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Endothelium-Dependent Vasoconstrictor Signals Requiring Activation Of Soluble Guanylyl Cyclase In Isolated Arteries

Charlotte M Detremmerie[1], Susan WS Leung[1], Aimin Xu[1], Yuansheng Gao[2], Paul M Vanhoutte[1,2]. Dept of Pharmacol, Univ of Hong Kong[1], China; Dept of Physiol and Pathophysiol, Beijing Univ[2], Beijing, China

Introduction. Thymoquinone causes an endothelium-dependent augmentation of contraction in isolated arteries, similar to that evoked by hypoxia.
Aims. *Ex vivo* experiments were designed to study the mechanisms underlying this unexpected response.
Methods. Arterial rings with or without endothelium were suspended in organ chambers for isometric tension recording. Certain rings were incubated with inhibitors of nitric oxide synthase (L-NAME, 10^{-4} M), soluble guanylyl cyclase (sGC; ODQ, 10^{-5} M), rho-associated protein kinases (Y-27632, 10^{-5} M), L-type- (nifedipine, 10^{-5} M) or T-type voltage-gated calcium channels (ML-218, 10^{-4} M), while others were calcium-depleted (n=4-7). The rings were contracted with phenylephrine (10^{-6} M, rat aortae) or prostaglandin $F_{2\alpha}$ (10^{-7} – 10^{-5} M, porcine coronary arteries) and exposed to increasing concentrations of thymoquinone. Some rings were used to measure cyclic nucleotide level by HPLC-MS/MS (n=4-6).
Results. Thymoquinone caused a sustained further increase of tension in rings with endothelium, which was prevented by endothelium-removal, L-NAME and ODQ. Incubation with the NO-donor DETA NONOate (10^{-5} M) in L-NAME-treated rings restored and even increased the contractile response to thymoquinone, while treatment with 8-bromo cyclic GMP (10^{-4} M) or pyrophosphate (10^{-3} M) of ODQ-treated rings did not. HPLC-MS/MS measurements revealed that thymoquinone increased the production of cyclic IMP. Y-27632, nifedipine and calcium depletion inhibited the thymoquinone-induced contraction in porcine but not in rat arteries, while ML-218 reduced the phenomenon in rat but not in porcine arteries.
Discussion. The augmentation caused by thymoquinone requires endothelium-derived NO and activation of sGC, as described for hypoxia. In addition, both thymoquinone- and hypoxia-induced augmentation require production of cyclic IMP, altering intracellular calcium handling.
Thymoquinone can serve as a pharmacological tool to elicit endothelium-dependent vasoconstrictions that require activation of soluble guanylyl cyclase.

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Apolipoprotein A-I Restores Endothelial Function in Rats with Arthritis

Ben J. Wu, Sudichhya Shrestha, Yue Li, Kwok L. Ong, Philip J. Barter and Kerry-Anne Rye
Centre for Vascular Research, University of New South Wales, Sydney, NSW, Australia

Introduction. Endothelial dysfunction is a key event in the development of atherosclerosis and has been identified in patients with rheumatoid arthritis and in rats with experimental arthritis. We have recently shown that apolipoprotein A-I (apoA-I), the most abundant apolipoprotein in high density lipoproteins (HDL), and reconstituted HDL [(A-I)rHDL] consisting of apoA-I complexed with phosphatidylcholine inhibit streptococcal cell wall peptidoglycan-polysaccharide (PG-PS)-induced arthritis in female Lewis rats.
Aim. This study asks if apoA-I also improves endothelial dysfunction in rats with arthritis.
Methods and Results. A single intraperitoneal injection of PG-PS (15 mg/kg) or an equivalent volume of saline (control) was administered to female Lewis rats. After four days the PG-PS-treated animals had acute joint inflammation, elevated circulating inflammatory cytokine levels, and had aortic endothelial dysfunction. Intravenous infusions of lipid-free apoA-I (8 mg/kg) 24 h pre- and 24 h post-PG-PS administration decreased the acute joint inflammation, reduced plasma TNF-α, IL-6 and IL-1β levels, and restored aortic endothelial function with an improvement in aortic vasorelaxation and an increase in guanosine 3',5'-cyclic monophosphate (cGMP) production at day 4 post-PG-PS injection. In *ex vivo* studies, incubation of aortic rings from control female Lewis rats with TNF-α (10 ng/mL) for 6 h impaired aortic vasorelaxation and decreased cGMP production. Pre-incubation of the aortic rings for 16 h with (A-I)rHDL (final apoA-I concentration 0.5 and 1.0 mg/mL) improved the TNF-α-induced impaired aortic vasorelaxation, and cGMP production. In addition, (A-I)rHDL induced endothelial nitric oxide synthase (eNOS) expression in human coronary artery endothelial cells (HCAECs) in a time- and dose-dependent manner. Incubation of HCAECs with TNF-α (1 ng/mL) for 6 h reduced HCAEC eNOS expression. Pre-incubation of the HCAECs for 16 h with (A-I)rHDL restored the TNF-α reduced HCAEC eNOS expression.
Discussion. These findings establish that apoA-I improves endothelial dysfunction in rats with arthritis by, at least partly, inhibiting inflammatory cytokine induced endothelial dysfunction.