Cardiovascular pharmacology

The effect of etanercept on aortic nitric oxide-dependent vasorelaxation in an unpredictable chronic, mild stress model of depression in rats

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ABSTRACT

Stress has been recognized as a risk factor for cardiovascular disease and depression, but the correlation is not well understood. However, inflammation is known to have a crucial role in both cardiovascular disease and depression. Tumor necrosis factor alpha (TNF-α) is a major cytokine for the activation of neuroendocrine, immune and behavioral responses. Therefore, we aimed to explore the effects of etanercept, an anti-TNF-α fusion protein, on endothelium-dependent vascular reactivity, blood pressure and endothelial nitric oxide synthase (eNOS) immunoreactivity in a model of unpredictable chronic mild stress (UCMS). Male rats were exposed to UCMS for 8 weeks, and etanercept (0.8 mg/kg, weekly) was administered during UCMS induction. The systolic blood pressure was recorded by the tail cuff method, and the relaxant responses of the aorta induced by carbachol, sodium nitroprusside (SNP) and papaverine were evaluated in an isolated organ bath system. UCMS rats exhibited an impaired carbachol-induced relaxant response compared to control rats, but there were no significant differences in the SNP- and papaverine-induced relaxant responses between the control and stressed rats. Etanercept treatment improved the carbachol-induced endothelium dependent relaxations observed in rats that experienced UCMS. No significant change in the systemic blood pressure was observed, but decreased expression of eNOS was detected in the UCMS group. Moreover, there were no significant changes in the etanercept treatment group compared to the control rats. Our results suggest that TNF-α could be a mediator of vascular dysfunction associated with UCMS, which leads to decreased expression of eNOS.

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1. Introduction

Psychological stress and depression are well known to be associated with cardiovascular diseases (Rudisch and Nemeroff, 2003; Plante, 2005). Chronic psychological stress is also associated with systemic inflammatory response (Naldi and Mercuri, 2012; Michaud and Wolfe, 2007), but the precise mechanisms linking these two events remain largely unknown. Under stressful conditions, the body recognizes, evaluates and adapts to stressors by initiating an immune reaction that results in a chain of local and systemic events mediated by immune intercellular messengers (Mosovich et al., 2008). In this regard, TNF-α is a pivotal cytokine for the activation of neuroendocrine, immune and behavioral responses (Mosovich et al., 2008).

Unpredictable chronic mild stress (UCMS) is one of the most validated rodent models used to study depression in animals. Consistently, our recent study demonstrated that UCMS induces depression-like behavior (Yazir et al., 2012). Additionally, there have been many reports that focus on the involvement of inflammatory agents in depression, and we have previously shown the beneficial effects of infliximab in UCMS-induced depression (Karson et al., 2012).

The vascular endothelium plays a major role in maintaining vascular homeostasis and is involved in several physiological processes such as vascular tone and vasomotor function as well as the regulation of inflammation, platelet aggregation and thrombosis. Nitric oxide (NO) actively mediates many of the functions exerted by the intact endothelium (Behrendt and Ganz, 2002). Previous studies have confirmed that a decrease in NO...
bioavailability is the major mechanism of endothelial dysfunction in depression (Le Mellédo et al., 2004, Chrapko et al., 2004). Recently, it has been shown that chronic depressive symptoms have a strong correlation with poor vascular endothelial function in UCMS-exposed mice (d'Audiffret et al., 2010; Isingrini et al., 2011, 2012). Moreover, inflammatory stimuli are known to increase the vulnerability of the vessel wall to atherogenic stimuli (Nieuwdorp et al., 2009).

TNF-α is a proinflammatory cytokine proposed to be involved in the pathogenesis of vascular dysfunction (Arenas et al., 2004). In endothelial cell culture, TNF-α decreases the expression of eNOS, which is the primary enzyme involved in NO production in the vasculature (Yoshizumi et al., 1993). It has been reported that vascular inflammation induced by chronic stress contributes to the development of atherosclerosis (Lu et al., 2012).

Considering this background, we aimed to investigate the effects of etanercept, an anti-TNF-α fusion protein, on blood pressure and vascular reactivity as well as to analyze the expression of eNOS by immunohistochemical staining in UCMS model of depression in rats.

2. Material and methods

2.1. Animal preparation and experimental design

Adult male Wistar rats (Experimental Medical Research and Application Center, Kocaeli University, Kocaeli, Turkey) weighing 250–300 g were kept in an animal colony at a density of approximately 4 to 5 per cage for 2 weeks prior to the experiments. All experiments were conducted between 9:00 A.M. and 12:00 P.M. under standard laboratory conditions (22 ± 2 °C room temperature; 12 h light/dark cycle with lights on at 7:00 A.M.). Tap water and food pellets were provided ad libitum. All animals used in this study were naive to the experimental tests, and different rat groups were used for each condition.

The experiments reported in this study were conducted in accordance with the Regulation of Animal Research Ethics Committee in Turkey (July 6, 2006, Number 26220). Ethical approval was granted by the Kocaeli University Animal Research Ethics Committee (Project number: HAEK 2012/8, Kocaeli, Turkey). Rats were divided into the following four groups (n=8 in each group): control, control+etanercept, UCMS and UCMS+etanercept. Saline (sc) was administered to rats in the control and UCMS groups, whereas etanercept (0.8 mg/kg) was administered to the control +etanercept and UCMS+etanercept groups once per week. After 8 weeks, the indirect blood pressure was determined, and acetylcholine, SNP and papaverine cumulative concentration–response curves were obtained. Additionally, we analyzed the expression of eNOS by immunohistochemistry.

2.2. Unpredictable chronic mild stress procedure

UCMS was applied as previously described by Yazir et al. (2012). Briefly, the UCMS groups either with or without etanercept treatment were subjected to different types of stressors: restraint for 4 h, cage tilting for 24 h, wet bedding for 24 h, swimming in 4 °C cold water for 5 min, swimming in 45 °C hot water for 5 min, pairing with another stressed animal for 48 h, level shaking for 10 min, nip tail for 1 min and inversion of the light/dark cycle for 24 h. These nine stressors were randomly applied for 56 days, and each stressor was applied 6–7 times during this time period. Rats received one of these stressors per day, and the same stressor was not applied for 2 consecutive days so that the animals could not predict the occurrence of stimulation. The stress procedure did not involve any food or water deprivation. The control groups received no stress, and all of the animals had continuous access to food and water.

2.3. Blood pressure measurement

At the end of the 8-week period of UCMS, the systolic blood pressure was recorded by the tail cuff method. Indirect systolic blood pressure and heart rate were recorded once per week by tail cuff plethysmography (MAY-COM BPHR 200, COMMAT Iletisim Co., Ankara, Turkey). In this procedure, conscious rats were placed in a restraining holder from which the tail protruded. Vasodilatation was achieved by local warming of the tail with an infrared bulb. A cuff and pulse sensor was placed around the tail, and the cuff was inflated until the pulse disappeared. When the cuff was deflated, the point of the reappearance of the pulse indicated the value of systolic blood pressure. The average value of the blood pressure and heart rate in each rat were obtained from three sequential cuff inflation–deflation cycles.

2.4. Organ bath studies

After blood pressure measurement, the rats were sacrificed and the thoracic aorta from the aortic arch to the diaphragm was excised. After excision, the vessels were immediately placed in Krebs’s solution (composition described below) and were dissected carefully to prepare the rings. The rings were transferred to organ baths containing 20 ml of Krebs’s solution (pH 7.4) maintained at 37 °C by a thermoregulated water circuit and continuously aerated with 95% O2 and 5% CO2. All of the tissues were allowed to equilibrate for 60 min prior to initiating 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 ring was connected to a force-displacement transducer (MAY-COM FDT 10A; COMMAT Iletisim Co., Ankara, Turkey) to measure the isometric force, which was continuously displaced and recorded online on a computer via a four channel transducer data acquisition system (MP30B-CE, Biopac System Inc, Santa Barbara, CA, USA), using software (BSL Pro 3.7, Biopac System Inc, Santa Barbara, CA, USA) that was capable of analyzing the data.

2.5. Agonist-induced contractions

The tissues were exposed to 80 mM KCl for 5 min to test the viability of the preparation. The tissues were then washed, and the contractile responses to a range of phenylephrine concentrations (10⁻⁶–10⁻⁴ M) were obtained cumulatively.

2.6. Agonist-induced relaxation

Each aortic ring was contracted by treatment with 3 × 10⁻⁶–10⁻⁵ M phenylephrine. These concentrations produced 85–87% of the maximal response to phenylephrine. After the phenylephrine-induced contraction had reached a plateau, the concentration–response relationships for carbachol (10⁻⁸–10⁻⁵ M), sodium nitroprusside (10⁻⁸–10⁻⁴ M) and papaverine (10⁻⁵–10⁻⁴ M) were obtained by adding one of these agents to the bath in a cumulative manner. The concentration of the agonist in the bath was increased approximately threefold at each step after the response to the previous dose reached a plateau. Between successive concentration–response curves, the tissues were rinsed with fresh buffer and allowed to recover for 30 min, during which time the tension returned to basal levels.
2.7. Solutions and drugs

The ionic composition of the Krebs solution was as follows (mM): NaCl, 118; KCl, 4.71; MgCl₂, 1.05; NaH₂PO₄, 1.33; NaHCO₃, 25; CaCl₂, 2.7 and glucose, 5.6. In the high K⁺ solution, NaCl was replaced with an equimolar amount of KCl. Fresh solutions were prepared on the day of the experiments. The following drugs were used (all from Sigma Chemical Co., St. Louis, MO, USA): phenylephrine hydrochloride, carbachol chloride, sodium nitroprusside and papaverine hydrochloride. The drugs were prepared daily in distilled water and kept on ice during the course of the experiments. Etanercept was also purchased from Wyeth (Münster, Germany) and dissolved in physiological saline. It was prepared immediately prior to use and administered subcutaneously (s.c.) to the rats at a volume of 0.1 ml per 100 g body weight.

2.8. Immunohistochemical analyses

Tissues were taken from rats and fixed with 10% neutral buffered formalin. Routine histological tissue procedures were performed, and the tissues were embedded in paraffin. Paraffin-embedded sections at a thickness of 3 μm were placed onto poly-L-lysine slides. The sections were deparaffinized with xylene, hydrated with a descending series of ethanol, and washed with PBS. Then, the tissue sections were placed in 1 mM citrate buffer (pH 6.0), and antigen retrieval was performed in a microwave oven followed by incubation with 3% H₂O₂ in methanol to reduce the endogenous peroxidase activity. The sections were then directly incubated overnight with a polyclonal primary antibody against eNOS (sc654, Santa Cruz, California USA), followed by incubation with a secondary biotinylated antibody, streptavidin–peroxidase and a diaminobenzidine solution. After the sections were counterstained with hematoxylin, the slides were mounted. The slides were examined under a light microscope (Olympus BX 50, Tokyo Japan), and photomicrographs were taken with a Leica DM 100 system (Leica DFC290 HD, Wetzlar, Hessen, Germany). All of the samples were treated using the same protocol. Two independent observers who were blinded to this study graded the staining intensity on a semiquantitative scale ranging from no expression (−) to very weak (1+), moderate (2+), strong (3+) and very strong (4+) expression. The concordance between the grading of the two observers was 89%. Percentage of positive cells is defined as 0, <5%; 1, 6–15%; 2, 16–50%; 3, 41–60%; and 4, >80% positive cell.

2.9. Statistical analysis

All of the data were expressed as the mean value ± standard error of the mean (S.E.M.). The contractile force was expressed in milligrams of developed tension. The relaxant effects of the agonists were expressed as a percentage of the precontractile response to phenylephrine. Concentration–response curves were fitted by nonlinear regression with simplex algorithm, and Eₘ and pD₂ (–log EC₅₀) were calculated using the software of the transducer data acquisition system. Briefly, the cumulative concentration–response curve data were fit with a computer, as previously described, to a four parameter logistic equation: \( E = E_{\text{max}} \frac{1 + [D]}{[D]^{n}} \), where \( E \) is the observed effect in grams of tension, \( [D] \) is the concentration of agonist, \( E_{\text{max}} \) is the calculated maximal effect, EC₅₀ is the [D] at 0.5 \( E_{\text{max}} \), and \( n \) is the slope factor parameter. Significance was tested by one-way analysis of variance (ANOVA) with a post-hoc Tukey’s–Kramer test. The score of immunoreactivity was assessed by the Kruskal–Wallis Test. Probabilities of less than 5% (\( P < 0.05 \)) were considered significant.

3. Results

There were no significant differences in the body weight among the groups at the onset of the experiments (\( P > 0.05 \); Table 1). The average body weight after 8 weeks of UCMS was significantly lower than that of the animals receiving no stress (\( P < 0.001 \); Table 1). Etanercept treatment in rats undergoing UCMS significantly increased the body weight of the rats. The overall body weight change over the 8-week period was.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Control + etanercept</th>
<th>UCMS</th>
<th>UCMS + etanercept</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight (g)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onset</td>
<td>275 ± 7.5</td>
<td>269 ± 8.8</td>
<td>277.5 ± 8.7</td>
<td>278.1 ± 7.7</td>
</tr>
<tr>
<td>After</td>
<td>395 ± 12.2</td>
<td>390 ± 10.9</td>
<td>314.3 ± 5.3*</td>
<td>391.7 ± 12.5</td>
</tr>
<tr>
<td><strong>Carbachol</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eₘ (%)</td>
<td>66.49 ± 5.59</td>
<td>69.6 ± 4.8</td>
<td>19.79 ± 2.30*</td>
<td>58.17 ± 2.52</td>
</tr>
<tr>
<td>pD₂</td>
<td>6.42 ± 0.18</td>
<td>6.49 ± 0.02</td>
<td>5.87 ± 0.06</td>
<td>6.27 ± 0.16</td>
</tr>
<tr>
<td><strong>Sodium nitroprusside</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eₘ (%)</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
</tr>
<tr>
<td>pD₂</td>
<td>6.29 ± 0.16</td>
<td>6.31 ± 0.09</td>
<td>6.44 ± 0.04</td>
<td>6.51 ± 0.05</td>
</tr>
<tr>
<td><strong>KCl</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Eₘ (mg)</td>
<td>0.71 ± 0.03</td>
<td>0.76 ± 0.04</td>
<td>0.74 ± 0.06</td>
<td>0.79 ± 0.07</td>
</tr>
<tr>
<td><strong>Papaverine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eₘ (%)</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
</tr>
</tbody>
</table>

Values are arithmetic means ± S.E.M. n= number of animals or preparation used.

* P < 0.05 statistically different from control rats.

** P < 0.05, statistically different from the response of aortic rings from control rats.

Fig. 1. Representative light microscopy of the endothelium of the aorta from rats from the control, UCMS and UCMS + etanercept groups. Decreased eNOS (B) immunoreactivity in the aorta in UCMS rats compared to the control group (A) and increased eNOS (C) immunoreactivity in endothelial tissue from the UCMS + etanercept group compared to the UCMS groups (B).
significantly different from that of rats undergoing UCMS without etanercept ($P < 0.001$) but not significantly different from that of either the control or control-etanercept rats ($P > 0.05$; Table 1).

No significant changes were observed in the systemic blood pressure of animals (control group $= 102.2 \pm 19$ mmHg; control+etanercept group $= 104.8 \pm 2.1$; UCMS group $= 105.1 \pm 2.4$ mmHg; UCMS + etanercept $= 103.1 \pm 1.6$ mmHg; $n=8$ for each group, $p > 0.05$).

In the control group, eNOS immunoreactivity was detected in the cytoplasm of the endothelial cells of the aorta (Fig. 1A). The immunopositivity was decreased in the UCMS groups compared to the control groups ($P < 0.01$, Fig. 1B). In the etanercept treatment group, eNOS immunoreactivity was similar to that of the control group (Fig. 1C, Table 2).

The contractile responses to 80 mM KCl also did not change among all of the groups. As shown in Table 1, there were no significant differences between the maximum responses of the rings from etanercept-treated UCMS, UCMS or control rats ($P > 0.05$).

The ability of carbachol and SNP to relax arteries precontracted with a submaximal concentration of phenylephrine was determined. The precontractile tone was similar in all of the groups (Table 1). In thoracic aortic rings precontracted with phenylephrine at submaximal concentration ($3 \times 10^{-6}$--$10^{-5}$ M), carbachol ($10^{-5}$--$10^{-3}$ M) evoked relaxation in a concentration-dependent manner. The endothelium-dependent relaxation to carbachol was significantly decreased in the UCMS group compared to the control and etanercept treated-UCMS groups ($P > 0.05$, Fig. 2). The concentration–response curve for carbachol was shifted to the right with significantly lower $E_m$ and $pD_2$ values in the UCMS group compared to the control group ($P < 0.05$, Table 1, Fig. 2). Treatment with etanercept corrected the impairment of relaxation to the same level as the control groups. There was no significant difference in $pD_2$ and $E_m$ values between the control and etanercept treatment groups ($P > 0.05$, Table 1).

In the aortic rings precontracted with phenylephrine, the NO donor SNP ($10^{-8}$--$10^{-4}$ M) caused concentration-dependent relaxation (Fig. 3), but there were no significant differences in $E_m$ and $pD_2$ values among the groups (Table 1). Additionally, the relaxation response to papaverine was similar among the vascular tissues from all of the groups (Table 1).

### Table 2

<table>
<thead>
<tr>
<th>Animal</th>
<th>Control</th>
<th>UCMS</th>
<th>Etanercept-UCMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3+</td>
<td>1+</td>
<td>2+</td>
</tr>
<tr>
<td>2</td>
<td>2+</td>
<td>2+</td>
<td>3+</td>
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<tr>
<td>3</td>
<td>3+</td>
<td>1+</td>
<td>2+</td>
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<tr>
<td>4</td>
<td>2+</td>
<td>1+</td>
<td>3+</td>
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<tr>
<td>5</td>
<td>4+</td>
<td>2+</td>
<td>3+</td>
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<tr>
<td>6</td>
<td>3+</td>
<td>1+</td>
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</tr>
<tr>
<td>7</td>
<td>3+</td>
<td>2+</td>
<td>3+</td>
</tr>
<tr>
<td>8</td>
<td>3+</td>
<td>1+</td>
<td>2+</td>
</tr>
</tbody>
</table>

The staining intensity was classified no expression (−), very (1+), moderate (2+), strong (3+) to very strong (4+) expression. The immunopositivity was decreased in the UCMS groups compared to the control groups ($P < 0.01$, Kruskal–Wallis Test.). In the etanercept treatment group, eNOS immunoreactivity was similar to that of the control group.

**4. Discussion**

In the present study, carbachol-induced NO-mediated vasodilatation was attenuated in the aortas of rats exposed to UCMS compared to unstressed rats, whereas this effect was abolished by etanercept treatment. However, the systemic blood pressure and induced relaxation after treatment with SNP (an NO donor) were unchanged in stressed rats. Moreover, we found that UCMS elicited a decrease in eNOS expression. These effects of UCMS were abolished by etanercept treatment, and the expression levels were restored to those observed in unstressed rats.

The results concerning altered vascular reactivity in the UCMS model are compatible with previous studies showing that UCMS attenuated endothelium-dependent dilatation but had no effect on constriction in response to phenylephrine (Isingrini et al., 2011, 2012; Audiffret et al., 2010). These data are consistent with our study in which UCMS caused an impairment of carbachol-induced vasodilatation. It is known that the relaxation of vascular smooth muscle cells in response to either acetylcholine or carbachol requires the presence of an intact endothelium. In this study, the observation that UCMS impairs endothelium-dependent relaxation of vascular smooth muscle appear to be related to one of three causes: the alteration of the NO/cGMP pathway, the impairment of the relaxation of vascular smooth muscle or the reduction of NO sensitivity. However, these possibilities are unlikely because the vascular strips relaxed in response to both sodium nitroprusside and papaverine. Because sodium nitroprusside donates NO directly to smooth muscle (Moncada et al., 1991) and NO causes the activation of guanylate cyclase and the intracellular accumulation of cGMP, this finding indicates that the smooth muscle response to NO is not altered by UCMS. Currently, the mechanism underlying this impaired relaxation is unknown; however, UCMS-induced dysfunction of vascular tissue does not appear to involve alterations in the cGMP-dependent...
relaxation of vascular smooth muscle. It has also been speculated that the decrease in the endothelium-dependent relaxation response to carbachol in UCMS-exposed rats likely occurs at the level of the endothelium rather than the smooth muscle cells and is possibly due to the endothelial cell response to muscarinic receptor-mediated activation. Additionally, there were no differences in the KCI-induced contractile responses between the two groups; thus, the contractile mechanisms were intact in the vascular smooth muscle. In addition, at the concentrations of phenylephrine used, the developed tension was similar for vascular strips from both unstressed and stressed animals, thus confirming that any difference in relaxation between the stressed and unstressed preparations was not due to differences in the degree of precontraction. Therefore, it is possible that UCMS impairs either the synthesis or availability of NO in vascular tissue. This is consistent with the results of previous reports on UCMS-exposed rats (d’Audiffret et al., 2010). The authors also found that UCMS leads to impairment of the endothelium-dependent relaxation, but no changes were observed in the endothelium-independent relaxation induced by SNP (d’Audiffret et al., 2010). Because NO reflects the endothelium-dependent relaxation of smooth muscle cells, situations in which the carbachol-induced relaxation is altered suggests the possibility of impaired NO bioavailability. For this reason, the UCMS model may have a direct effect on the endothelium-dependent relaxation induced by NO release by impairing vasorelaxation. It has been reported that UCMS exposure results in a reduction in vascular basal NO synthesis (Isingrini et al., 2011). It has been well documented that the major causative factor contributing to vascular dysfunction in stressed animals is the reduction of NO synthesis in the aorta, resulting in alterations of normal vascular homeostasis (Neves et al., 2009).

Previous studies have reported that the endothelial dysfunction associated with the loss of NO bioavailability is due to the increased degradation of NO and the reduced formation of NO (Behrendt and Ganz, 2002). Both excessive generation of reactive oxygen species (including superoxide anions) and decreased antioxidant defense mechanisms contribute to enhanced degradation of NO (Kojda and Harrison, 1999). In experimental models of vascular disease, increased superoxide production is critically involved in reduced NO bioactivity and endothelial dysfunction (Ohara et al., 1993). In rats with UCMS, increased superoxide production has also been associated with decreased eNOS production (Kamper et al., 2009). Moreover, an increasingly popular theory is that formation of the proinflammatory cytokine products may have an important pathophysiological role in vascular dysfunction in UCMS. Alterations of endothelium-mediated relaxation may be the result of quenching and inactivation of NO by cytokines. Previously, proinflammatory cytokines TNF-α and interleukin-1 beta have been found to be elevated in both the plasma and the central nervous system of rats subjected to UCMS. In that same study, the cytokine levels also correlated with the degree of anhedonia; that is, a higher degree of anhedonia was correlated with higher levels of TNF-α and interleukin-1 beta (Grippo et al., 2005). These data provide new insights into the pathogenesis of vascular dysfunction associated with UCMS. It is possible that a reduction in endothelial NO availability may be responsible for endothelial dysfunction in UCMS.

We have demonstrated that chronic etanercept treatment for 8 weeks prevented UCMS-induced endothelial dysfunction. Our results are consistent with the previous findings in blood vessels from aged rats. Etanercept treatment has been shown to protect aged rats from endothelial dysfunction, suggesting that increased expression of the inflammatory markers iNOS and TNF-α may be pathogenically important in aging (Csiszár et al., 2007). In addition to this evidence, previous studies have demonstrated that anti-TNF-α therapies might improve endothelial dysfunction in various pathophysiological conditions (Hürlimann et al., 2002; Fichtlscherer et al., 2001). The mechanisms of the beneficial effects of etanercept on impaired relaxation in isolated vascular smooth muscle from UCMS rats are unknown. Recently, the endothelial glycocalyx (intraluminal layer consisting primarily of heparin sulfate and hyaluronan) has been claimed to be an orchestrator of vascular homeostasis, and TNF-α is one of the crucial factors that has been shown to disrupt the endothelial glycocalyx. Ince et al. (2009) showed that etanercept attenuated the loss of the microvascular glycocalyx thickness by changing the hyaluronan thickness and coagulation activation in healthy male volunteers receiving endotoxin. Their results revealed that inflammatory activity partially mediated by TNF-α leads to perturbation of the endothelial glycocalyx and contributes to the vascular vulnerability induced by inflammation (Ince et al., 2009). It is well known that TNF-α reduces NO availability by either decreasing eNOS expression (Yoshizumi et al., 1993) or increasing NO inactivation (Murphy et al., 1992). It has been reported that inhibition of TNF-α with etanercept improves agonist-mediated vasorelaxation and increased eNOS expression in fructose-fed rats (Tian et al., 2009).

Overall, previous studies and findings from the current study suggest that this protective effect of etanercept on the endothelium-dependent relaxation of vascular smooth muscle may result in decreased expression of inflammatory markers as well as increased production and/or availability of NO.

Lack of measurements of serum TNF-α levels might be considered a major limitation of our study. The mechanisms of action of TNF-α inhibitors, including etanercept, are dependent on the neutralization of TNF-α bioactivity via binding to the membrane-bound and soluble forms of TNF instead of affecting circulating levels of TNF-α (Tracey et al., 2008). It was previously shown that anti-TNF treatments led to increased levels of this cytokine in blood (Charles et al., 1999, Barrera et al., 2001); thus, serum TNF-α levels could not accurately reflect the efficacy of these agents.

5. Conclusions

We propose that our data are in accord with those of a previous study demonstrating that chronic mild stress-induced endothelial dysfunction observed in rats is primarily related to a decrease in NO production by the endothelium (Neves et al., 2009) and that etanercept treatment prevents the development of this endothelial dysfunction by restoring eNOS expression in rats that experienced UCMS.

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References


