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Effect of a free radical scavenger on nitric oxide release in microvessels

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ABSTRACT

Background: Superoxides impair nitric oxide (NO) bioactivity; however, the dynamics of NO release in the peripheral microcirculation remain unknown. We investigated the effect of a free-radical scavenger (edaravone) on dynamic NO release and the expression of eNOS and iNOS in microvessels.
Methods and results: An electrochemical microsensor was positioned at the iliac artery bifurcation of the rat abdominal aorta, and NO release was measured in response to edaravone. A bio-imaging model was also used to obtain images of NO release from microvessels. Moreover, eNOS expression and iNOS expression were investigated in inflammatory and non-inflammatory models. NO was observed in association with microvessels in the mesentery. NO release in the aorta was significantly greater with edaravone than with placebo in the non-inflammatory model ($P<0.05$). Acetylcholine-induced NO release with edaravone was greater than with placebo in both models. Bio-imaging showed greater NO release from arterioles than from venules. eNOS expression with edaravone was greater than with placebo with or without inflammation. iNOS expression was increased by inflammation, but edaravone inhibited this increase.
Conclusion: These results support the critical role of NO in the microcirculation and suggest that free-radical scavenging increases the bioavailability of NO in the microcirculation *via* eNOS upregulation.

1. Introduction

The majority of reactive oxygen species (ROS) generated *in vivo* are removed by antioxidants or antioxidative enzymes. However, excessive generation of ROS can result in oxidation of critical biogenic substances such as DNA, lipids, enzymes, and proteins (Lau et al., 2008). Many reports show that oxidative damage of these biogenic substances can lead to problems such as the development of atherosclerosis (Barry-Lane et al., 2001; Griendling et al., 2000; Ozono et al., 2007). The level of oxidized low-density lipoproteins (LDLs) is believed to increase with increased oxidative stress and these oxidized LDLs contribute to the development of human coronary artery plaque, disturb vascular endothelial cells, and affect the accumulation of inflammatory cells (Ehara et al., 2001; Meisinger et al., 2005). They may also contribute to plaque destabilization (Ehara et al., 2001; Meisinger et al., 2005). Atherosclerotic progress is followed by damage to the microcirculation, which results in impaired blood flow and the formation of basic arteriosclerotic lesions. This damage to the microcirculation has a major impact on the production and release of vasorelaxation factors such as prostacyclin (Fleming et al., 1996; Gryglewski et al., 2001), nitric oxide metabolites (NOx) (Gryglewski et al., 2001), and endothelium-derived hyperpolarizing factors (Fleming et al., 1996). Nitric oxide (NO) is an essential gas mediator that regulates blood flow in the microcirculation in response to tissue metabolism (*i.e.*, the consumption of oxygen) *via* vasorelaxation. It also has a platelet-antiaggregating effect (Arnal, 1997).

3-Methyl-1-1-phenyl-2-pyrazolin-5-one (edaravone), a free-radical scavenger, has been used clinically in Japan for the treatment of cerebral infarction since 2001, and it has been reported to improve clinical outcomes in patients with ischemic stroke (Group, 2003; Tanaka, 2002; Xi et al., 2007). Experimental studies have revealed that edaravone decreases oxidative stress (Zhang et al., 2005), and its neuroprotective effects are indisputable (Watanabe et al., 1994; Yoneda et al., 2003).

We examined the influence of ROS on NO release and on nitric oxide synthase (NOS) expression in endothelial cells *in vivo* by using the free-radical scavenger edaravone.

2. Material and methods

2.1. Experimental animals

Male Wistar–ST rats weighing 250 to 330 g were obtained from Japan SLC, Inc. (Hamamatsu, Japan). All animals were maintained in air-conditioned rooms (temperature: 22.5±0.5 °C; humidity: 50%±5%) with a 12-h light–dark cycle. Animals had free access to food and drinking water. All procedures were conducted in compliance

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with the guiding principles for the care and use of animals in the field of physiological science of the Physiological Society of Japan.

2.2. Free-radical scavenger

Edaravone (MCI-186; [3-Methyl-1-1-phenyl-2-pyrazolin-5-one]), a free-radical scavenger, was kindly donated by Mitsubishi Pharma Corporation (Tokyo, Japan). Edaravone was administered to rats by infusion into the femoral vein with an infusion pump over a period of 60 min (10.5 mg/kg/h′).

2.3. DSS-induced rat colitis model

Three-percent DSS (dextran sulfate sodium salt; Nacalai Tesque, Inc. Japan) in water was orally administered to rats for 5 days after pre-feeding to induce colitis (Barros et al., n.a.). Oral administration of DSS solution to rodents is widely employed as a model of human ulcerative colitis because it causes acute inflammatory reactions and ulceration along the entire colon that are similar to those observed in human colitis (Cooper et al., 1993; Okayasu et al., 1990).

2.4. Measurement of peripheral NO release

Peripheral NO release was measured by using an electrochemical sensor (Malinski and Taha, 1992). The working electrode was introduced through a femoral artery and positioned close to the branch point of the abdominal aorta and the common iliac artery, without preventing blood flow. Cannulae were implanted in both the left and right femoral veins of anesthetized 8-week-old Wistar–ST rats and positioned at the iliac artery bifurcation (Fig. 1). Edaravone (10.5 mg/kg/4 mL) or saline was administered to rats through the femoral vein over a period of 60 min by using an infusion pump. The change in potential was recorded for 30 min after the start of dosing to find the baseline potential. Acetylcholine (20 μg/kg BW; Daiichi Sankyo Co., Ltd, Japan) or saline was intravenously injected to the femoral vein (Fig. 1) 30 min after the start of edaravone infusion, and the change in potential was recorded. The control experiments in the presence of L-NAME (NG-nitro-L-arginine methyl ester, hydrochloride, Dojindo, Japan) were conducted to confirm the NO specificity of this method. L-NAME (1–10 mg/kg BW) was administered in advance and all other procedures were done in the same way. The results of the control experiments showed that the fluorescence derived from NO was not detected at 10 mg/kg L-NAME. The sensitivity and selectivity of the electrodes were confirmed each time before use using S-nitroso-N-acetyl-D,L-penicillamine (SNAP, Sigma). A stable standard solution of 1 mM SNAP was prepared by dissolving 2.2 mg SNAP in 10 mL of phosphate-buffered saline (PBS) and then aerating the solution with 95% O_2–5% CO_2 (Wang and Zweier, 1996). The electrodes were immersed in PBS (pH 7.4) in a small chamber and calibrated using a graded series of SNAP concentrations from 1×10^{-3} M to 1×10^{-5} M. The SNAP solutions were stored in a cool, dark place and used within 2 to 5 h.

2.5. Intravital NO imaging in the mesenteric microcirculation with DAF-2DA

Rats were anesthetized by intramuscular injection with pentobarbital (65 mg/kg) and then used in a bio-imaging model. The bio-imaging system consisted of a biological microscope (Olympus BX51), a confocal scanner unit (CSU-X1; Yokokawa Electric Corporation, Japan), an EMCCD camera (electron multiplying CCD camera YHQ-CascadeII512B; Nippon Roper, Japan), a DPSS (diode-pumped solid-state) laser combiner system (Yokokawa Electric Corporation, Japan), and a shutter controller (Yokokawa Electric Corporation). Images were acquired along the z-axis by connecting objects of the microscope with a piezo system (Nippon Roper). The femoral artery and vein were cannulated with a polyethylene catheter. The abdomen was opened *via* a midline incision, and the ileocecal portion of the mesentery was exposed. The mesentery was continuously perfused at 2.0 mL/min with Krebs–Henseleit buffer (37 °C) saturated with 95% N_2–5% CO_2, and then perfused with 3,6-diacetoxy-5′,6′-diaminospiro[9H-xanthene-9,1′-isobenzofuran]-3′-one (DAF-2DA; Sekisui Medical Co., Ltd, Japan) (Patel et al., 2008) at 3.0 μmol/L for 20 min. Edaravone (10.5 mg/kg/4 mL) or saline was administered as described for the electrochemical sensor approach. DAF-2DA was loaded 30 min after the start of edaravone infusion. DAF-2DA is a commercially available fluorescent probe that has been used as a tool to measure intracellular NO (Kojima et al., 1998). It is non-toxic to living cells and does not impair cellular function (Jourd'heuil, 2002; Kojima et al., 1998). NO forms a quantifiable fluorescent

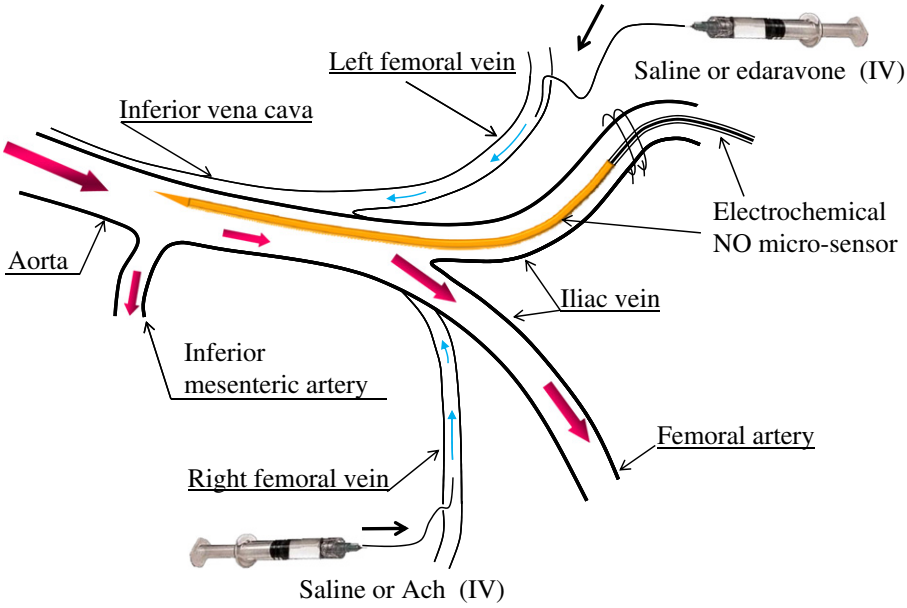

Fig. 1. Measurement of peripheral nitric oxide (NO) release *in vivo* using electrochemical sensors. Ach: acetylcholine, 20 μg/kg BW; IV: intravenous injection; red arrows: direction of blood flow.