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1,4-Anhydro-4-seleno-D-talitol (SeTal) protects endothelial function in the mouse aorta by scavenging superoxide radicals under conditions of acute oxidative stress

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ABSTRACT

Hyperglycaemia increases the generation of reactive oxidants in blood vessels and is a major cause of endothelial dysfunction. A water-soluble selenium-containing sugar (1,4-Anhydro-4-seleno-D-talitol, SeTal) has potent antioxidant activity *in vitro* and is a promising treatment to accelerate wound healing in diabetic mice. One possible mechanism of SeTal action is a direct effect on blood vessels. Therefore, we tested the hypothesis that SeTal prevents endothelial dysfunction by scavenging reactive oxidants in isolated mouse aorta under conditions of acute oxidative stress induced by hyperglycaemia. Aortae were isolated from C57BL/6 male mice and mounted on a wire-myograph to assess vascular function. In the presence of a superoxide radical generator, pyrogallol, 300 μM and 1 mM of SeTal effectively prevented endothelial dysfunction compared to other selenium-containing compounds. In a second set of *ex vivo* experiments, mouse aortae were incubated for three days with either normal or high glucose, and co-incubated with SeTal at 37 °C in 5% CO_2. High glucose significantly reduced the sensitivity to the endothelium-dependent agonist, acetylcholine (ACh), increased superoxide production and decreased basal nitric oxide ($NO^{\cdot}$) availability. SeTal (1 mM) co-treatment prevented high glucose-induced endothelial dysfunction and oxidative stress in the mouse aorta. The presence of a cyclooxygenase inhibitor, indomethacin significantly improved the sensitivity to ACh in high glucose-treated aortae, but had no effect in SeTal-treated aortae. Our data show that SeTal has potent antioxidant activity in isolated mouse aortae and prevents high glucose-induced endothelial dysfunction by decreasing superoxide levels, increasing basal $NO^{\cdot}$ availability and normalising the contribution of vasoconstrictor prostanoids.

1. Introduction

Diabetes is a metabolic disease that affects 415 million people worldwide; it is estimated that 642 million people will be living with diabetes by the year 2040 [1]. This chronic disorder is a major risk factor in the development of cardiovascular diseases such as stroke, myocardial infarction and peripheral vascular disease. Macro and microvascular complications are also a leading cause of mortality in people with diabetes [2]. These vascular complications are derived from chronic elevation in blood glucose levels, and affect both conduit and resistance arteries, leading to endothelial dysfunction. This is characterised by impaired endothelium-dependent relaxation due, at least in part, to increased oxidative stress, increased non-enzymatic glycation of proteins and over activation of inflammatory molecules [3].

Among the factors implicated in diabetes-induced vascular complications, increased generation of reactive oxygen species (ROS) is particularly apparent in experimental animal models [4,5]. Clinically, diabetic patients have increased plasma levels of ROS markers such as 8α-isoprostane, oxidised low density lipoprotein (LDL) and lipid-derived oxidation products (detected as species that react with thiobarbituric acid), as well as reduced levels

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of endogenous antioxidant molecules such as bilirubin, and decreased activity of the antioxidant enzymes superoxide dismutase and glutathione peroxidase [6,7]. In the vasculature, diabetes induces oxidative stress by promoting ROS generation including superoxide radicals from the mitochondrial electron transport chain, and via the activation of NADPH oxidases including NOX1, 2 and 4 [8]. Superoxide radicals react rapidly with the vasodilator nitric oxide ($NO^{\cdot}$) to form the powerful oxidant peroxynitrous acid. The latter further increases superoxide radical production via disruption of the zinc-sulfur cluster within endothelial $NO^{\cdot}$ synthase (eNOS) and uncoupling of the functional dimer [9]. Such uncoupling of eNOS results in further generation of superoxide radicals and peroxynitrous acid, and the oxidation of the eNOS co-factor tetrahydrobiopterin (BH_4) [10]. Diabetic patients treated with BH_4 supplements show a significant improvement in endothelium-dependent vasorelaxation, consistent with a link between damage to eNOS, increased superoxide radical and diabetes-induced endothelial dysfunction [11]. Therefore, a reduction in ROS generation might be a therapeutic approach to reduce the impact of diabetes on endothelial dysfunction and associated vascular complications.

Inorganic and organic selenide compounds have been investigated as potential drugs to treat atherosclerosis via their ability to improve endothelium-dependent vasorelaxation and maintain $NO^{\cdot}$ bioavailability in the rat aorta [12]. A positive correlation has also been reported between glucose metabolism and selenium treatment in diabetic animals [13,14] and humans [15]. Selenium treatment prevents diabetes-induced myocardial dysfunction through a reduction in cardiomyocyte size and limits myofibrils degradation [16], as well as prevents platelet aggregation by reducing thromboxane B_2 levels [17] in streptozotocin-induced diabetic rats. We recently demonstrated that topical application of the low-molecular mass water-soluble selenium-containing sugar (1,4-Anhydro-4-seleno-D-talitol, SeTal) but not the water-soluble glutathione peroxidase mimetic, D,L-*trans*-3,4-dihydroxy-1-selenolane (DHS_{red}) accelerates wound closure in diabetic *db*/*db* mice (J.C. Kwan et al., 2016 unpublished results). These effects were partially explained by improved vascular perfusion at the wound site. SeTal reacts more rapidly than its sulfur and oxygen analogues with both peroxynitrous acid and other potent oxidants such as hypochlorous acid, HOCl, generated by myeloperoxidase (MPO) [18–20]. MPO-derived oxidants including HOCl, chloramines and HOCl-modified proteins can induce endothelial dysfunction and reduce $NO^{\cdot}$ bioavailability in the vasculature [21–23]. Additionally, MPO-derived HOCl amplifies ROS-induced injury and subsequently leads to impaired endothelium-dependent vasorelaxation [22]. Therefore, compounds that effectively scavenge MPO-derived species, such as SeTal [24,25], could be used to alleviate systemic endothelial dysfunction associated with hyperglycaemia.

In the present study, we tested the hypothesis that improvements in local vascular perfusion at the wound site in SeTal-treated mice are associated with direct actions of this compound on blood vessels, likely by enhancing endothelial function. The overall aim was to investigate the potential vasoprotective effects of SeTal and other selenium-containing compounds in the mouse aorta. Specifically, we investigated whether or not SeTal is more potent and efficacious compared to other selenium-containing compounds in preventing endothelial dysfunction under conditions of acute oxidative stress. Secondly, based on the promising effects of SeTal in promoting wound healing in diabetic mice, we utilised an *ex vivo* mouse model of hyperglycaemia [26] to determine the optimum concentration of SeTal, as well as examine the mechanisms of action of this compound in preventing high glucose-induced endothelial dysfunction.

2. Materials and methods

2.1. Animals

This study used male C57BL/6 mice aged 3–5 months old (n = 103) obtained from the School of BioSciences Animal Facility (University of Melbourne). All mice were housed in rooms with a 12 h day/night cycle at 20 ± 2 °C, in the School of BioSciences Animal Facility (The University of Melbourne). The mice were given *ad libitum* access to standard rodent chow (Barastock, VIC, Australia) and water. All experimental procedures were approved by The University of Melbourne Animal Experimental Ethics Committee (1513579.1) and conform to the Australian Code of Practice & National Health and Medical Research Council guidelines for the care and humane use of animals for scientific purposes.

2.2. Isolation of aortae for in vitro and tissue culture experiments

Mice were anaesthetized with 2% isoflurane (Univentor 400, Agnthos, AB, Sweden) in oxygen via inhalation followed by cervical dislocation. The whole aorta was isolated and immediately placed in ice cold Krebs bicarbonate solution (120 mM NaCl, 5 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 11.1 mM D-glucose, 2.5 mM $CaCl_2$) and then cleared of fat and connective tissues. For tissue culture experiments, the aorta was cut into 2 mm long rings and incubated in Medium 199 (M7528 Sigma, Castle Hill, NSW, Australia) supplemented with 1% L-Glutamine-Penicil lin-Streptomycin solution (G1146 Sigma, Castle Hill, NSW, Australia) for three days in either normal glucose (5.5 mM) or high glucose (30 mM) and co-incubated with different concentrations of SeTal (10 μM, 300 μM or 1 mM) at 37 °C in 5% CO_2 [26].

2.3. Functional experiments

Vascular function was assessed in isolated abdominal aorta as previously described [27,28] with the following modifications. Briefly, aortic rings were mounted on a multi wire myograph system 620 M (Danish Myo Technology, Aarhus, Denmark) and allowed to stabilize at zero tension for 15 min followed by a 30 min equilibration period at 5 mN. All experiments were performed at 37 °C in the presence of 95% O_2 and 5% CO_2. Changes in isotonic tension were recorded using Powerlab/LabChart data acquisition system (AD Instruments, Bella Vista, NSW, Australia). Thirty minutes after equilibration at 5 mN, aortic rings were maximally contracted with the thromboxane A_2 mimetic, U46619 (1 μM). Once the U46619-induced contraction reached a plateau (F_{max}), aortic rings were washed with Krebs solution to regain their basal tension. Aortic rings were then pre-contracted with titrated concentrations of U46619 (0.1–100 nM), followed by a single concentration of acetylcholine (ACh, 10 μM) to assess the endothelium integrity. Concentration-response curves obtained from functional experiments were fitted to a sigmoidal curve using nonlinear regression (Prism version 6.0, GraphPad Software, San Diego, CA, USA) to calculate the sensitivity of each agonist (pEC_{50}). Maximal relaxation (R_{max}) was measured as a percentage of U46619 contraction.

2.4. Vascular reactivity in pyrogallol or xanthine/xanthine oxidase (X-XO) system

The aortic rings were incubated in the absence (control) or presence of the superoxide radical generator, pyrogallol (100 μM) [29] for 20 min. In the presence of pyrogallol, aortic rings were co-incubated with 3 different concentrations of SeTal (10 μM, 300 μM or 1 mM) or 3 different selenium-containing molecules (300 μM or 1 mM), namely selenomethionine (SeMet),