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Negative inotropic and hypotensive effects of the superoxide dismutase mimetic tempol in pigs

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ABSTRACT

Through interference with free radicals, the nitroxide tempol potentially increases bioavailability of nitric oxide (NO) and along with modulation of potassium channels reduces blood pressure (BP). We studied whether tempol in pigs lowers BP by mechanisms sensitive to inhibition of NO synthase or large conductance calcium-activated potassium channels (BK_{Ca}). The cardiovascular effects of intravenous tempol (25–50 mg/kg) were examined in anesthetized pigs with myocardial function being evaluated by echocardiography. While saline-treated animals remained hemodynamically stable, tempol induced fast, dose-dependent and transient reductions in BP lasting 5–10 min with a simultaneous impairment of left ventricular contraction. Pretreatment with the NO synthase (NOS) inhibitor N^G-nitro-L-arginine methyl ester (L-NAME, 4 mg/kg) or a blocker of BK_{Ca} (tetraethylammonium (TEA), 100 mg/h) increased baseline BP but also enhanced BP reductions to tempol. Isolated myocardial trabeculae subjected to an identical protocol also demonstrated dose-related reductions in contractility to tempol. This effect was not affected by L-NAME, but attenuated by TEA. In isolated mesenteric resistance arteries contracted with noradrenaline, tempol caused small postjunctional L-NAME sensitive relaxations, while neurogenic contractions were inhibited by tempol by TEA-sensitive mechanisms and mechanisms insensitive to TEA and L-NAME. In conclusion intravenous tempol induces fast transient reductions in BP associated with simultaneous reductions in myocardial contraction. Tempol exerts direct negative inotropic effects which are partly sensitive to BK_{Ca}-blockade but independent of NOS inhibition. In addition tempol has direct vasodilatory effects despite NOS and potassium channel blockade. The negative inotropic and hypotensive effects raise concerns using tempol, or structurally similar drugs, for intravenous use.

1. Introduction

Tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine 1-oxyl) belongs to a class of relatively simple molecules, nitroxides, which possess antioxidant effects. Tempol scavenges superoxide *in vitro* as well as *in vivo* through a superoxide dismutase (SOD) mimicking activity (Simonsen et al., 2009; Wilcox 2010). Cardiovascular diseases are associated with oxidative stress caused by an increased formation of reactive oxygen species in the vascular wall (Forstermann, 2010). Superoxide, a reactive oxygen species, may transform into the harmful substance peroxynitrite in a nitric oxide (NO) consuming process decreasing the bioavailability of NO (Papaharalambus and Griendling, 2007) and possibly increasing the bioavailability and vasodilatory effects of NO (Christensen et al., 2007). Tempol also interacts with potassium channels as the vasodilatory effect can be reduced by simultaneous blockade of vascular large-conductance calcium-activated potassium channels (BK_{Ca}) (Xu et al., 2005). Furthermore, tempol may lower peripheral sympathetic nervous activity, in part mediated by opening of ATP-regulated potassium channels (Chen et al., 2007). Numerous studies with tempol treatment in rats have shown improvement in biomarkers of oxidative stress (Wilcox, 2010), improved vasodilation and a reduction in peripheral vascular resistance resulting in a well-documented antihypertensive effect as recently reviewed (Wilcox and Pearlman, 2008; Simonsen et al., 2009).

Tempol has also shown promising results as a radio-protective drug in terms of reduced mortality and carcinogenesis following whole body ionizing radiation (Mitchell et al., 2012) as well as mucosa protection after local radiotherapy (Cotrim et al., 2005).

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Furthermore, due to its paramagnetic properties tempol may be used as a marker of organ redox state using magnetic resonance imaging (Davis et al., 2011). These applications may require infusion of high doses of tempol with possible effects on hemodynamic parameters (Hahn et al., 1998). However, in regards to the use of tempol as a potential radio-protective drug, the cardiovascular effects have only been investigated in rodents.

Merely one study has sought to investigate the hemodynamic effects of tempol treatment in a large animal model, and in this study using pigs, tempol caused an immediate reduction of BP followed by reflex tachycardia and increased skin temperature also suggesting peripheral vasodilation (Hahn et al., 1999). However, the effect on the myocardial function has never been investigated in vivo neither in rats nor in other animals. As cardiovascular regulation in small rodents differ substantially from larger animals and humans, especially concerning heart rate (HR) and sympathetic activity, evaluation of potentially new drugs in larger species is pivotal.

The present study investigated whether tempol lowers BP by a mechanism sensitive to NO synthase inhibition or blockade of BK_{Ca} in a large animal model. To address the hypothesis increasing doses of tempol were infused into pigs and the effects on the systemic circulation were investigated in the presence of an NO synthase inhibitor and a blocker of BK_{Ca}, while the function of the heart was evaluated by echocardiography. Furthermore in vitro investigations were made in order to study myocardial contractility in isolated ventricular trabeculae and vasoactivity in isolated resistance arteries.

2. Materials and methods

2.1. Laboratory animals

Female swine of Danish Landrace, 8–9 weeks old and weighing approximately 20 kg, were used for *in vivo* experiments and experiments on myocardial trabeculae, while 40 kg Landrace–Yorkshire hogs were used for experiments on isolated resistance arteries. Animal handling and experimental procedures conformed to the most recent "Guide for the Care and Use of Laboratory Animals" published by the US National Institutes of Health and were performed with approval from the Danish Institutional Animal Care and Use Committee.

2.2. Pre-experimental procedure and anesthesia

The pigs used for *in vivo* experiments were sedated with intramuscular ketamine (0.25 mg/kg) and midazolam (0.5 mg/kg) and an intravenous line was placed in an ear vein. Induction of anesthesia was performed with pentobarbital (Mebumal®, 15 mg/kg) and maintained at a dose of 12.5 mg/kg/h. Analgesia was induced by fentanyl (Haldid®, 40 ug/kg) with a subsequent infusion of 60 μg/kg/h. The pigs were intubated and ventilated mechanically with a respirator (S/5 Datex-Ohmeda Avance, GE Health Care, Horten, Norway) for the duration of the experiment using an O_2 inspiratory fraction of 30% and a fixed tidal volume of 250 ml. The respiratory rate was adjusted to maintain an expiratory CO_2 value of 5.3–5.7 kPa.

The pigs used for experiments on myocardial trabeculae were premedicated with an intramuscular injection of midazolam (0.5 mg/kg) and azaperone (4 mg/kg). Anesthesia was induced with an intravenous bolus of etomediate (0.5 mg/kg) followed by intubation and ventilation with 50% O_2 and 2.5–3% sevoflurane. We performed a median thoracotomy and the right ventricle was rapidly excised from the beating heart and placed in oxygenated modified Krebs–Henseleit (mKH) buffer ($NaCl_2$: 118 mM, KCl: 4.8 mM, $NaHCO_3$: 27.2 mM, $MgCl_2$: 1.2 mM, KH_2PO_4: 1.0 mM, $CaCl_2$: 2.0 mM, glucose: 10 mM, pyruvic acid: 10 mM) at room temperature.

Pigs used for experiments on isolated resistance arteries were obtained at a local slaughterhouse. Immediately after sacrificing the animal, a 10 cm long segment of the jejunum was excised and kept in a physiological salt solution (PSS, NaCl 119 mM, $NaHCO_3$ 25 mM, KCl 4.7 mM, KH_2PO_4 1.18 mM, $MgSO_4$ 1.17 mM, CaCl 2.5 mM, EDTA 0.026 mM, and glucose 5.5 mM), bubbled with 5% CO_2 in O_2, and buffered with HEPES.

2.3. In vivo experiments

2.3.1. Instrumentation and data acquisition

Vascular sheaths were inserted into the right and left external jugular veins and the left carotid artery, which allowed for (1) placement of a Swan Ganz catheter in the pulmonary artery for measurement of cardiac output (CO) using a Vigilance Monitor (Edwards Lifesciences, CA, USA), (2) measurement of central venous pressure and (3) measurement of mean arterial pressure (MAP). All pressures were recorded via connection of fluid filled tubing to pressure transducers.

Hemodynamic and respirator data were collected continuously and stored on a PC using the S5 collect software suit (Datex-Ohmeda, Finland). Echocardiography was performed at selected times throughout the experiment (see below). CO was measured continuously using a thermo-dilution method in which a fixed amount of energy was added to the bloodstream as heat with recording of the downstream increase in temperature.

2.3.2. Echocardiography

Echocardiography was performed before infusion of tempol, immediately after the third infusion of tempol (35 mg/kg) and immediately after the final infusion of tempol (50 mg/kg) as illustrated in Fig. 1. The left ventricle (LV) was visualized in the parasternal short-axis view using a Vivid Q ultrasound system equipped with a M4S probe (GE Health Care, Horten, Norway). Imaging obtained at baseline served as a guide for subsequent scans, and uniform projection of the LV throughout the experiment was achieved by comparing the current scan to baseline using the split-screen view on the scanner. Cine loops of three successive cardiac cycles, as defined by the corresponding ECG, were stored during brief pauses in ventilation for off-line analyses.

All echocardiography analyses were performed using commercially available software (Echopac, GE Health Care, Horten, Norway). The short axis end-diastolic area (EDA) and end-systolic area (ESA) of the LV was traced manually and the relative area change (RAC) calculated as (EDA-ESA)/EDA was expressed as a percentage. The RAC represents a two-dimensional equivalent of ejection fraction. In addition, myocardial systolic deformation was quantified by means of speckle tracking ultrasound as radial strain. The method is based on tracking of natural acoustic markers in the image of the myocardium from frame to frame, and is expressed as a percentage of change in tissue length during the cardiac cycle. The software processing is semiautomatic and based on manually placed regions of interest as previously described (Sivesgaard et al., 2009; Frederiksen et al., 2012). Analyses were performed by a single observer blinded from the point of acquisition and protocol allocation and repeated by a second observer, blinded as well.

2.4. Study protocols

2.4.1. Pilot experiments

To establish which dose of tempol resulted in significant changes in MAP, three pigs received bolus infusions of tempol in a dose of 5 mg/kg repeated every 15 min with a total of 10 infusions. This produced only insignificant changes in MAP, and there was no correlation in time between the administration of the drug and the