Title: Effects of resveratrol on ileal smooth muscle reactivity in polimicrobial sepsis model

Article Type: Regular Article

Keywords: Resveratrol, sepsis, organ bath, in vitro, ileum

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Reviewers’ comments:

Reviewers #1: This is generally well conducted research with interesting findings. The authors have administered resveratrol immediately after CLP which is essentially a prophylactic administration. It would have been more valuable if the authors can show whether resveratrol is effective 4-8 h after CLP.

Authors Answers: in this study We wanted to show whether resveratrol has protective effect in sepsis. However this is a very good idea requires further study.

Although the current findings are a proof of concept, the suggested studies will be more clinically relevant. What did the authors base their dose of resveratrol? Do they have a dose response information?

Authors Answers: Resveratrol dose were given according to references. However dose range changes in many other studies.

The figures have no Figure numbers which need to be corrected.

Authors Answers: Figure numbers were corrected

Reviewer #2: Gacar and coworkers present the results of their animal study regarding the effect resveratrol on the ileal smooth activity in a rat model 24h after cecal ligation and puncture. Accordingly, resveratrol has a beneficial effect in a way that has not been reported yet and reporting these results is certainly of importance. The manuscript presents however with several limitations which do not allow to assess the relevance of the results in their full extent.

The statistical analysis would certainly benefit from professional support. Moreover, it appears that not all individuals of all groups could undergo a complete analysis.

Authors Answers: Statistical analysis were overwieved. Animal numbers were corrected according to our results in tables and graphics due to typing errors. All individuals of the groups underwent complete analysis in the study.

The manuscript would also certainly benefit from the revision by a native English speaker.

Authors Answers: Revised manuscript were corrected by native English speaker.(see labeled sentences)

Detailed comments:

Abstract
Twenty-four hours after the operation, the rats sacrificed and the...
Comment: Check spelling

Authors Answers: The rats sacrificed and the ileum was excised twenty-four hours after the operation.

Improved ileal smooth muscle reactivity after sepsis were observed...
Comment: Check spelling

Authors Answers: Ileal smooth muscle reactivity was improved after resveratrol treatment in rats with sepsis.

Introduction
In this background, we aimed to investigate whether resveratrol protects ileal smooth muscle reactivity in an experimental CLP model of polimicrobial sepsis.
In this background, we investigated whether resveratrol has protective effects on ileal smooth muscle reactivity in polimicrobial sepsis after cecal ligation and perforation (CLP).

Material and Methods
After the washout, cumulative concentration-response curve was obtained with carbachol (10^-8-10^-4 M) were recorded.

Authors Answers: After the washout, cumulative carbachol (10^-8-10^-4 M) concentration-response curve was obtained.

Then the relaxant responses to SNP (10^-8-10^-4 M) and papaverine (10^-5 M) were obtained.

AuthorsAnswers: SNP (Sodium nitroprusside)

Statistics:
Results are expressed as mean±SEM.

Authors Answers: Standard deviation were used instead of SEM.

Obviously, repeated pairwise testing was performed. Then however, the level of significance has to be adjusted e.g. according to Bonferroni.

Regarding the repeated, dose dependent measurement of relaxation / concentration an adequate test design is also missing, consider an analysis of variance for repeated measures. Explain all statistical methods used.

Authors Answers: We did not use repetitive measurements from the same tissue (or animal). All measurements were done from different tissues. For this reason we used unpaired t Test.

Results
What happened to the animals? Did all animals survive? What kind of septic symptoms occur? Did the behavior change?

Data presented in tables and graphs is contradictory regarding the number of included and analyzed animals per group.

Authors Answers: They survived until they sacrificed (24 hours). Cecal ligation and perforation was successful in 16 of 23 cases which showed obvious clinic and postmortem findings (with piloerection, nasal exudation, diarrhea, tachypnea, cecal necrosis, and ascites); were underwent testing. The remaining animals were not used in these experiments. The number of the animals were corrected according to our results.

Display cytokine levels as graph applying mean and standard deviation.

Authors Answers: cytokine levels was displayed as a graphic (Figure 4a 4b) with mean and Standard deviation

Discussion
Previously it was shown that sepsis induced rats showed decreased contractile and relaxant responses

Authors Answers: Decreased ileal smooth muscle reactivity were recorded in the sepsis model.

In this background, we investigated the healing effects of resveratrol on ileal smooth muscle reactivity in a different sepsis model.

Authors Answers: The sentence was erased according to your suggestion
Is it justified to talk about healing in this regard? The data might allow to draw the conclusion that resveratrol has a protective or preserving effect. We believe it is protective effect. What is meant by "different" regarding the model? Authors Answers: This sentence was changed regarding previous suggestion.

Additionally inflammatory cytokines such as TNF-α, and IL-1 levels were measured in the study. Comment: Do not repeat what has been studied. Rather give a very concise summary of the main findings which are subject to the discussion. Authors Answers: This paragraph was corrected according to your suggestion.

Furthermore, increased TNF-α and IL-6 levels decreased to control values after resveratrol treatment.

This observation is very similar to previous study which showed the contractile responses to KCl and phenylephrine decreased after sepsis in isolated rat aorta [9]. Comment: What exactly is meant by similar? Please be specific in the discussion of the result in order to give the reader exact information about the relevance of your findings. Authors Answers: This paragraph was corrected according to your suggestion.

This result is consistent with a previous study which 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 Ca^{2+} influx, calcium storage or secondary mechanisms during contraction.

Check all references. Regarding (9) it should be 10: Tasatargil A, Dalakloglu S, Sadan G. Inhibition of poly(ADP-ribose) polymerase... Authors Answers: Reference 10 changed to 9. Reference 10 changed.

It was shown that electrically evoked non-adrenergic non-cholinergic-mediated relaxation is mediated by NO in rat ileum

It is possible that impaired NO-mediated relaxation appears to be related to the decreased production of cGMP. Comment: Give a reference Authors Answers: Please go to new reference 10.

However, according to our results this possibility is unlikely. Because the relaxations induced by SNP, a nonreceptor mediated relaxant that relaxes the ileal smooth muscle by a mechanism similar to that of NO, did not significantly changed among the groups. Thus we suggest that responsiveness of the smooth muscle to NO is not altered by sepsis may related to other mechanisms requires further study.

Comment: This statement is somewhat a contradiction to the statement above: " We observed diminished NANC relaxation induced by EFS in ileal tissues obtained from septic rats compared to control rats. The mechanism for impaired NO-mediated response in ileal smooth muscle is yet unknown". SNP application is either a marker for the NO/ NANC pathway or not.
SNP and EFS cause relaxation response by different mechanisms. SNP activates guanylate cyclase and leading to accumulation of cGMP. EFS causes NO release from NANC and this NO activates guanylate cyclase. We found no changes in SNP responses while decreased EFS responses. Therefore we believe that cGMP production did not change in sepsis. However NO production is decreased during sepsis. Therefore our statements is not contradictory one another.

Authors Answers: These sentences were corrected according to your instructions

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 changed among the groups. We interpret the SNP data as evidence that this sepsis model failed to effect either the ability of NO to activate guanylyl cyclase-cGMP or the sensitivity of ileal smooth muscle contractile apparatus to cGMP-mediated actions.

Based on these statements, it can be suggested that the functional impairment of the ileal tissues were due to an oxidative stress and detrimental effects of sepsis on ileal contractility can be prevented by antioxidant activity of resveratrol.

Comment: This conclusion is not based on the provided data and should be omitted.

In conclusion, we found impaired NO-mediated relaxation and carbachol induced contraction in septic rats.

Authors Answers: This sentence was ommited according to your instructions

In conclusion, we found impaired EFS-mediated relaxation and carbachol-induced contraction in septic rats.

Alltogether, a discussion of the model and the limits of this investigation is missing.


* How was the dose of resveratrol established? Have any pre-studies been performed.
Authors Answers: Resveratrol dose were given according to references. Preliminary studies performed regarding sepsis and resveratrol dose and effects.

* Which doses have been used by other investigators?
Authors Answers: Various doses of resveratrol were used in different studies however we considered 100mg/kg is most suitable for the study due to prevalent effects. (30-100mg)
Protective effects of resveratrol on spleen and ileum in rats subjected to ischemia-reperfusion.
Karabulut AB, Kirimlioglu V, Kirimlioglu H, Yilmaz S, Isik B, Isikgil O.
15 mg/kg/d-5 day

Effect of resveratrol on peritoneal macrophages in rats with severe acute pancreatitis.
Ma ZH, Ma QY, Wang LC, Sha HC, Wu SL, Zhang M.
10 mg/kg after severe acute pancreatitis-single dose

Protective effect of resveratrol in endotoxemia-induced acute phase response in rats.
Sebai H, Ben-Attia M, Sani M, Aouani E, Ghanem-Boughanmi N.
acute: 40 mg/kg-ip- single dose

* What is actually known about resveratrol.
Authors Answers: Resveratrol has potent antioxidant and anti-inflammatory effects promotes vascular endothelial function, enhanced lipid metabolism, and has anticancer activity. Also resveratrol exerts antiaging effects in animals.

* A discussion of the cytokine results is missing.
Authors Answers: Cytokine results were added to discussion section.

Recently, Pearson et al. found resveratrol treatment of mice decreased expression of the inflammatory markers TNF-alpha, Interleukin-6, Interleukin 1-Beta, Intercellular adhesion molecules-1, and i-NOS [13]. In the present study in accordance to Pearson et al.; we observed increased TNF-alpha 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.
Figure 1
Comment: Omit figure 1 as it provides no additional data when compared to table 1. In the table, 8 animals are indicated. In the graph however, only 6 and 7 are listed, but the results remain the same. What happened to the missing animals. Did all animals survive?

Author answers: figure 1 is erased. They survived until they sacrificed (24 hours). Cecal ligation and perforation was successful in 16 of 23 (%) cases (with piloerection, nasal exudation, diarrhea, tachypnea, cecal necrosis, and ascites); the remaining animals were not used in these experiments. The number of the animals were corrected according to our results.

Figure 2
Comment: Why were only 5 and 6 animals included. Sepsis group is marked with an * indicating several significant differences. This should have been tested using an adequate test design, e.g. an analysis of variance for repeated measures.

Numbers for the figures are missing.

Authors Answers: we don't have any repeated measures. All the measures were taken from different animals and different analysis. Therefore ANOVA was used to test the study. The number of animals (typing error) were corrected according to our results.

Figure 4: Sodium nitroprusside concentration-response curves in isolated rat ileal smooth muscles. Each point is expressed as a percentage of the relaxation induced by 10^-4 M papaverine and is given as the mean±SE mean. Number of rats in each group is shown in parantheses. * significant difference from the corresponding control value (p<0.05, test).

Comment: There is no mark indicating a difference between groups. Altogether, the legends seem to be a mere copy-paste product.

Authors Answers: There is no difference between the groups. The sentence was corrected according to our results.

Tables
Table 1: Values are arithmetic means ± S.E.M. n= the number of preparations used. * P<0.05, statistically different from the other groups.

Comment: Accordingly, at least two paired tests were performed, maybe three. According to Bonferroni, p value should be adjusted by dividing 0.05 by two or three accordingly.

What are the units when compared to graph 1 (mg or %)? What is the meaning of abbreviations

Authors Answers: we don't have any results taken from same animal (repeated measurements) So we used unpaired t Test. Figure 1 is erased according to your suggestions.

Table 2: What is the use of this table when compared to table 1. Explain any abbreviation in the legend.

Authors Answers: This table is concern measuring tissue response to drugs such as carbachol, SNP. In order to evaluate the effects of agonists, maximum responses ($E_{max}$) and pD$_2$ values (apparent agonist affinity constants; $-\log ED_{50}$) were calculated. Agonist pD$_2$ value was calculated from each agonist dose–response curve by linear portion of the curve and taken as a measure of the sensitivity of the tissues to each agonist.
Effects of resveratrol on ileal smooth muscle reactivity in polimicrobial sepsis model

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Subject category: Sepsis
Running title: effects of resveratrol on smooth muscle reactivity during sepsis

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Abstract

Aim: To determine the effects of resveratrol on the ileal smooth muscle reactivity during polymicrobial sepsis.

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

Results: Contractile responses to carbachol and KCl and relaxant responses to EFS were significantly decreased in the sepsis group compared to control and resveratrol groups. No significant changes were observed for smooth muscle reactivity responses in the resveratrol and control groups. In contrast, sodium nitroprusside (SNP) or papaverine-induced relaxations were similar in the all groups. Resveratrol treatment suppressed increased TNF alpha and IL-6 levels in blood seen in sepsis group.

Conclusion: Improved ileal smooth muscle reactivity after sepsis were observed following resveratrol treatment. The results of the present study indicated that the beneficial effects of resveratrol might be, at least in part, attributed to its effects on non-adrenergic non-cholinergic (NANC) pathway and/or anti-inflammatory and antioxidant activity.

Introduction
Gastrointestinal stasis or ileus commonly accompanies sepsis and multiorgan dysfunction in critically ill patients and may contribute to their pathophysiology. Nonmechanical 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 also to modulate lipoprotein mechanism. Resveratrol supresses iNOS expression and NO production in culture cells [4]. Moreover, it was observed that the production of inflammatory cytokines such as TNFα, IL-1β, IL-6 was supressed by resveratrol [5]. Although antiinflammatory and antioxidant activity of resveratrol has been well documented there is no report on its activity in sepsis. In this background, we aimed to investigate whether resveratrol protects ileal smooth muscle reactivity in an experimental CLP model of polimicrobial sepsis. We also observed inflammatory cytokines TNFα and IL-1 levels affected by resveratrol in the study.

Materials and Methods

The experiments were carried out in accordance with regulation of Animal Research Ethics Committee in Turkey (6 July, 2006, Number 26220). Ethical approval was granted by the Kocaeli University Animal Research Ethics Committeee (Kocaeli, Turkey). Adult male Wistar rats weighing 200-250g 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 (Resveratrol group).

**Polimicrobial sepsis model**

The experimental polimicrobial sepsis model was induced by CLP as previously described [6]. Briefly, under ketamine (50 mg/kg ip) and xylazine (25 mg/kg ip) anesthesia, an approximately 2 cm midline incision was made in the abdomen. The cecum was isolated carefully and then ligated just below the ileocecal 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 two layers. All rats were then resuscitated with 1 ml saline injected subcutaneously. Sham-operated control underwent the similar surgical procedure except that the cecum was neither ligated nor punctured. Animals received resveratrol (100 mg/kg) or saline immediately after the induction of sepsis. Afterwards, we observed the rats in a recovery cage for 24 h. The rats had free access to food and water after the operation.

**Organ Bath Studies**

24 h 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, 255 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 full-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, Turkey) for the measurement of isometric force, which was continuously displaced and recorded on-line on a computer via a four-channel transducer data acquisition system (TDA 94, COMMAT Iletisim Co, Turkey). After 1 h equilibration, at the beginning of each experiment 80 mM KCl was added to the organ bath and the contraction was considered as reference response. After the washout, cumulative concentration-response curve was obtained with carbachol ($10^{-8}$-$10^{-4}$ M) were recorded. Electrical stimulation was provided by a stimulator (ST 95 PT; Commat Iletisim, Turkey) and applied via two platinum wire electrodes set vertically within the opposite organ bath sides of the suspended tissue. Prior to EFS, the tissue was treated with guanethidine ($3.10^{-6}$ M) (adrenergic nerve blocker) and atropine ($10^{-6}$ M) (muscarinic receptor blocker) for 30 min. Square-wave pulses of 10 V (0.5 ms duration) in 10-s trains of varying frequency (1–10 Hz) were applied at 5 min intervals. The SMS were allowed to return to baseline tension between the tests at each frequency. Then the relaxant responses to SNP ($10^{-8}$-$10^{-4}$ M) and papaverine ($10^{-5}$ M) were obtained. Relaxant responses were expressed as percentage of the maximal relaxation produced by papaverine ($10^{-5}$ M).

Blood Analysis

Blood was drawn via the heart (1.0 ml each). The serum was immediately separated by centrifugation at 4000 rotation per min for 15 min at 4 °C, divided into aliquots and stored at -70 °C until assayed. The proinflammatory cytokine which are secretion of rat interleukin 1β (IL-1β), IL-6 and tumor necrosis factor-α (TNF-α), levels of serum
were quantified using enzyme-linked immunosorbent assays (ELISA) (Biosource, Invitrogen) according to the manufacturer’s recommendations then analyzed with Versa Max Molecular Devices Microplate reader using SoftMax Pro 5 software.

**Statistical Analysis**

Results are expressed as mean±SEM. The differences between means were assessed with one-way analysis of variance (ANOVA). Probability levels than 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 isolated from rats (Fig. 1, 2 and Table 1, 2). Resveratrol treatment (100 mg/kg, ip) significantly reversed the decreased contractile responses of both KCl and carbachol compared to sepsis group (Fig. 1, 2 and Table 1, 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 to responses obtained from that of control group (Figure 3 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 to control group (Figure 4 and Table 1, 2).

TNF alpha blood levels were significantly increased in sepsis group (158,84±21,46 pg/ml) compared to resveratrol (45,63±5,13 pg/ml) and control (5,05±1,81 pg/ml) groups (One-way Anova Tukey test. p<0,01).
IL-6 levels in blood were significantly increased in sepsis group (0,377±0,0569 pg/ml) compared to resveratrol group (0,098±0,010 pg/ml) and control group (0,084±0,008 pg/ml). (One-way Anova Tukey test. p<0,01).

Discussion
The intestine is one of the major organs that are involved in sepsis. Decreased intestinal motility is a well known complication of sepsis which may lead to bacterial translocation and worsen organ damage. Previously it was shown that sepsis induced rats showed decreased contractile and relaxant responses [2-7]. In this background, we investigated the healing effects of resveratrol on ileal smooth muscle reactivity in a different sepsis model. Additionally inflammatory cytokines such as TNF-α, and IL-1 levels were measured in the study.

The present study showed that impaired ileal smooth muscle reactivity markedly improved after resveratrol treatment in sepsis. Furthermore, increased TNF-α 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 Ca$^{+2}$ channel and increasing intracellular Ca$^{+2}$ [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 observation is very similar to previous study which showed the contractile responses to KCl and phenylephrine decreased after sepsis in isolated rat aorta [9]. However in the present study, impaired contractile response to KCl and carbachol increased to control values after resveratrol treatment. Therefore resveratrol treatment in sepsis may work by restoration of physiologic signal transmission such as altered Ca$^{+2}$ influx, calcium storage or secondary mechanisms during contraction.
It’s well established that NO is the major inhibitory neurotransmitter in the GI tract and impairments in nitrergic innervation lead to intestinal motility disorder [10]. It was shown that electrically evoked NANC-mediated relaxation is mediated by NO in rat ileum [11]. Neuronal nitric oxide synthase (nNOS) is responsible for this relaxation [12]. NO diffuses to smooth muscle cells and activates soluble guanylate cyclase so that the intracellular cGMP concentration increases. Various pathological conditions that inhibit the synthesis of NO, enhance its degradation, or inhibit guanylyl cyclase are accompanied with the gastrointestinal dysfunction. We observed diminished NANC relaxation induced by EFS in ileal tissues obtained from septic rats compared to control rats. The mechanism for impaired NO-mediated response in ileal smooth muscle is yet unknown. It is possible that impaired 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 changed among the groups. Thus we suggest that responsiveness of the smooth muscle to NO is not altered by sepsis may related to other mechanisms requires further study.

In the present study treatment with resveratrol restored the contractility of the ileal smooth muscle reactivity. Several studies in recent years have shown that resveratrol exhibits strong antioxidant and antiinflammatory effects [4,13]. 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 antiinflammatory effects were account for
cardioprotective actions. Similar protective effect of resveratrol on endotoxemia-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]. Based on these statements, it can be suggested that the functional impairment of the ileal tissues were due to an oxidative stress and detrimental effects of sepsis on ileal contractility can be prevented by antioxidant activity of resveratrol.

In conclusion, we found impaired NO-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, antiinflammatory and NANC mechanism related effects. To gain a better understanding of the mechanisms by which resveratrol induces these effects, further studies must be performed.

**Figure 1:** The contractions elicited by 80 mM KCl on ileum smooth muscles isolated from rats in control, sepsis and resveratrol groups. Values are mean±SE mean from six to seven experiments. * significant difference from the corresponding control value (p<0.05,  \textit{t} test).

**Figure 2:** Carbachol concentration-response curves in isolated rat ileal smooth muscles. Each point is expressed as a percentage of the contraction induced by 80 mM KCl and is given as the mean±SE mean. Number of rats in each group is shown in parantheses. * significant difference from the corresponding control value (p<0.05, \textit{t} test).
**Figure 3:** 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 $10^{-4}$ M papaverine and is given as the mean±SE mean. Number of rats in each group is shown in parantheses. * significant difference from the corresponding control value (p<0.05, test).

**Figure 4:** Sodium nitroprusside concentration-response curves in isolated rat ileal smooth muscles. Each point is expressed as a percentage of the relaxation induced by $10^{-4}$ M papaverine and is given as the mean±SE mean. Number of rats in each group is shown in parantheses. * significant difference from the corresponding control value (p<0.05, test).

References


Effects of resveratrol on ileal smooth muscle reactivity in polimicrobial sepsis model

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Abstract

Aim: To determine the effects of resveratrol on the ileal smooth muscle reactivity in polimicrobial sepsis.

Material and Methods: Polimicrobial sepsis was induced by the cecal ligation and perforation (CLP) procedure. Sprague Dawley rats divided into three groups. Rats in resveratrol group received resveratrol after CLP (100 mg/kg, ip). Rats received saline immediately after CLP in the sepsis group. Control group rats underwent sham operation. The rats sacrificed and the ileum was excised twenty-four hours after the operation. Contractile and relaxant responses in isolated smooth muscle strips (SMS) were determined using an in vitro muscle technique. TNF alpha 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 to 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 the all groups. Resveratrol treatment supressed increased TNF alpha 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 indicated that the beneficial effects of resveratrol might be, at least in part, attributed to its effects on non-adrenergic non-cholinergic pathway and/or antiinflammatory and antioxidant activity.

Introduction
Gastrointestinal stasis or ileus commonly accompanies sepsis and multiorgan dysfunction in critically ill patients and may contribute to their pathophysiology. Nonmechanical 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 also 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.

Materials and Methods

The experiments were carried out in accordance with regulation of Animal Research Ethics Committee in Turkey (6 July, 2006, Number 26220). Ethical approval was granted by the Kocaeli University Animal Research Ethics Committee (Kocaeli, Turkey). Adult male Wistar rats weighing 200-250g 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 (Resveratrol group).

**Polimicrobial sepsis model**

The experimental polimicrobial sepsis model was induced by CLP as previously described [6]. Briefly, under ketamine (50 mg/kg ip) and xylazine (25 mg/kg ip) anesthesia, an approximately 2 cm midline incision was made in the abdomen. The cecum was isolated carefully and then ligated just below the ileocecal 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 resuscitated with 1 ml saline injected subcutaneously. **Sham-operated control underwent the similar surgical procedure except that the cecal ligature and puncture.** Animals were 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**

24 h 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, 255 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 full-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, Turkey) for the measurement of isometric force, which was continuously displaced and recorded on-line on a computer via a four-channel transducer data acquisition system (TDA 94, COMMAT Iletisim Co, Turkey). After 1 h equilibration, at the beginning of each experiment 80 mM KCl was added to the organ bath and the contraction was considered as reference response. After the washout, cumulative carbachol (10^{-8}-10^{-4} M) concentration-response curve was obtained. Electrical stimulation was provided by a stimulator (ST 95 PT; Commat Iletisim, Turkey) and applied via two platinum wire electrodes set vertically within the opposite organ bath sides of the suspended tissue. Prior to transmural electrical field stimulation (EFS), the tissue was treated with guanethidine (3.10^{-6} M) (adrenergic nerve blocker) and atropine (10^{-6} M) (muscarinic receptor blocker) for 30 min. Square-wave pulses of 10 V (0.5 ms duration) in 10-s trains of varying frequency (1–10 Hz) were applied at 5 min intervals. The SMS were allowed to return to baseline tension between the tests at each frequency. Then the relaxant responses to SNP (Sodium nitroprusside) (10^{-8}-10^{-4} M) and papaverine (10^{-5} M) were obtained. Relaxant responses were expressed as percentage of the maximal relaxation produced by papaverine (10^{-5} M). In order to evaluate the effects of agonists, maximum responses (Emax) and pD2 values (apparent agonist affinity constants; -log ED50) were calculated. Agonist pD2 value was calculated from each agonist dose–response curve by linear portion of the curve and taken as a measure of the sensitivity of the tissues to each agonist.
Blood Analysis

Blood was drawn via the heart (1.0 ml each). The serum was immediately separated by centrifugation at 4000 rotation per min for 15 min at 4 ºC, divided into aliquots and stored at -70 ºC until assayed. The proinflammatory cytokine which are secretion of rat interleukin 1β (IL-1β), IL-6 and tumor necrosis factor-α (TNF-α), levels of serum were quantified using enzyme-linked immunosorbent assays (ELISA) (Biosource, Invitrogen) according to the manufacturer’s recommendations then analyzed with Versa Max Molecular Devices Microplate reader using SoftMax Pro 5 software.

Statistical Analysis

Results are expressed as mean±S.D. The differences between means were assessed with one-way analysis of variance (ANOVA). Probability levels lower than 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 isolated from rats (Fig. 1 and Table 1, 2). Resveratrol treatment (100 mg/kg, ip) significantly reversed the decreased contractile responses of both KCl and carbachol compared to sepsis group (Fig. 1 and Table 1, 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 to responses obtained from that of control group (Figure 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 to control group (Figure 3 and Table 1, 2).

TNF alpha and IL-6 blood levels were significantly increased in sepsis group compared to resveratrol and control groups (Fig 4a and 4b, One-way Anova Tukey test p<0.01).

**Discussion**

The intestine is one of the major organs that are 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 TNF-alpha 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 Ca\(^{+2}\) channel and increasing intracellular Ca\(^{+2}\) [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 which 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 Ca\(^{+2}\) influx, calcium storage or secondary mechanisms during contraction.

It's well established that NO is the major inhibitory neurotransmitter in the GI tract and impairments in nitrergic 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 to smooth muscle cells and activates soluble guanylyl cyclase so that the intracellular cGMP concentration increases. Various pathological conditions that inhibit the synthesis of NO, enhance its degradation, or inhibit guanylyl cyclase are accompanied with the gastrointestinal dysfunction. We observed diminished non-adrenergic non-cholinergic-mediated relaxation induced by EFS in ileal tissues obtained from septic rats compared to 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 changed among the groups. We interpret the SNP data as evidence that this sepsis model failed to effect either the ability of NO to activate guanylyl cyclase-cGMP or the sensitivity of ileal smooth muscle contractile apparatus to cGMP-mediated actions.

Recently, Pearson et al. found resveratrol treatment of mice decreased expression of the inflammatory remarkers TNF-alpha, Interleukin-6, Interleukin 1-Beta, intercellulary adhesion molecules-1, and i-NOS [13]. In the present study in accordance to Pearson et al.; we observed increased TNF-alpha 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
which resveratrol induced effects. To gain a better understanding of the mechanisms by
due to its antioxidant, anti-inflammatory and non-adrenergic non-cholinergic smooth muscle reactivity induced by sepsis. Reversal effect of resveratrol may be
According to our study, resveratrol has some protective effects on impaired ileal
contraction in septic rats. The mechanism of this result is not completely known.

In conclusion, we found impaired EFS-mediated relaxation and carbachol-induced such as superoxide dismutase. We can not draw any conclusion at the present time.

In a previous study, it was shown that resveratrol has a protective effect on endotoxemia-dependent intestinal motility responses induced by ischemia/reperfusion [14]. They showed cardiotrope effects that its antioxidant and anti-inflammatory effects were accounted for

Additionally, Shigematsu et al. demonstrated that resveratrol prevents superoxide-dependent mitochondrial oxidative stress in rats subjected to ischemia-reperfusion [14]. They concluded that the adrenergic and antioxidant activities of resveratrol were independent of EFS-mediated relaxation and carbachol-induced.

In conclusion, we found impaired EFS-mediated relaxation and carbachol-induced such as superoxide dismutase. We can not draw any conclusion at the present time.

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In conclusion, we found impaired EFS-mediated relaxation and carbachol-induced such as superoxide dismutase. We can not draw any conclusion at the present time.
**Figure 1:** Carbachol concentration-response curves in isolated rat ileal smooth muscles. Each point is expressed as a percentage of the contraction induced by 80 mM KCl and is given as the mean±S.D. Number of rats in each group is shown in parantheses. * significant difference from the corresponding control value (p<0.05).

**Figure 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^{-4} M papaverine and is given as the mean±S.D. Number of rats in each group is shown in parantheses. * significant difference from the corresponding control value (p<0.05).

**Figure 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^{-4} M papaverine and is given as the mean±S.D. Number of rats in each group is shown in parantheses.

**Figure 4a:** TNF alpha blood levels. Each point is given as the mean±S.D. * significant difference from the corresponding control value (p<0.05). # significant difference from the sepsis value (p<0.05).

**Figure 4b:** IL-6 levels blood levels. Each point is given as the mean±S.D. * significant difference from the corresponding control value (p<0.05).
References


Effects of resveratrol on ileal smooth muscle reactivity in polimicrobial sepsis model

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Subject category: Sepsis
Running title: effects of resveratrol on smooth muscle reactivity during sepsis

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Abstract

Aim: To determine the effects of resveratrol on the ileal smooth muscle reactivity in polimicrobial sepsis.

Material and Methods: Polimicrobial sepsis was induced by the cecal ligation and perforation (CLP) procedure. Sprague Dawley rats divided into three groups. Rats in resveratrol group received resveratrol after CLP (100 mg/kg, ip). Rats received saline immediately after CLP in the sepsis group. Control group rats underwent sham operation. The rats sacrificed and the ileum was excised twenty-four hours after the operation. Contractile and relaxant responses in isolated smooth muscle strips (SMS) were determined using an in vitro muscle technique. TNF alpha 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 to 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 the all groups. Resveratrol treatment supressed increased TNF alpha 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 indicated that the beneficial effects of resveratrol might be, at least in part, attributed to its effects on non-adrenergic non-cholinergic pathway and/or antiinflammatory and antioxidant activity.

Introduction
Gastrointestinal stasis or ileus commonly accompanies sepsis and multiorgan dysfunction in critically ill patients and may contribute to their pathophysiology. Nonmechanical 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 also to modulate lipoprotein mechanism. Resveratrol supresses 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 supressed by resveratrol [5]. Although antiinflammatory 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 polimicrobial 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.

Materials and Methods

The experiments were carried out in accordance with regulation of Animal Research Ethics Committee in Turkey (6 July, 2006, Number 26220). Ethical approval was granted by the Kocaeli University Animal Research Ethics Committee (Kocaeli, Turkey). Adult male Wistar rats weighing 200-250g 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 (Resveratrol group).

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Statistical Analysis

Results are expressed as mean±S.D. The differences between means were assessed with one-way analysis of variance (ANOVA). Probability levels lower than 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 isolated from rats (Fig. 1 and Table 1, 2). Resveratrol treatment (100 mg/kg, ip) significantly reversed the decreased contractile responses of both KCl and carbachol compared to sepsis group (Fig. 1 and Table 1, 2). In the presence of adrenergic (guanethidin, 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 to responses obtained from that of control group (Figure 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
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**Discussion**

The intestine is one of the major organs that are 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 TNF-alpha 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 Ca$^{+2}$ channel and increasing intracellular Ca$^{+2}$ [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 which 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 Ca$^{+2}$ influx, calcium storage or secondary mechanisms during contraction. It’s well established that NO is the major inhibitory neurotransmitter in the GI tract and impairments in nitrergic 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 to smooth muscle cells and activates soluble guanylyl cyclase so that the intracellular cGMP concentration increases. Various pathological conditions that inhibit the synthesis of NO, enhance its degradation, or inhibit guanylyl cyclase are accompanied with the gastrointestinal dysfunction. We observed diminished non-adrenergic non-cholinergic-mediated relaxation induced by EFS in ileal tissues obtained from septic rats compared to 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 changed among the groups. We interpret the SNP data as evidence that this sepsis model failed to effect either the ability of NO to activate guanylyl cyclase-cGMP or the sensitivity of ileal smooth muscle contractile apparatus to cGMP-mediated actions.

Recently, Pearson et al. found resveratrol treatment of mice decreased expression of the inflammatory markers TNF-alpha, Interleukin-6, Interleukin 1-Beta, intercellulary adhesion molecules-1, and i-NOS [13]. In the present study in accordance to Pearson et al.; we observed increased TNF-alpha 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 antiinflammatory effects were account for cardioprotective actions. Similar protective effect of resveratrol on endotoxemia-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, in this study, we did not measure antioxidant activity such as superoxide dismutase, we can not draw any conclusion at the present time.

In 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, antiinflammatory 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.
Figure 1: Carbachol concentration-response curves in isolated rat ileal smooth muscles. Each point is expressed as a percentage of the contraction induced by 80 mM KCl and is given as the mean±S.D. Number of rats in each group is shown in parentheses. * significant difference from the corresponding control value (p<0.05).

Figure 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^-4 M papaverine and is given as the mean±S.D. Number of rats in each group is shown in parentheses. * significant difference from the corresponding control value (p<0.05).

Figure 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^-4 M papaverine and is given as the mean±S.D. Number of rats in each group is shown in parentheses.

Figure 4a: TNF alpha blood levels. Each point is given as the mean±S.D. * significant difference from the corresponding control value (p<0.05). # significant difference from the sepsis value (p<0.05).

Figure 4b: IL-6 levels blood levels. Each point is given as the mean±S.D. * significant difference from the corresponding control value (p<0.05).
References


Table 1: $E_{\text{max}}$ (% of $10^{-5}$ M papaverine) values for sodium nitroprusside (SNP) and electrical field stimulation (EFS), $E_{\text{max}}$ (% of 80 mM KCl) values for carbachol, and $E_{\text{max}}$ values (mg) for 80 mM KCl in ileal strips obtained from control, sepsis and resveratrol-treatment groups.

<table>
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<tr>
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<th>Carbachol</th>
<th>n</th>
<th>SNP</th>
<th>n</th>
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<th>n</th>
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<td>3258.7±330.79</td>
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<td>65.4±11.84</td>
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Values are arithmetic means±S.D. n= the number of preparations used. * P<0.05, statistically different from the response of strips from sham-operated control rats.
Table 2: pD$_2$ values (-log M EC50) for carbachol, sodium nitroprusside (SNP) in corpus cavernosum strips obtained from neurotomy and sham operation groups.

<table>
<thead>
<tr>
<th></th>
<th>Carbachol</th>
<th>SNP</th>
</tr>
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<tr>
<td>Control (n=8)</td>
<td>6.44±0.62</td>
<td>5.21±0.76</td>
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<td>Sepsis (n=8)</td>
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<tr>
<td>Resveratrol (n=8)</td>
<td>6.47±0.68</td>
<td>5.21±0.68</td>
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</table>

Values are arithmetic means±S.D. n= the number of preparations used. * P<0.05, statistically different from the response of strips from sham-operated control rats.
Figure 1

- Control (n=8)
- Septis (n=8)
- Resveratrol (n=8)

Contraction (KCl %) vs. Carbachol (M)

Control (n=8)
Septis (n=8)
Resveratrol (n=8)
Figure 2
Figure 3
Figure 4a

![Figure 4a Image]

- **Control (n=8)**
- **Sepsis (n=8)**
- **Resveratrol (n=8)**

**TNF-alpha (pg/ml)**

* and # symbols indicate statistical significance between groups.
Figure 4b

Interleukine-6 (pg/ml)
Control (n=8)
Sepsis (n=8)
Resveratrol (n=8)