 2001. Manganese intoxication and parenteral nutrition. Nutrition, 17: 689-693.
32. Fridovich, I., 1995. Superoxide radical and superoxide dismutases. Ann. Rev. Biochem., 64: 97-112.
33. Karcioglu, Z.A., G. Karcioglu, R.M. Sarper and M. Hargovcic, 1980. Zinc and Copper in Neoplastic Diseases. In: Zinc and Copper in Medicine, Karcioglu, Z.A. and R.M. Sarper (Eds.). C.C. Thomas, Springfield, IL., ISBN: 9780398039776, pp: 464-534.
34. Saner, G., S.U. Baysal, E. Unuvar and T. Ozden, 2000. Serum zinc, copper levels and copper/zinc ratios in infants with sepsis syndrome. J. Trace Elem. Exp. Med., 13: 265-270.
35. Gartner, R., W. Albrich and M.W. Angstwurm, 2001. The effect of a selenium supplementation on the outcome of patients with severe systemic inflammation, burn and trauma. BioFactors, 14: 199-204.
36. Hawker, F.H., H.E. Ward, P.M. Stewart, L.A. Wynne and P.J. Snitch, 1993. Selenium deficiency augments the pulmonary toxic effects of oxygen exposure in the rat. Eur. Respir. J., 6: 1317-1323.
37. Forceville, X., B. Laviolle, D. Annane, D. Vitoux and G. Bleichner *et al*., 2007. Effects of high doses of selenium, as sodium selenite, in septic shock: A placebo-controlled, randomized, double-blind, phase II study. Critical Care, Vol. 11. 10.1186/cc5960.
38. Lee, C.Y., W.C. Jan, P.S. Tsai and C.J. Huang, 2011. Magnesium sulfate mitigates acute lung injury in endotoxemia rats. J. Trauma-Injury Infect. Crit. Care, 70: 1177-1185.
39. Durlach, J., 1988. Magnesium in Clinical Practice. J. Libbey, London, ISBN: 9780861961443, Pages: 360.
40. Sakaguchi, S., H. Ibata and K. Yokota, 1990. Participation of calcium ion on depletion mechanism of liver glycogen by purified glucocorticoid antagonizing factor released in blood during endotoxemia. Microbiol. Immunol., 34: 985-994.

41. Zhou, X., W. Yang and J. Li, 2006. Ca^{2+} -and protein kinase C-dependent signaling pathway for nuclear factor-κB activation, inducible nitric-oxide synthase expression and tumor necrosis factor-α production in lipopolysaccharide-stimulated rat peritoneal macrophages. J. Biol. Chem., 281: 31337-31347.
42. Brodie, C., A. Tordai, J. Saloga, J. Domenico and E.W. Gelfand, 1995. Ouabain induces inhibition of the progression phase in human T-cell proliferation. J. Cell. Physiol., 165: 246-253.
43. Chiao, J.J., W.G. Jones, G.T. Shires, A.E. Barber and G.T. Shires, 1992. Effect of sepsis on intracellular sodium activity, sodium concentration and water content in thermal injured rat. Circ. Shock, 38: 42-49.
44. Illner, H. and G.T. Shires, 1982. Changes in sodium, potassium and adenosine triphosphate contents of red blood cells in sepsis and septic shock. Circ. Shock, 9: 259-267.
45. Shimizu, K., M. Aikawa, K. Takayama, P. Libby and R.N. Mitchell, 2003. Direct anti-inflammatory mechanisms contribute to attenuation of experimental allograft arteriosclerosis by statins. Circulation, 108: 2113-2120.
46. Rikitake, Y., S. Kawashima, S. Takeshita, T. Yamashita and H. Azumi *et al*., 2001. Anti-oxidative properties of fluvastatin, an HMG-CoA reductase inhibitor, contribute to prevention of atherosclerosis in cholesterol-fed rabbits. Atherosclerosis, 154: 87-96.
47. Wassmann, S., U. Laufs, K. Muller, C. Konkol and K. Ahlbory *et al*., 2002. Cellular antioxidant effects of atorvastatin *in vitro* and *in vivo*. Arterioscler. Thromb. Vasc. Biol., 22: 300-305.