Effects of Resveratrol on Ileal Smooth Muscle Reactivity in Polymicrobial Sepsis Model

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INTRODUCTION

Gastrointestinal stasis or ileus commonly accompanies sepsis and multiorgan dysfunction in critically ill patients and may contribute to their pathophysiology. Non-mechanical ileus is one of the most frequent complications within the gastrointestinal tract during sepsis [1]. The mechanism of ileus may be associated with impaired contractile function of gastrointestinal smooth muscle [2]. Resveratrol (3,5,4’-trans-trihydroxystilbene) is a natural phytoalexin present in grapes and red wine, which possesses a variety of biological activities including anti-inflammatory and antioxidative effects [3]. It has also been shown to display potent inhibitory effect on lipid peroxidation and to modulate lipoprotein mechanism. Resveratrol suppresses inducible nitric oxide synthase (iNOS) expression and nitric oxide (NO) production in culture cells [4]. Moreover, it was observed that the production of inflammatory cytokines such as TNFα, IL-1β, IL-6 was suppressed by resveratrol [5]. Although anti-inflammatory and antioxidant activity of resveratrol has been well documented, there is no report on its smooth muscle reactivity in sepsis. In this background, we investigated whether resveratrol has protective effects on ileal smooth muscle reactivity in polymicrobial sepsis after cecal ligation and perforation (CLP). We also observed blood levels of inflammatory cytokines TNFα and IL-1 after resveratrol treatment in the study.

Objective. To determine the effects of resveratrol on the ileal smooth muscle reactivity in polymicrobial sepsis.

Material and Methods. Polymicrobial sepsis was induced by the cecal ligation and perforation (CLP) procedure. Sprague Dawley rats were divided into three groups. Rats in resveratrol group received resveratrol after CLP (100 mg/kg, i.p.). Rats received saline immediately after CLP in the sepsis group. Control group rats underwent sham operation. The rats were sacrificed and the ileum was excised 24 h after the operation. Contractile and relaxant responses in isolated smooth muscle strips (SMS) were determined using an in vitro muscle technique. TNFα and IL-6 levels were measured in blood samples.

Results. Contractile responses to carbachol and KCl and relaxant responses to transmural electrical field stimulation (EFS) were significantly decreased in the sepsis group compared with control and resveratrol groups. No significant changes were observed for smooth muscle reactivity in the resveratrol and control groups. Sodium nitroprusside (SNP) or papaverine-induced relaxations were similar in all groups. Resveratrol treatment suppressed increased TNFα and IL-6 levels in blood seen in sepsis group.

Conclusion. Ileal smooth muscle reactivity was improved after resveratrol treatment in rats with sepsis. The results of the present study indicate that the beneficial effects of resveratrol might be, at least in part, attributed to its effects on non-adrenergic non-cholinergic pathway and/or anti-inflammatory and antioxidant activity. Crown Copyright © 2012 Published by Elsevier Inc. All rights reserved.

Key Words: resveratrol; sepsis; organ bath; in vitro; ileum.
MATERIALS AND METHODS

The experiments were carried out in accordance with regulation of Animal Research Ethics Committee in Turkey (6 July, 2006, number 202229). Ethical approval was granted by the Kocaeli University Animal Research Ethics Committee (Kocaeli, Turkey). Adult male Wistar rats weighing 200–250 g obtained from Kocaeli University Experimental Medical Research Center (DETAB, Kocaeli, Turkey) were placed in a quiet, temperature- and humidity-controlled room (22 ± 3°C and 62% ± 7%, respectively) in which a 12–12 h light–dark cycle was maintained (07:00–19:00 light). The rats were divided into three groups, each consisting of eight rats: sham-operated group (control group), saline-treated CLP group (sepsis group), and resveratrol-treated CLP group (sepsis group), and resveratrol.

Polymicrobial Sepsis Model

The experimental polymicrobial sepsis model was induced by CLP as previously described [6]. Briefly, under ketamine (50 mg/kg i.p.) and xylazine (25 mg/kg i.p.) anesthesia, an approximately 2 cm midline incision was made in the abdomen. The cecum was isolated carefully and then ligated just below the illeocecal valve with 3-0 silk ligature. The cecum was punctured twice with a sterile 20-gauge needle, gently squeezed to extrude the fecal material and placed back into the peritoneal cavity. The incision was closed in layers. All rats were then reuscinated with 1 mL saline injected subcutaneously. Sham-operated control underwent the similar surgical procedure except the cecal ligature and puncture. Animals received resveratrol (100 mg/kg) or saline immediately after the induction of sepsis. Afterwards, the rats were kept in a recovery cage for 24 h. The rats had free access to food and water after the operation.

Organ Bath Studies

Twenty-four hours after the surgery, the rats were killed by cervical dislocation. The abdomen was immediately opened and ileum was removed and placed in previously aerated (95% O2 and 5% CO2) Krebs bicarbonate solution composed of: 118.5 mM NaCl, 4.85 mM KCl, 1.25 mM MgSO4, 1.25 mM KH2PO4, 2.55 mM NaHCO3, 1.95 mM CaCl2, and 10.15 mM glucose. The contents of the excised segment were gently flushed out with Krebs bicarbonate solution. Whole thickness segments of ileum were suspended in a four-channel organ bath containing 20 mL of oxygenated O2/CO2. Krebs bicarbonate solution maintained at 37°C. All tissues were allowed to equilibrate for 1 h prior to beginning the experiments. During this period, the bath fluid was routinely changed every 15 min. Resting tension was set at 1 g by repeat adjustments and remained unchanged throughout the experiment. Each ileal segment was connected to a force-displacement transducer (MAY-COM, FDT 10 A; COMMAT Iletisim Co.), divided into aliquots and stored at –70°C and 62% for 4000 rotation per min for 15 min at 4°C, divided into aliquots and stored at –70°C until assayed. The proinflammatory cytokine, which is a secretion of rat interleukin 1β (IL-1β), IL-6, and tumor necrosis factor α (TNFα), levels of serum were quantified using enzyme-linked immunosorbent assays (ELISA) (Biosource, Invitrogen, Carlsbad, CA) according to the manufacturer’s recommendations, then analyzed with VersaMax microplate reader (Molecular Device, Sunnyvale, CA, USA), using SoftMax Pro 5 software.

Statistical Analysis

Results are expressed as mean ± SD. The differences between means were assessed with one-way analysis of variance (ANOVA). Probability levels < 0.05 were considered to be significant.

RESULTS

The contractions elicited by 80 mM KCl and carbachol (10^-9–10^-4 M) were significantly reduced in sepsis group compared to control and resveratrol groups in ileal SMS (Fig. 1 and Tables 1 and 2). In the presence of adrenergic (guanethidine, 3.10^-6 M) and cholinergic (atropine 10^-6 M) blockade, EFS (1–10 Hz, 10 s train) evoked frequency-dependent relaxation. Relaxations to field stimulation in the SMS of sepsis group were significantly reduced compared with responses obtained from that of control group.
The intestine is one of the major organs involved in sepsis. Decreased intestinal motility is a well known complication of sepsis, which may lead to bacterial translocation and worsen organ damage. Decreased ileal smooth muscle reactivity was recorded in the sepsis group (Fig. 2 and Table 1). The impaired EFS-induced relaxation of SMS was markedly improved by treatment of resveratrol. Relaxant responses to SNP (10⁻⁸–10⁻⁴ M) or papaverine (10⁻⁵ M) in sepsis and resveratrol groups were not significantly changed compared with control group (Fig. 3 and Tables 1 and 2).

TNFα and IL-6 blood levels were significantly increased in sepsis group compared with resveratrol and control groups (Fig. 4A and B, one-way ANOVA Tukey test P < 0.01).

**DISCUSSION**

The intestine is one of the major organs involved in sepsis. Decreased intestinal motility is a well known complication of sepsis, which may lead to bacterial translocation and worsen organ damage. Decreased ileal smooth muscle reactivity was recorded in the sepsis model [2–7]. The present study showed that impaired ileal smooth muscle reactivity markedly improved after resveratrol treatment in sepsis. Furthermore, increased TNFα and IL-6 levels decreased to control values after resveratrol treatment.

It is well known that KCl contracts smooth muscle by opening the voltage dependent Ca²⁺ channel and increasing intracellular Ca²⁺ [8]. Moreover carbachol causes smooth muscle contraction by inducing influx of extracellular calcium through L-type voltage gated calcium channels. In this study, we found decreased contractile response to carbachol and KCl in rats with sepsis. This result is consistent with a previous study that showed decreased aortic smooth muscle reactivity to KCl and phenylephrine in sepsis [9]. Additionally, resveratrol treatment revealed improved ileal smooth muscle contractile response to KCl and carbachol. Therefore, resveratrol treatment in sepsis may work by restoration of physiologic signal transmission such as altered Ca²⁺ influx, calcium storage, or secondary mechanisms during contraction.

It is well established that NO is the major inhibitory neurotransmitter in the GI tract and impairments in nitricergic innervation lead to intestinal motility disorder [10]. It was shown that electrically evoked non-adrenergic non-cholinergic-mediated relaxation is mediated by NO in rat ileum [11]. Neuronal nitric oxide synthase (nNOS) is responsible for this relaxation [12]. NO diffuses smooth muscle cells and activates soluble guanylyl cyclase so that the intracellular cGMP concentration increases. Various pathologic conditions that inhibit the synthesis of NO, enhance its degradation, or inhibit guanylyl cyclase are accompanied by gastrointestinal dysfunction. We observed diminished non-adrenergic non-cholinergic-mediated relaxation induced by EFS in ileal tissues obtained from septic rats compared with control rats. The mechanism for impaired EFS response in ileal smooth muscle is yet unknown. It is possible that impaired EFS-induced NO-mediated relaxation appears to be related to the decreased production of cGMP. However, according to our results, this possibility is unlikely because the relaxations induced by SNP, a non-receptor mediated relaxant that relaxes the ileal smooth muscle by a mechanism similar to that of NO, did not significantly change among the groups. We interpret the SNP data as evidence that this sepsis model failed to affect either the ability of NO to activate guanylyl cyclase-cGMP or the sensitivity of ileal smooth muscle contractile apparatus to cGMP-mediated actions.

<table>
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<th>TABLE 1</th>
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<tr>
<td><strong>E₉₀ (% of 10⁻⁵ M Papaverine) Values for Sodium Nitroprusside (SNP) and Electrical Field Stimulation (EFS), E₉₀ (% of 80 mM KCl) Values for Carbachol, and E₉₀ Values (mg) for 80 mM KCl in Ileal Strips Obtained from Control, Sepsis, and Resveratrol-Treatment Groups</strong></td>
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<tr>
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<th>Carbachol</th>
<th>SNP</th>
<th>KCL</th>
<th>EFS</th>
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<tr>
<td>Control</td>
<td>99.6 ± 8.46</td>
<td>105.1 ± 29.33</td>
<td>3234.9 ± 471.79</td>
<td>75.28 ± 9.627.07</td>
</tr>
<tr>
<td>Sepsis</td>
<td>82.1 ± 6.20*</td>
<td>93.5 ± 12.97</td>
<td>2249.5 ± 251.26*</td>
<td>50.3 ± 14.66*</td>
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<tr>
<td>Resveratrol</td>
<td>105.7 ± 11</td>
<td>101.3 ± 6.20</td>
<td>3258.7 ± 330.79</td>
<td>65.4 ± 11.84</td>
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Values are arithmetic means ± SD.  
ₙ = the number of preparations used.  
*P < 0.05, statistically different from the response of strips from sham-operated control rats.

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<th>TABLE 2</th>
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<tr>
<td><strong>pD₂ Values (-Log M EC50) for Carbachol, Sodium Nitroprusside (SNP) in Corpus Cavernosum Strips Obtained from Neurotomy and Sham Operation Groups</strong></td>
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<tr>
<th></th>
<th>Carbachol</th>
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<tr>
<td>Control (ₙ = 8)</td>
<td>6.44 ± 0.62</td>
<td>5.21 ± 0.76</td>
</tr>
<tr>
<td>Sepsis (ₙ = 8)</td>
<td>5.21 ± 0.85*</td>
<td>5.40 ± 1.05</td>
</tr>
<tr>
<td>Resveratrol (ₙ = 8)</td>
<td>6.47 ± 0.68</td>
<td>5.21 ± 0.68</td>
</tr>
</tbody>
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Values are arithmetic means ± SD.  
ₙ = the number of preparations used.  
*P < 0.05, statistically different from the response of strips from sham-operated control rats.
Recently, Pearson et al. found resveratrol treatment of mice decreased expression of the inflammatory markers TNFα, interleukin-6, interleukin 1β, intercellular adhesion molecules-1, and i-NOS [13]. In the present study, in accordance to Pearson et al., we observed increased TNFα and interleukin-6 levels in septic animals which that of decreased levels detected after resveratrol treatment. Based on this statement it can be suggested the functional impairment of the ileal tissues were due to inflammatory response and detrimental effects of sepsis on ileal contractility can be prevented by anti-inflammatory effect of resveratrol.

In a previous study, it was shown that resveratrol has a protective effect on ileum mitochondrial oxidative stress in rats subjected to ischemia-reperfusion [14]. Additionally, Shigematsu et al. demonstrated that resveratrol prevents superoxide-dependent inflammatory responses induced by ischemia/reperfusion [14]. They concluded that its antioxidant and anti-inflammatory effects were account for cardioprotective actions. Similar protective effect of resveratrol on endotoxia-induced acute phase responses in rats has recently been described. They showed that all deleterious LPS effects were reversed by resveratrol treatment via a NO independent way [15]. Since we did not measure antioxidant activity such as superoxide dismutase in this study, we can not draw any conclusion at the present time.

FIG. 2. The relaxation responses evoked by electrical field stimulation (EFS) of isolated rat ileal muscles. Each point is expressed as a percentage of the relaxation induced by $10^{-5}$ M papaverine and is given as the mean ± SD. Number of rats in each group is shown in parentheses. *Significant difference from the corresponding control value ($P < 0.05$). (Color version of figure is available online.)

FIG. 3. Sodium nitroprusside concentration-response curves in isolated rat ileal smooth muscles. Each point is expressed as a percentage of the relaxation induced by $10^{-5}$ M papaverine and is given as the mean ± SD. Number of rats in each group is shown in parentheses. (Color version of figure is available online.)

FIG. 4. (A) TNFα blood levels. Each point is given as the mean ± SD. *Significant difference from the corresponding control value ($P < 0.05$). †Significant difference from the sepsis value ($P < 0.05$). (B) IL-6 levels blood levels. Each point is given as the mean ± SD. *Significant difference from the corresponding control value ($P < 0.05$).

CONCLUSION

We found impaired EFS-mediated relaxation and carbachol-induced contraction in septic rats. The mechanism of this result is not completely known. According to our study, resveratrol has some protective effects on impaired ileal smooth muscle reactivity induced by sepsis. Reversal effect of resveratrol may be due to its antioxidant, anti-inflammatory, and non-adrenergic non-cholinergic mechanism related effects. To gain a better understanding of the mechanisms by which resveratrol induces these effects, further studies must be performed.
REFERENCES