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ORIGINAL ARTICLE

The effect of alpha lipoic acid in a porcine in-stent restenosis model

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Stents;
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Summary
Background: The aim of this study was to investigate the effect of alpha lipoic acid (α-LA) on a porcine in-stent restenosis (ISR) model.
Methods: In protocol 1, porcine vascular smooth muscle cells (PVSMC) were stimulated by granulocyte-colony stimulating factor (G-CSF) in the presence or absence of α-LA. MTT (3-[4,5-dimethylthiazole-2-yl] 2,5-diphenyl tetrazolium bromide) assay and western blotting were used to determine the cell growth inhibitory rate and anti-inflammatory effect associated with nuclear factor-κb (NF-κb) and extracellular signal-regulated kinase (ERK). In protocol 2, 28 days after balloon overdilation injuries, 24 bare metal stents were placed in coronary artery of 12 pigs. The pigs were randomly divided to receive control diet with or without α-LA (100 mg/kg). In protocol 3, 8 control stents and 8 α-LA coated stents were randomly implanted in 2 coronary arteries of 8 pigs and follow-up coronary angiogram and histopathologic assessment were performed 4 weeks after stenting.
Results: Protocol 1. The proliferation of PVSMC was inhibited and protein expression of NF-κb and ERK were attenuated by α-LA pretreatment. Protocol 2. On histopathologic analysis, the neointimal area ($4.0 \pm 1.0\,mm^2$ vs. $1.5 \pm 0.7\,mm^2$, $p < 0.001$) and histopathologic area of stenosis ($66.7 \pm 10.7\%$ vs. $24.2 \pm 9.7\%$, $p < 0.001$) were reduced in the α-LA feeding group compared to controls. Protocol 3. On histopathologic analysis, the neointimal area ($3.9 \pm 0.8\,mm^2$ vs. $1.0 \pm 0.4\,mm^2$, $p < 0.001$), and

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the histopathologic area of stenosis (67.1 ± 8.8% vs. 17.4 ± 10.0%, $p < 0.001$) were reduced in the α-LA coated stent group compared to the control stent group.
Conclusions: α-LA feeding and α-LA coated stents inhibit neointimal hyperplasia in porcine ISR, possibly through inhibiting the activation of NF-κb pathway and proliferation of PVSMC.

Introduction

Despite improvements in coronary interventions, in-stent restenosis (ISR) remains a major problem [1—3]. Drug-eluting stents (DES) have been considered the most successful tool in preventing ISR. Unfortunately, it has been reported that DES may result in delayed arterial healing and cause late stent thrombosis when compared with bare metal stent implantation [4,5]. In addition, the US Food and Drug Administration has warned that DES may be a cause of systemic and intrastent hypersensitivity reactions, late thrombosis, and death [6]. Therefore, a new generation of DES to overcome the inflammation, delayed endothelial healing, and late stent thrombosis is needed.

α-Lipoic acid (α-LA) is a potent antioxidant, and acts as a cofactor of key mitochondrial enzymes such as pyruvate dehydrogenase and α-ketoglutarate dehydrogenase [7]. α-LA exists endogenously and improves diabetic-induced endothelial dysfunction, probably due to antioxidant effects and direct free-radical scavenging properties [8]. Moreover, α-LA inhibits the inflammatory pathway and prevents neointimal hyperplasia after stenting in the carotid artery [9,10].

The present study aimed to investigate whether α-LA feeding and α-LA coated stents prevent ISR in the porcine coronary artery restenosis model.

Methods

Protocol 1

We pretreated porcine aorta smooth muscle cells (PASMCs; Modern Cell & Tissue Technologies, Seoul, Korea) with α-LA (100, 250, and 500 mM) 24 h before stimulation by granulocyte-colony stimulating factor (G-CSF) (100 ng/ml, CJ, Ichon, Korea). After 24 h of G-CSF stimulation, we collected cell extracts and performed immunoblotting for phospho-p65 (Cell Signaling, Danvers, MA, USA), extracellular signal-regulated kinases (ERK) (Cell Signaling), phosphorylated ERK (Cell Signaling), signal transducers and activators of transcription (STAT)-3 (Cell Signaling), phosphorylated STAT-3 (Cell Signaling), and β-actin (Sigma, St Louis, MO, USA).

Next, we performed the proliferation rate assay. We pretreated α-LA (100 mM) with PASMCs 2 h before stimulation by G-CSF (100 ng/ml). After 48 h of G-CSF stimulation, MTT (3-[4,5-dimethylthiazole-2-yl] 2,5-diphenyl tetrazolium bromide, 5 mg/ml in PBS, Sigma, Seoul, Korea) was added to cells, and they were incubated further for 4 h at 37 °C. Supernatants were removed by aspiration, and then dimethyl sulfoxide (DMSO) was added to solubilize the precipitated dyes. Absorbance was measured at a wavelength of 570 nm.

Animal preparation

The animal study was approved by the Ethics Committee of Chonnam National University Medical School and Chonnam National University Hospital, and conformed to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Study animals were female swine weighing 25—35 kg. To prevent acute thrombosis after stenting, premedication with aspirin 100 mg and clopidogrel 75 mg per day was given for 7 days before the procedure. On the procedure day, pigs were anesthetized with ketamine (20 mg/kg intramuscularly) and xylazine (2 mg/kg intramuscularly). They received intramuscular ketamine every 30 min and supplemental oxygen continuously through oxygen mask. Subcutaneous 2% lidocaine at the cut-down site was administered, left carotid artery was surgically exposed, and a 7 French sheath was inserted. Continuous hemodynamic and surface electrocardiographic monitoring was maintained throughout the procedure. Then 5000 units heparin was administered intravenously as a bolus prior to the procedure, the target coronary artery was engaged using standard 7 F guide catheters and control angiograms of both coronary arteries were performed using nonionic contrast agent in two orthogonal views. The stent was deployed by inflating the balloon and the resulting stent-