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Etanercept restores vasocontractile sensitivity affected by mesenteric ischemia reperfusion

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ABSTRACT

Background: The aim of the study is to evaluate *in vivo* and *in vitro* effects of etanercept, a soluble tumor necrosis factor receptor, on the contractile responses of superior mesenteric artery in an experimental mesenteric ischemia and reperfusion model.

Material and methods: After obtaining animal ethics committee approval, 24 Sprague–Dawley rats were allocated to three groups. Control group (Gr C, $n = 6$) underwent a sham operation, whereas ischemia/reperfusion and treatment groups underwent 90 min ischemia and 24-h reperfusion (Gr I/R, $n = 12$; Gr I/R+E, $n = 6$). The treatment group received 5 mg/kg etanercept intravenously at the beginning of reperfusion. At the end of reperfusion, all animals were sacrificed, and third branch of superior mesenteric artery was dissected for evaluation of contractile responses. *In vitro* effects of etanercept on vasocontractile responses were also evaluated. The excised ileums were analyzed under light microscope. Two-way analysis of variance following Bonferroni post hoc test was used for evaluation of contractile responses.

Results: Endothelin-1 and phenylephrine-mediated vasocontractile sensitivity were found increased in Gr I/R when compared with Gr C. Both intravenous administration and organ bath incubation of etanercept decreased the sensitivity of contractile agents for Gr I/R. Mucosal injury, lamina propria disintegration, and denuded villous tips were observed in Gr I/R, whereas the epithelial injury and the subepithelial edema were found to be milder in Gr I/R+E.

Conclusions: Etanercept can be a promising agent in mesenteric ischemic reperfusion injury as it does not only inhibit inflammation by blocking tumor necrosis factor-α in circulation but also restores vascular contractility during reflow. These findings support an unexplained recuperative effect of drug beyond its anti-inflammatory effects.

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Introduction

Ischemic reperfusion injury (IRI) is a complex process involving a number of cytokines, chemokines, complement factors, lipid mediators, and oxygen radicals. The ischemic phase of the injury is related to the parenchymal damage due to intracellular acidosis resultant of hypoxia and adenosine triphosphate depletion. The postischemic reperfusion paradoxically causes additional injury due to the inflammatory response at tissue level. Also, postischemic reperfusion is generally related to capillary perfusion failure due to endothelial cell swelling, intravascular hemoconcentration, capillary narrowing as a result of increased interstitial pressure due to edema, and especially the sensitivity to vasoconstriction.[1] Mesenteric IRI is a life-threatening problem that occurs in a variety of clinical situations like mesenteric arterial embolism, mesenteric arterial or venous thrombosis, intestinal strangulation, and intestinal transplantation. Even though the resultant of local and systemic effects of the mesenteric IRI is related to numerous mediators, tumor necrosis factor (TNF)-α has shown to have a pivotal role in this setting. TNF-α triggers the production of cytokines in which return amplifies and propagates its biological effects. Overproduction of TNF-α is related to endothelial dysfunction, inflammatory genes induction, immune cells recruitment and activation, apoptosis, and cellular survival.[2]

Etanercept is a recombinant soluble TNF receptor that binds TNF-α and inhibits TNF-α-endogenous TNF receptors interaction. It is mostly known for its anti-inflammatory effects and is generally used to block the inflammatory and autoimmune effects of TNF-α in clinical practice. It has the Food Drug and Administration approval in the United States to treat rheumatoid arthritis, juvenile rheumatoid arthritis, psoriatic arthritis, plaque psoriasis, and ankylosing spondylitis.

Previous studies have demonstrated that IRI increases α1-adrenoceptor and endothelin-1 (ET-1) receptor–mediated vasocontractile responsiveness in various arteries.[3,4] Thus, we wanted to question whether TNF-α has any role in vasocontractile responsiveness during IRI and if so, do TNF-α blockers restore the altered vasocontractile responses due to IRI. This study is designed to evaluate *in vivo* and *in vitro* effects of etanercept, the soluble TNF receptor, on contractile responses of superior mesenteric artery in an experimental model of mesenteric IRI.

Material and methods

The study protocol was approved by the animal ethics committee (2013-7-53/Ankara University Animal Experiments Local Ethics Committee) and performed according to the guidelines of the Research Committee of the Faculty of Medicine at Ankara University. The study comprised 24, 12 wk- to 14-wk-old male Sprague–Dawley rats weighing 300 to 360 g (mean: 335 g). All animals were kept under controlled temperature ($21 \pm 2^{\circ}C$) and humidity (55.5%), with a 14 h light and 10 h dark cycle. They were fed with commercial food, and they had free access to water. There were no water and light restrictions throughout the experiment. All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources published by the National Institutes of Health. Two surgeons performed all surgical procedures in absolute sterile conditions. Every surgical intervention was performed under anesthesia using intraperitoneal injection of ketamine hydrochloride (Ketalar, Eczacibasi, Turkey) and xylazine hydrochloride (Alfazyne, Ege Vet, Turkey) with doses of 80-100 mg kg^{-1} and 10-12.5 mg kg^{-1}, respectively.

Twenty-four Sprague–Dawley rats were allocated to 3 groups. The rats in control group (Gr C, $n = 6$) underwent a median laparotomy and dissection of superior mesenteric artery with no further intervention. The rats in ischemia reperfusion group (Gr I/R, $n = 12$) underwent a median laparotomy, dissection of superior mesenteric artery, and occlusion of the artery adjacent to its root by a microclamp for 90 min. After clamping the artery, the paleness of the jejunoileal segments along with pulseless mesenteric artery confirmed the ischemia. The rats in the treatment group (Gr I/R+E, $n = 6$) underwent the same surgical procedure and received etanercept intravenously. The caudal caval veins of the rats were cannulated with 24G catheter, and 5 mg/kg of etanercept in distilled water was started to infuse via catheter at the beginning of reperfusion. The infusion of each animal was completed in an hour. All animals were sacrificed at the 24th h of reperfusion. Bowel mesentery as well as ileum was excised. Third branch of superior mesenteric artery (SMA) was immediately dissected to evaluate the contractile responses. *In vitro* effects of etanercept on vasocontractile responses were also investigated by using organ bath incubation of the drug for animals in Gr I/R. The excised ileums were analyzed under light microscope.

Pharmacological evaluation

To dissect the SMA apart from superior mesenteric vein and evaluate the mesenteric arterial branching, bowel mesentery was separated from bowel segments, placed in an ice-cold, modified Krebs' solution composed of NaCl (118 mM/L), KCl (4.7 mM/L), NaH_2PO_4 (1.2 mM/L), $NaHCO_3$ (25 mM/L), $MgSO_4.7H_2O$ (1.2 mM/L), glucose (11.2 mM/L), and $CaCl_2$ (2.5 mM/L). Connective tissue of the mesenteric artery was cleaned out and cut into four segments using Cold Lighted Loop (Leica). One stainless steel wire was passed through arterial lumen and attached to chamber of Myograph System (610M; Danish Myo Technology, Aarhus, Denmark) in a temperature-controlled environment that was 37°C and aerated with a mixture of 95% O_2 and 5% CO_2. Second wire was also passed parallel to the wire and fixed transducer side of chamber. Parallel wires were separated from each other through micrometric screw. After 40 min of incubation period, all rings were gradually adjusted to a pressure of 100 mm Hg. To reach 100 mm Hg tension, all rings were stretched in four steps, each step lasting for 2 min. All rings were prestimulated with 100 mM KCl and 1 μM Noradrenalin mixture to check vascular contractile property and washed at least three times to return to baseline tension. Contractile experiments were