The effects of β-glucan on iron levels and lipid peroxidation in intra-abdominal sepsis in rats

Semra Ozdemir¹, Selmin Toplan¹, Gamze Tanriverdi² and Oguzhan Sunamak³

¹ Department of Biophysics, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey
² Department of Histology, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey
³ General Surgery Clinics, Inebolu State Hospital, Kastamonu, Turkey

Abstract. Sepsis is defined as a systemic response of organisms to microorganisms and toxins. Sepsis is associated with the enhanced generation of reactive oxygen metabolites, leading to multiple organ dysfunctions. β-glucan is accepted to be one of the most powerful immune response modifiers. The aim of this study was to investigate the putative protective effect of β-glucan on changes of iron and malondialdehyde (MDA) levels in various tissue and blood after experimental sepsis in rats. Sepsis was induced by cecal ligation and perforation (CLP) in 32 male Wistar albino rat. To evaluate this, rats were divided into four groups as sham operated, β-glucan treated sham operated, CLP and β-glucan treated CLP. Sixteen hours after operation, rats were decapitated and MDA and iron levels were measured in the liver, kidney, heart, diaphragm tissues and blood. Also, whole tissue histopathology was evaluated by a light microscope. The results demonstrate that sepsis significantly decreased iron levels of all tissues and blood. The decrease in tissue iron levels and the increase MDA levels demonstrate the role of trace elements and free radicals in sepsis-induced tissue damage. Our results indicate that the given dose of β-glucan was probably insufficient to prevent sepsis-induced organ injury.

Key words: Sepsis — β-Glucan — Iron — Rat — Tissue

Introduction

Sepsis and related syndromes, as the multiple organs dysfunction are the most frequent causes of morbidity and mortality in intensive care units. The mortality rate in sepsis patients remains as high as 40–70% even with aggressive treatment, and has not changed significantly over the past 30 years despite advances in modern technology (Tekin et al. 2008).

Iron is an essential trace element for mammals and most other aerobic organisms, where it plays a vital role as a catalyst in oxidase, oxygenase and antioxidant enzymes, and as a transporter of oxygen and electrons. When iron is not functionally or tightly bound to a protein it can, as part of a low molecular mass complex, catalyse unwanted electron transfer reactions. This leads to formation of reactive and damaging species such as the hydroxyl radical (Symons and Gutteridge 1998). In its unbound form, iron is also available to act as a microbial growth promoter (Festa et al. 2002). Most bacteria also require iron for growth, and iron availability is therefore a determinant of microbial virulence. Iron withdrawal mechanisms are therefore seen as a key defense during the development of sepsis (Quinlan et al. 2002).

Oxidative stress is described as impairment of equilibrium between prooxidant and antioxidant systems. Under physiological conditions, there is an equilibrium between oxidants and antioxidants that are generated during normal aerobic metabolism and their detoxification. Lipid peroxidation is the automatical chain reaction that produces radicals in cell membranes (Yucel et al. 2009). The physiologic generation of the products of the partial reduction of oxygen, O₂⁻ and H₂O₂, constitute the biological basis of the process of lipid peroxidation in mammalian cells. From a molecular point of view hydroxyl radical (HO•) generation, formed from H₂O₂ and Fe²⁺ by the Fenton reaction, has been considered for a long time as the likely rate-limiting step for physiological
lipid peroxidation (Repetto et al. 2010). Lipid peroxidation can be easily determined in plasma and tissue and accepted as an indicator of the oxidative stress related to sepsis induced tissue damage. The most widely measured lipid peroxidation product is malondialdehyde (MDA) (Sener et al. 2005a).

Glucans are natural product biological response modifiers that are composed of (1–3)-β-D-linked polymers of glucose. In animal studies glucans have been shown to be effective in preventing experimental peritonitis. Clinical studies suggest that administration of glucans to trauma or surgical patients will stimulate conversion from energy, decrease the incidence of septic complications, and improve survival. The mechanism(s) by which glucans alter the septic state is unknown (David et al. 1999). Several mechanisms were proposed for the protective effect of β-glucan, one of them is related to antioxidant capacity of the molecule (Sener et al. 2005b). There are several studies in which the relationship between sepsis and trace elements has been investigated (Koksal et al. 2004; Ozdemir et al. 2009). However, the effects of β-glucan on the iron metabolism or its alterations have not been studied.

Antioxidants might counteract the toxicity of oxygen radicals and that free radical ablation for the treatment of sepsis could be useful in the clinical setting of sepsis (Sener et al. 2005b). It has been shown that the increase in tissue and plasma oxidative stress correlates in intra-abdominal sepsis. However, trace element status needs to be taken into account in regard to the involvement of trace elements in the antioxidant responses, inflammation, wound healing and immune responses (Agay et al. 2005).

In this study, we investigated the possible protective effects of β-glucan on the alteration of iron and MDA levels of blood and liver, kidney, heart, diaphragm tissues in experimental model of intra-abdominal sepsis established by cecal ligation and puncture in rats.

Materials and Methods

All experimental protocols were performed in accordance with the National Institutes of Health guidelines and the approval of the Istanbul University Animal Care and Use Ethics Committee.

Animals and experimental design

Thirty-two male Wistar albino rats, weighing 200–250 g were kept in individual wire-bottom cages, in a room at a constant temperature (22 ± 2°C) with 12-h light/dark cycles, and fed standard rat chow. The rats were divided into the following four groups of eight rats each: sham operated control, sham operated+β-glucan treated group, cecal ligation and perforation (CLP) group, and CLP+β-glucan treated (CLP+β-glucan) group. Sepsis was induced by CLP technique (Figure 1) (Wichterman et al. 1980; Ozdemir et al. 2009). General anesthesia was induced by injection of intraperitoneal ketamine hydrochloride (Ketalar, Parke-Davis, USA). All procedures were performed under sterile conditions. The cecum was exposed, ligated just distally to the ileocecal valve to avoid intestinal obstruction, punctured twice with a 22-gauge needle, squeezed gently to force out a small amount of feces, and then returned to the abdominal cavity and the laparotomy was closed with 4.0 silk sutures. At the end of the operation, all rats were resuscitated with saline, 3 ml/100 g body weight given subcutaneously. Post-operatively, the rats were then returned to their cages with free access to food and water. The sham operated group rats underwent laparotomy, but the cecum was neither ligated nor perforated.

β-Glucan treatment

The β-glucan (Mustafa Nevzat Company, Turkey) used in this study is 1,3-1,6 β-D-glucan in the microparticulate form which is prepared from Saccharomyces cerevisiae yeast. β-glucan was suspended in saline and was given per oral by intragastric gavage once a day for 10 days in a dose of 50 mg/kg (Sener et al. 2005b; Ozdemir et al. 2009). The rats were decapitated 16 h after the CLP procedure. Kidney, liver, heart, diaphragm tissue samples and blood were taken and tissue samples were stored at –70°C.

Measurement of trace element

In order to eliminate adsorbed metals on the glassware being use, all the glassware was kept in 10% (v/v) nitric acid solution
before use. These were then cleaned with distilled water and dried in an oven overnight at 100°C (Cosan et al. 2006). The tissue samples were weighed and transferred into metal-free glass tubes for digestion. The samples were first digested with 2 ml of concentrated nitric acid at 100°C in the furnace for 1 h and 2 ml of perchloric acid (60%) was added to the cooled materials. The materials were then completely digested at 120°C until the materials diminished to the half of the original total volume. Digested materials were diluted with deionized water to 10 ml. The last dilutions of the samples were mixed on a shaker for 15 min just before measurement. Iron levels of the serum and kidney, liver, heart, diaphragm were measured by flame atomic absorption spectrophotometer (Shimadzu AA-680). Results were calculated as µg/g wet weight and µg/dl (Karakoc et al. 2003).

**Measurement of thiobarbituric acid reactive species (TBARS)**

Thiobarbituric acid (TBA) test was applied for MDA level measurement that is the final product of lipid peroxidation. Measurements were done according to Uchiyama and Mihera method (Uchiyama and Mihera 1978). Absorbances were read at 532 nm. 1,1,3,3-Tetra-ethoxypropane was used as the standard. Results were calculated as nmol MDA/ml in plasma and nmol MDA/g in tissues.

**Histopathological examination**

Samples of kidney, liver, heart and diaphragm tissue were fixed in 10% formaldehyde and processed routinely for embedding in paraffin. Paraffin sections were stained with hematoxylin and eosin and examined under a light microscope. The histopathological examination was performed by a histologist who was unaware of the groups of the specimens. Histopathological analysis was based on the scoring system described by Sener et al. (2005c). Histological changes were scored from 0 to 4 and the means of the scores were taken. The following scale was used: 0, no pathological findings; 1, mild (fewer than three fields); 2, moderate (three to six fields); 3, severe (more than six fields). Criteria for the microscopic scoring for liver are: vacuolization of hepatocytes, congestion, Kupffer cell infiltration, enlargement of sinusoids; for kidney: congestion, degeneration of proximal and distal tubules, interstitial edema; for diaphragm and heart: degeneration of muscle fibers and inflammatory cell infiltration.

**Statistical Analysis**

All data are expressed as mean ± standard deviation (SD). Statistical analyses were performed with Statistical Products and Service Solution package (SPSS, for Windows, 10.0.1 version, Chicago, Ill., USA). Statistical significance was defined by a p value less than 0.05.

**Results**

The iron levels in the liver tissue were significantly lower in the CLP group than in the controls (p < 0.001). In the CLP group, kidney, diaphragm and heart tissues iron levels were significantly lower than in controls (p < 0.001, p < 0.001 and p < 0.01, respectively). In the CLP+β-glucan group, iron levels of liver (p < 0.01), kidney (p < 0.05), heart (p < 0.01) and diaphragm (p < 0.001) were found to be significantly lower than those of controls (Table 1). No significant difference was found in the serum and liver, kidney, heart, diaphragm tissues iron levels between the CLP and CLP+β-glucan groups (p > 0.05) (Table 1). The iron levels in serum were significantly lower in the CLP and CLP+β-glucan groups than in the controls (p < 0.01).

In the CLP group, plasma and liver, kidney, heart, diaphragm tissues MDA levels were found to be significantly higher than in control group (p < 0.001). In the CLP+β-glucan group, MDA levels of plasma (p < 0.001), liver (p < 0.01), kidney (p < 0.01), heart (p < 0.001) and diaphragm (p < 0.001) were found to be significantly higher than those of controls (Table 2). When CLP group compared with CLP+β-glucan group, no significant statistical differences were observed in MDA levels of the plasma, liver, kidney, heart and diaphragm tissues (p > 0.05) (Table 2).

**Table 1. Iron levels of serum and liver, kidney, heart, diaphragm tissues in control, β-glucan, cecal ligation and perforation (CLP) and CLP+β-glucan groups**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>β-glucan</th>
<th>CLP</th>
<th>CLP+β-glucan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>110.86 ± 33.15</td>
<td>80.41 ± 23.27</td>
<td>54.99 ± 16.48**</td>
<td>63.25 ± 15.24**</td>
</tr>
<tr>
<td>Liver</td>
<td>377.20 ± 76.10</td>
<td>288.70 ± 53.50</td>
<td>148.65 ± 27.80***</td>
<td>162.63 ± 94.10**</td>
</tr>
<tr>
<td>Kidney</td>
<td>402.52 ± 140.16</td>
<td>228.34 ± 22.30</td>
<td>110.56 ± 21.80***</td>
<td>209.70 ± 13.67*</td>
</tr>
<tr>
<td>Heart</td>
<td>418.95 ± 179.10</td>
<td>272.23 ± 133.05</td>
<td>169.21 ± 55.45**</td>
<td>140.60 ± 22.73**</td>
</tr>
<tr>
<td>Diaphragm</td>
<td>493.05 ± 92.85</td>
<td>272.90 ± 34.11</td>
<td>116.05 ± 36.54***</td>
<td>144.29 ± 69.93***</td>
</tr>
</tbody>
</table>

Results were calculated as µg/dl in serum, µg/g wet weight in tissue. Values are expressed as means ± SD. * p < 0.05, ** p < 0.01, *** p < 0.001 versus control group.
Effect of β-glucan on iron levels in sepsis

Histological evaluation in liver tissue of control group are indicated in Figure 2A. Histological assessment in the liver tissue samples of CLP group showed vacuolization of hepatocytes, mononuclear cell infiltration and fibrosis (Figure 2B). The histological changes seen in the CLP group was similar to that of CLP+ β-glucan group and there was no significant difference. Histological analysis in kidney tissues of control group are indicated in Figure 3A. There was no significant difference between the control and β-glucan groups in terms of histological findings. Sepsis-induced histological damage was seen in the kidney tissue demonstrating degeneration of Bowman space and glomeruli, vascular congestion and interstitial edema and degeneration of proximal and distal tubules (Figure 3B). There was no significant difference between the CLP and CLP+β-glucan groups.

Discussion

Sepsis has been important causes of mortality in intensive care units for several decades. In the presence of sepsis the failing organ is not necessarily directly injured or involved in the primary disease process (Oter et al. 2005). Infection-induced changes in host metabolism are often accompanied by an extensive and simultaneous flow of trace elements between blood and tissues (Ilback et al. 2003; Frisk et al. 2007). Trace elements are required for the activity of a number of acute-phase proteins and immune cells that directly participate and interact in host defense processes (Beisel 1998). During infection there is a flux of both free and protein bound, essential as well as non-essential, trace elements between blood and the tissues involved by the disease (Goyer 1997). Recent studies suggest that intracellular zinc, copper and iron participate in the activation of immune cells (Failla 2003). For proliferation microbes desperately need iron for which they have to compete with the host. When trace elements are used as diagnostic tools during disease, it is important to know whether the balance is changed in free or bound elements. Although acute infections are associated with changed trace elements balance in serum/plasma, it is not known whether changes occur concomitantly in serum and tissues (Frisk et al. 2007). Determination of blood iron, copper and zinc can be used to

Table 2. MDA levels of plasma and liver, kidney, heart, diaphragm tissues in control, β-glucan, cecal ligation and perforation (CLP) and CLP+β-glucan groups

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>β-glucan</th>
<th>CLP</th>
<th>CLP+β-glucan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>3.45 ± 0.31</td>
<td>3.27 ± 0.34</td>
<td>7.30 ± 0.54***</td>
<td>7.26 ± 0.30***</td>
</tr>
<tr>
<td>Liver</td>
<td>80.9 ± 6.10</td>
<td>82.9 ± 3.47</td>
<td>102.4 ± 8.90***</td>
<td>97 ± 8.11**</td>
</tr>
<tr>
<td>Kidney</td>
<td>31.6 ± 1.4</td>
<td>30 ± 1.84</td>
<td>40.5 ± 4.66***</td>
<td>37.9 ± 3.76**</td>
</tr>
<tr>
<td>Heart</td>
<td>46.0 ± 2.7</td>
<td>44.7 ± 3.72</td>
<td>64.9 ± 4.97***</td>
<td>60.6 ± 4.32***</td>
</tr>
<tr>
<td>Diaphragm</td>
<td>20.2 ± 2.90</td>
<td>22.90 ± 3.07</td>
<td>38.3 ± 6.29***</td>
<td>34.10 ± 4.90***</td>
</tr>
</tbody>
</table>

Results were calculated as nmol MDA/ml in plasma and nmol MDA/g in tissues. Values are expressed as means ± SD. ** p < 0.01, *** p < 0.001 versus control group.

Figure 2. A. Normal liver histology in control group. B. Liver histology in sepsis group. The view of liver vaculaziation of hepatocytes, mononuclear cell infiltration and fibrosis. Magnification ×10; Hematoxylin eosin stain.
indicate ongoing infectious and inflammatory disease, but blood levels cannot be used to predict levels of these elements in inflammatory tissues (Rosander et al. 2004). Our previous study results demonstrated that sepsis significantly decreased zinc and copper levels of all tissues (Ozdemir et al. 2009).

According to the findings of the present study, the iron levels in the serum, liver, kidney, heart and diaphragm were significantly decreased when compared to control groups (Table 1). The reduced level of serum iron found during inflammatory disease is known. This may be a part of the normal defence against infections, since iron is vital to most microorganisms (Srinivas et al. 1988; Bullen et al. 2000; Quinlan et al. 2002). In animals with sepsis, Zager et al. (2004) have demonstrated that intravenous administration of iron can compound the inflammatory response and increase mortality rates. In addition, trace metal overload, like deficiency, suppresses immune cell function and increases the risks of morbidity and mortality due to infectious disease (Failla 2003). Galley and colleagues reported increased redox reactive iron concentrations in patients with sepsis or septic shock, coupled with lowered plasma levels of vitamin C and elevated lipid peroxides (Galley et al. 1997). Later work has, however, disputed the presence of redox reactive iron in the plasma of patients with septic shock (Macdonald et al. 2003). Gunaydin et al. (1995) showed that the determination of initial serum level may help to differentiate bacterial infection from viral infection and the low levels of serum iron should not be considered as iron deficiency. The iron needed for Fenton reaction may be derived from plasma or damaged cells, in particular from the microorganisms themselves serving as a “Trojan horse”. The unleashed chain of toxic activities, including production of oxygen radicals, may finally kill both the micro-organisms and the phagocytes.

It has been demonstrated that iron-mediated production of oxygen metabolites caused lipid peroxidation of granulocyte and monocyte membranes and of cells in the environment, and too much iron impaired granulocyte function. Iron may therefore be involved in the tissue damage that is associated with host response during infection (Marks 2002). During the course of severe sepsis, several biochemical abnormalities develop, which are secondary to alterations in metabolic pathways within the affected cells and in various organs. MDA is the breakdown product of the major chain reactions leading to oxidation of polyunsaturated fatty acids, and thus serves as a reliable marker of oxidative stress-mediated lipid peroxidation. An increased concentration of MDA reflects the level of lipid peroxidation in tissues and is considered a marker of tissue injury (Tekin et al. 2008). Our results showed that sepsis causes oxidative stress in the liver, kidney, heart, diaphragm as demonstrated by the increased MDA levels. The close relationship between lipid peroxidation and iron has been described a long time (Andraes et al. 2004; Agarwal 2008). But there are no studies to compare lipid peroxidation and iron levels in sepsis and β-glucan treatment.

Sepsis is associated with heightened oxidative stress. There is increasing evidence that oxidative stress has an important role in the development of sepsis-induced multiorgan failure. Diminished antioxidative defenses, superoxide dismutase, catalase and glutathione, also contribute to oxidative stress. Recent studies have reported increased levels of lipid peroxides and decreased antioxidant enzyme activity in experimental sepsis, indicating an exhaustion of the antioxidant system (Andraes et al. 2004; Liaw et al. 2005). Ritter et al. (2003) showed that MDA and plasma superoxide dismutase levels are markers of early mortality in septic rats. Batra and colleagues found that MDA levels were also increased in

Figure 3. A. Normal kidney histology in control group. B: Kidney histology in sepsis group. The view of kidney vascular congestion, degeneration of Bowman space and glomeruli, interstitial edema, degeneration of proximal and distal tubules. Magnification ×40; Azan stain.
neonates with sepsis suggesting that the elevations of these antioxidant enzymes were not so effective as to prevent cellular damage (Batra et al. 2000). Enhancement of antioxidant status in animal models of sepsis has demonstrated beneficial effect and administration of antioxidants to septic patients may be protective.

The beneficial effects on the immune system and the lack of toxic or adverse effect had focused the studies on β-glucan molecule. Some researchers have claimed that protective effect was due to the antioxidant capacity. Furthermore, it has been suggested that β-glucan binds to scavenger receptors (Brown and Gordon 2003). Lazarová et al. (2006a) observed protective effects of carboxymethyl chitin-glucan (dose approximately 200 mg/kg body weight for 21 days) supplementation in terms of the decreased level of DNA damage measured by comet assay in different primary rat cell lines damaged after isolation either with genotoxic carcinogens requiring metabolic activation or oxidative stresses induced by visible light-excited methylene blue. Lazarová et al. (2006b) also showed immunopotentiating activity of β-D-glucans, efficiently inhibiting the genotoxicity of carcinogens requiring metabolic activation. Sener et al. (2005b) showed that treatment with β-glucan significantly reversed the elevations in MDA levels in the liver, kidney, heart, lung, diaphragm and brain tissues. However, the relationship between the kidney, liver, heart and diaphragm tissues iron levels and β-glucan administration in sepsis have not been defined.

We examined the effects of β-glucan therapy on the iron and MDA levels in experimental sepsis. The results of the present study demonstrate that sepsis causes decrease of iron in the serum, liver, kidney, heart, diaphragm tissues, and these data suggest that the sepsis-induced damages in these tissues have not returned to control levels with given β-glucan dose (50 mg/kg) treatment. The control and β-glucan groups showed similarity in terms of histological changes in comparisons of both the liver tissues and the kidney tissues (Figures 2A and 3A). β-glucan treatment was not effective in correcting histological changes seen in the subjects of the sepsis group (Figures 2B and 3B).

Our results indicated that β-glucan administration did not return the iron levels to the control group level. It seems likely that the given dose of β-glucan was insufficient to prevent sepsis-induced organ injury. Iron has been known to cause peroxidation of lipids by means of Fenton reaction. According to our results, we can say that it is not the iron that causes increase in MDA level but may be other mechanisms which may cause this increment. We think that the use of β-glucan which we used as antioxidant agent on variable doses and between variable time intervals may have a role in explaining the relationship between lipid peroxidation and iron in sepsis.

Acknowledgements. This work was supported by the Research Fund of the Istanbul University (project number BYP 3938).

References

doi:10.1002/jtra.10023


doi:10.1207/s15327914nc5601_1

doi:10.1097/01.shk.0000145937.70085.89

doi:10.1093/bja/aeg034


doi:10.1007/s00134-005-2701-6

doi:10.1007/s12011-009-8385-y

doi:10.1016/S0891-5849(02)00903-6

doi:10.1007/s00204-009-0487-y

doi:10.1007/s00134-003-1789-9

Rosander C. N., Lindh U., Friman G., Lindqvist O. (2004): Trace element changes in sclerotic heart valves from patients are expressed in their blood. BiomMetals 17, 121–128
doi:10.1023/B:BIOM.0000018374.99902.bc


doi:10.1016/j.intimp.2005.03.007


doi:10.1016/j.intimp.2005.03.007

doi:10.1016/0003-2697(78)90342-1

doi:10.1016/0022-4804(80)90037-2

doi:10.1007/s12020-009-9251-6

doi:10.1111/j.1523-1755.2004.00742.x

Received: August 19, 2010
Final version accepted: November 9, 2010