Vardenafil protects against myocardial and endothelial injuries after cardiopulmonary bypass

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Abstract

Objectives: Phosphodiesterase-5 inhibitors and elevated myocardial cyclic guanosine monophosphate levels can induce potent cardioprotection-like effects against ischaemia—reperfusion injury. We investigated the effects of vardenafil, a selective phosphodiesterase-5 inhibitor on myocardial and endothelial functions during reperfusion in a canine model of cardioplegic arrest and extracorporeal circulation. Methods: Vehicle-treated (control, n = 8) and vardenafil-treated (30 µg kg⁻¹ intravenous; n = 8) anaesthetised dogs underwent hypothermic cardiopulmonary bypass with 60 min of hypothermic cardiac arrest. Left and right ventricular end-systolic pressure—volume relationship (Esv) was measured by a combined pressure—volume conductance catheter at baseline and after 60 min of reperfusion. Left anterior descending coronary blood flow and endothelium-dependent vasodilation to acetylcholine were determined. Isolated coronary arterial rings were investigated for vasomotor function using an in vitro organ bath system. Results: Pharmacological preconditioning with vardenafil led to significantly higher plasma cyclic guanosine monophosphate levels and myocardial adenosine triphosphate content to a better recovery of left and right ventricular function using an endothelium-dependent vasodilatation to acetylcholine were determined. Isolated coronary arterial rings were investigated for vasomotor function using an in vitro organ bath system. Results: Pharmacological preconditioning with vardenafil led to significantly higher plasma cyclic guanosine monophosphate levels and myocardial adenosine triphosphate content to a better recovery of left and right ventricular Esv (Δ left ventricular Esv given as percent of baseline: 74.2 ± 4.5% vs 50.4 ± 5.0%, p < 0.05) and to a higher coronary blood flow (58 ± 12 vs 24 ± 7 ml min⁻¹, p < 0.05). Endothelium-dependent vasodilatory responses to acetylcholine — measured both in vivo and in vitro — were improved in the vardenafil group. Conclusions: Application of vardenafil improves myocardial and endothelial functions after cardiopulmonary bypass with hypothermic cardiac arrest. The observed protective effects imply that phosphodiesterase-5 inhibition could be a novel therapeutic option in the protection against ischaemia—reperfusion injury in cardiac surgery.

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Keywords: Cardiopulmonary bypass; Ischaemia—reperfusion; Contractility; Endothelium; Vardenafil

1. Introduction

Vardenafil is a selective inhibitor of phosphodiesterase-5 (PDE-5), an enzyme, which catalyses the breakdown of cyclic guanosine monophosphate (cGMP), an essential second messenger involved in smooth muscle relaxation. PDE-5 inhibitors cause an accumulation of nitric oxide (NO)-driven cGMP and subsequent vasodilatation in the corpus cavernosum and in pulmonary vasculature; therefore pharmacological PDE-5 inhibition has become a widely used treatment for erectile dysfunction [1] and pulmonary arterial hypertension [2]. In recent years it has been demonstrated that not only vascular smooth muscle, but also myocardial tissue contains PDE-5 [3], and there has been considerable interest in the role of the NO—cGMP—protein kinase G pathway in cardioprotection [4]. The research group of Kukreja reported about a powerful protection against regional myocardial ischaemia—reperfusion injury by pretreatment with the PDE-5 inhibitors sildenafil [5] and vardenafil [6] in rabbits, which were mediated by cGMP-induced opening of mitochondrial KATP channels. A recent human study demonstrated the improvement of endothelial dysfunction (endothelium-dependent vasodilatation) after ischaemia—reperfusion with pharmacological PDE-5 inhibition [7]. Moreover, intracellular cGMP-accumulation has been shown to reduce tissue injury in conditions associated with increased free radical release and oxidative stress [8,9]. The preconditioning-like and cytoprotective effects of sildenafil and vardenafil raised the possibility of the new therapeutic strategy of pharmacological preconditioning by PDE-5 inhibition in myocardial protection [10].

Routine cardiac surgery with extracorporeal circulation and cold cardioplegic arrest leads to a global cardiac...
ischaemia—reperfusion injury, thus to an impaired cardiac performance. Even if cardiac dysfunction is not clinically evident, a reduction of myocardial contractility may occur as described in a human study using pressure—volume relationships [11]. In addition, coronary endothelial and peripheral vascular dysfunction may further complicate the postoperative course [12]. Extracorporeal circulation is also known to induce a systemic inflammatory reaction with leucocyte activation and free radical release [13], leading to secondary organ injury which may affect all vital organs including the brain, kidneys, lungs and gut.

Based upon the concept of cardioprotection by PDE-5 inhibition we have designed experiments to test the hypothesis that pharmacological preconditioning with vardenafil, a highly selective and biochemically potent inhibitor of PDE-5, improves myocardial and endothelial recovery in a clinically relevant canine model of cardiopulmonary bypass (CPB) with hypothermic cardiac arrest.

2. Materials and methods

2.1. Animals and experimental groups

Twenty dogs (foxhounds) weighing 26.6–40.0 kg (33.1 ± 1.8 kg) were used in this experiment. All animals received humane care in compliance with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86—23, revised 1996). The experiments were approved by the Ethical Committee of the Land Baden-Württemberg for Animal Experimentation. After the dogs were randomly assigned to the experimental groups, animals of the Württemberg for Animal Experimentation. After the dogs were approved by the Ethical Committee of the Land Baden-Württemberg for Animal Experimentation. After the dogs were randomly assigned to the experimental groups, animals of the treatment group (n = 8) received vardenafil (30 μg kg⁻¹ intravenous (IV) bolus, a dose comparable to that of previous cardiovascular studies [6]) before CPB and after completing the baseline measurements. Vehicle-treated animals undergone CPB (n = 8) served as controls. Additional dogs (n = 4) were used as non-CPB controls only for removal of the coronary arteries for in vitro functional measurements (see below).

2.2. General management and cardiopulmonary bypass

The dogs were premedicated with propionylpromazine and anaesthetised with pentobarbital (15 mg kg⁻¹ initial bolus and then 0.5 mg kg⁻¹ h⁻¹ IV), paralysed with pancuronium bromide (0.1 mg kg⁻¹ as a bolus and then 0.2 mg kg⁻¹ h⁻¹ IV) and endotracheally intubated. The dogs were ventilated with a mixture of room air and O₂ (FIO₂ = 60%) at a frequency of 12–15 min⁻¹ and a tidal volume starting at 15 ml kg⁻¹ min⁻¹. The settings were adjusted to maintain arterial partial carbon dioxide pressure levels between 35 and 40 mmHg. The femoral artery and vein were cannulated to record aortic pressure (AoP) and to take blood samples for the analysis of blood gases, electrolytes, pH and further biochemical measurements (see below). Basic intravenous volume substitution was carried out with Ringer’s solution (1 ml min⁻¹ kg⁻¹). According to the values of potassium, bicarbonate and base excess, substitution included administration of potassium chloride and sodium bicarbonate (8.4%). Neither catecholamines nor other hormonal or pressor substances were administered. After left anterolateral thoracotomy in the fourth intercostal space and pericardiectomy, the great vessels were dissected. After systemic anticoagulation with sodium heparin (300 U kg⁻¹), the left subclavian artery was cannulated for arterial perfusion. The venous cannula was placed in the right atrium. The extracorporeal circuit consisted of a heat exchanger, a venous reservoir, a roller pump and a membrane oxygenator primed with Ringer’s lactate solution (1000 ml) supplemented with heparin (150 U kg⁻¹) and 20 ml sodium bicarbonate (8.4%). After initiation of CPB, the body temperature was cooled to 28 °C. After cross clamping of the aorta, the heart was arrested with 25 ml kg⁻¹ histidine tryptophane ketoglutarate (HTK) solution (Custodiol®, Dr Franz Köhler Chemie GmbH, Alsbach-Hähnlein, Germany). During cardiac arrest the pump flow was set at 100 ml kg⁻¹ min⁻¹ to maintain perfusion pressure above a value of 35–40 mmHg at any time point, and alpha-stat management was applied. Twenty minutes before cross-clamp removal, re-warming was initiated. After 60 min of cardiac arrest, the aorta was declamped, and the heart was reperfused with normothermic blood in the bypass circuit. If necessary, ventricular fibrillation was counteracted with DC cardioversion of 40 J. Ventilation was re-started with 100% oxygen. All animals were weaned from CPB without inotropic support 20 min after the release of the aortic cross clamp. Each animal of the CPB groups underwent 90 min of CPB with 60 min of cardiac arrest.

2.3. Measurements of cardiac and coronary vascular function in vivo

Left and right ventricular systolic and diastolic pressures and volumes were measured by a combined 6F Millar pressure—volume conductance catheter with 6 mm spacing, which was inserted through the apex. Stroke volume (SV) was calculated from the integrated flow signal measured by an aortic ultrasonic flow probe (Transonic Systems Inc., Ithaca, NY, USA) and was used to calibrate the volume signal from the conductance catheter. Parallel conductance was estimated by rapid injection of 1 ml of hypertonic saline into the left and right atrium, respectively. The pressure and volume signal provided by the conductance catheter was registered continuously and computed by the Iox software (EMKA Technologies, Paris, France). Vena cava occlusions were performed to obtain a series of pressure—volume loops. The slope (Em) of the left and right ventricular end-systolic pressure—volume relationship and preload recruitable stroke work (PRSW) were calculated as load-independent indices of myocardial contractility. Coronary blood flow was measured on the left anterior descending (LAD) coronary artery with a perivascular ultrasonic flow probe (Transonic Systems Inc., Ithaca, NY, USA). Endothelium-dependent coronary vaso dilatation was assessed after intracoronary administration of a single bolus of acetylcholine (10⁻⁷ mol). The vasoresponse was expressed as percentage change of CBF. As severe hypotension has been described at simultaneous application of PDE-5 inhibitors and NO donors [14], such as sodium nitroprusside, endothelium-independent vasodilatation has...
not been assessed in vivo. At the end of the experiments, left ventricular myocardial tissue samples were taken and immediately immersed in fluid nitrogen (−196 °C) and stored frozen at −80 °C until the biochemical measurements.

2.4. In vitro vascular function

After the end of the in vivo experiments in both CPB groups and immediately after anaesthesia of the non-CPB control animals, the hearts were excised and the LAD coronary arteries were isolated and placed in cold (−4 °C) Krebs–Henseleit solution (118 mM NaCl, 4.7 mM KCl, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 1.77 mM CaCl₂, 25 mM NaHCO₃, 11.4 mM glucose; pH 7.4). The coronary arteries were prepared and cleaned from periadventitial fat and surrounding connective tissue and cut transversely into 4-mm-width rings using an operation microscope.

Isolated coronary arterial rings were mounted on stainless steel hooks in individual organ baths (Radnoti Glass Technology, Monrovia, CA, USA), containing 25 ml of Krebs–Henseleit solution at 37 °C and aerated with 95% O₂ and 5% CO₂. Special attention was paid during the preparation to avoid damaging the endothelium.

Isometric contractions were recorded using isometric force transducers (Radnoti Glass Technology, Monrovia, CA, USA), digitised, stored and displayed with the IOX Software System (EMKA Technologies, Paris, France).

The coronary arterial rings were placed under a resting tension of 3.5 g and equilibrated for 60 min. During this period, tension was periodically adjusted to the desired level and the Krebs–Henseleit solution was changed every 30 min. Potassium chloride was used in these experiments to prepare vessels for stable contractions and reproducible dose–response curves to other vasoactive agents. Coronary rings were contracted with potassium chloride (80 mM) and rinsed after the contraction until resting tension was again obtained. Thromboxane A₂-receptor agonist U46619 (5 × 10⁻⁷ M) was used to precontract the rings until a stable plateau was reached, and relaxation responses were examined by adding cumulative concentrations of endothelium-dependent dilator acetylcholine (10⁻⁹–10⁻⁶ M) and the endothelium-independent dilator sodium nitroprusside (10⁻⁸–10⁻⁵ M). Contractile responses are expressed as grams of tension; relaxation is expressed as percent of contraction induced by U46619. EC⁵₀ values were obtained from individual concentration–response curves by fitting experimental data to a sigmoidal equation using the Origin software (OriginLab, Northampton, MA, USA).

2.5. Biochemical measurements

Plasma cyclic guanosine monophosphate (cGMP) levels were determined by enzyme immunoassay (EIA) using a commercial kit (Amersham cGMP EIA Biotrak System, GE Healthcare, Buckinghamshire, UK).

For determination of the myocardial energetic state, myocardial adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine monophosphate (AMP) contents were assessed from myocardial tissue with standard photometry using an enzyme-kinetic assay, as described previously [15].

Plasma nitrate/nitrite levels (as an index of in vivo total NO production) were determined by a colourimetric assay according to the Griess method using a commercial kit (Cayman Chemical Co., Ann Arbor, MI, USA).

Plasma myeloperoxidase level (as a marker for systemic inflammation and leucocyte activation) was measured by enzyme-linked immunosorbent assay (ELISA) using a commercial kit (EMD Biosciences Inc., La Jolla, CA, USA).

2.6. Drugs

Vardenafil was provided by Bayer HealthCare, Wuppertal, Germany. Thromboxane A₂-receptor agonist U46619 (9,11-dideoxy-11α,9α-epoxy-methanoprostaglandin F₂α), acetylcholine and sodium nitroprusside were from Sigma, Aldrich, Germany.

2.7. Statistical analysis

All values were expressed as mean ± standard error of the mean (SEM). In the case of in vitro experiments on vessel rings, means between the groups were compared by one-way analysis of variance (ANOVA) followed by an unpaired t-test with Bonferroni correction for multiple comparisons. In all other cases, a paired t-test was used to compare two means within a group (comparison of ‘before’ and ‘after’ values). Means between the groups were compared by an unpaired two-sided Student’s t-test (comparison of control and vardenafil groups). A p-value less than 0.05 was considered statistically significant.

3. Results

3.1. In vivo haemodynamic effects of vardenafil prior to ischaemia

Infusion of vardenafil resulted in an initial drop of blood pressure up to 15–20% (data not shown). After the infusion, the animals were allowed to recover with regard to blood pressure values to baseline levels. The myocardial contractility parameters +dP/dt, ESPVR and PRSW at this time point showed no significant changes when compared to that at baseline (data not shown).

3.2. Cardiovascular functions in vivo in the setting of CPB

All in vivo cardiovascular parameters are shown as baseline values (‘before CPB’, at the beginning of the experiment, before injection of vehicle/vardenafil) and values at 60 min of reperfusion (‘after CPB’).

3.2.1. Haemodynamics

Heart rate (HR), mean arterial pressure (MAP), cardiac output (CO) and coronary blood flow (CBF) are shown in Table 1. Baseline HR was to some extent higher in the treatment group, otherwise no differences could be documented. MAP showed decreased values in both groups after CPB. CO and CBF levels were comparable in both groups at baseline and decreased significantly after CPB in the control group. CO level remained unchanged in the treat-
3.2.1. Coronary blood flow (CBF)

When compared to control, CBF was significantly higher in the vardenafil group after 60-min reperfusion.

3.2.2. Left ventricular function

Baseline values did not differ between the groups. After CPB, both left ventricular $E_{es}$ and PRSW decreased significantly in the control group, which could be partly reversed by vardenafil (Fig. 1).

3.2.3. Right ventricular function

Baseline $E_{es}$ values did not differ between the groups. After CPB, right ventricular $E_{es}$ decreased significantly in the control group, which could be completely reversed by vardenafil pretreatment (Fig. 2).

3.2.4. Endothelial function in vivo

Prior to CPB no differences have been observed. After CPB the response to acetylcholine was significantly reduced in the control group, which was completely eliminated by vardenafil (Fig. 3).

3.3. Vascular function in vitro

The net maximal forces developed by the coronary arterial rings during precontraction with the thromboxane A$_2$-receptor agonist U46619 ($5 \times 10^{-7}$ M) showed no differences among the groups (Fig. 4A). A significant impairment of endothelium-dependent vasorelaxation of precontracted coronary arterial rings has been found in the CPB-control group when compared to non-CPB controls, which was demonstrated by the rightward shift of the acetylcholine concentration–response curve (EC$_{50}$ values: $9.16 \times 10^{-8} \pm 2.56 \times 10^{-8}$ M CPB control vs $4.36 \times 10^{-8} \pm 2.10 \times 10^{-9}$ M non-CPB control, $p = 0.037$). Pretreatment with vardenafil resulted in significantly enhanced endothelium-dependent vasorelaxation after CPB (EC$_{50}$ values: $3.13 \times 10^{-8} \pm 3.93 \times 10^{-9}$ M CPB + vardenafil vs $9.16 \times 10^{-8} \pm 2.56 \times 10^{-8}$ M CPB control, $p = 0.0011$) (Fig. 4B). Endothelium-independent vasorelaxation to sodium nitroprusside was slightly increased in the vardenafil-treated group (EC$_{50}$ values: $5.94 \times 10^{-8} \pm 2.26 \times 10^{-9}$ M CPB + varden-

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**Table 1**

<table>
<thead>
<tr>
<th>Haemodynamic variables.</th>
<th>Before treatment and CPB</th>
<th>After CPB</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Vardenafil</td>
</tr>
<tr>
<td>Heart rate (HR) (min$^{-1}$)</td>
<td>110±8</td>
<td>135±5$^*$</td>
</tr>
<tr>
<td>Mean arterial pressure (MAP) (mmHg)</td>
<td>104±10</td>
<td>96±4</td>
</tr>
<tr>
<td>Cardiac output (CO) (l min$^{-1}$)</td>
<td>3.22±0.29</td>
<td>2.93±0.62</td>
</tr>
<tr>
<td>Coronary blood flow (CBF) (ml min$^{-1}$)</td>
<td>40±5</td>
<td>48±10</td>
</tr>
</tbody>
</table>

Heart rate (HR), mean arterial pressure (MAP), cardiac output (CO) and coronary blood flow (CBF) values are shown before and after cardiopulmonary bypass (CPB) in both groups.

* $p < 0.05$ versus control.

# $p < 0.05$ versus baseline.
**3.4. Biochemical measurements**

Plasma cGMP concentrations at 60 min of reperfusion were significantly higher in the vardenafil group when compared to the control (Fig. 5).

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**Fig. 2.** Right ventricular contractility. The slope of the right ventricular (RV) end-systolic pressure–volume relationship ($E_{es}$) before and after cardiopulmonary bypass (CPB) at 60 min of reperfusion. After CPB, $E_{es}$ is shown also as percentage of the baseline value (panel B). All values are given as mean ± SEM; *$p < 0.05$ versus before CPB, $^*p < 0.05$ versus control.

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**Fig. 3.** Coronary endothelial function in vivo. Endothelium-dependent vasodilatation after intracoronary administration of a single bolus of acetylcholine ($10^{-7}$ mol) expressed as percent change of coronary blood flow (CBF) before and after cardiopulmonary bypass (CPB) at 60 min of reperfusion. All values are given as mean ± SEM; *$p < 0.05$ versus before CPB, $^*p < 0.05$ versus control.

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**Fig. 4.** Coronary vascular function *in vitro*. Contraction forces induced by the thromboxane $A_2$-receptor agonist U46619 ($5 \times 10^{-7}$ M) (A); acetylcholine (ACh)-induced endothelium-dependent vasorelaxation (B); sodium nitroprusside (SNP)-induced endothelium-independent vasorelaxation (C) in the different groups. Each column and each point of the curves represents mean ± SEM of 12–28 experiments in coronary arterial rings. *$p < 0.05$ versus non-cardiopulmonary bypass (CPB) control, $^*p < 0.05$ versus CPB control.
Plasma nitrate/nitrite and myeloperoxidase values did not significantly differ between the groups at any time point. Nevertheless, there was a strong tendency towards higher myeloperoxidase values in the control group in comparison to the treatment group after CPB (Fig. 6).

Myocardial high-energy phosphate content is listed in Table 2. ATP values were significantly higher in the vardenafil group (Table 2).

### Table 2: Myocardial high energy phosphates.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Vardenafil</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP (µmol g⁻¹ drw)</td>
<td>9.6 ± 1.2</td>
<td>15.2 ± 1.1*</td>
</tr>
<tr>
<td>ADP (µmol g⁻¹ drw)</td>
<td>5.6 ± 0.3</td>
<td>6.4 ± 0.5</td>
</tr>
<tr>
<td>AMP (µmol g⁻¹ drw)</td>
<td>1.5 ± 0.2</td>
<td>2.1 ± 0.3</td>
</tr>
</tbody>
</table>

Myocardial levels of adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine monophosphate (AMP) are shown after cardiopulmonary bypass in both groups.

* $p < 0.05$ versus control.

4. Discussion

In this study, the benefits of the application of vardenafil, a selective PDE-5-inhibitor, were assessed in a canine model of crystalloid cardioplegia and extracorporeal circulation. In accordance with the literature [11,16], hypothermic cardioplegic arrest and reperfusion resulted in a decline in left and right ventricular contractile and endothelial function. We have shown that pretreatment with vardenafil improves biventricular and endothelial functional recovery after cardioplegic arrest.

Myocardial and endothelial damage with temporary cardiac dysfunction is a well-described phenomenon in the context of cardiac surgery. Hearts undergoing coronary bypass surgery or other surgical procedures requiring CPB and elective cardioplegia undergo episodes of global ischaemia and reperfusion, which leads to endothelial injury as well as contractile dysfunction and morphological injury despite the use of cardioprotective cardioplegic solutions and other strategies of myocardial protection [16]. Due to leucocyte activation in the extracorporeal circuit and during reperfusion after global myocardial ischaemia (as demonstrated by increased plasma myeloperoxidase levels in the present study, Fig. 6), high levels of reactive oxygen radicals and other related oxidants are produced and are central mediators of reperfusion injury. Although enhanced formation of reactive oxygen species (ROS) has been reported to occur in both cardiomyocytes and endothelial cells [17], leucocyte—endothelial cell interactions and increased release of ROS from leucocytes affect first and foremost mainly the endothelium, resulting in endothelial dysfunction. The damaged dysfunctional coronary endothelium is responsible for the impaired endothelial vasodilatory function of coronary arteries, which limits CBF (as demonstrated in the present experiments, Table 1) and triggers a range of problems, including platelet and leucocyte adhesion and aggregation, leading to impaired cardiac performance.

cGMP, a key intracellular second messenger in cardiovascular signaling, is synthesised by the soluble guanylate cyclase (sGC) in response to NO. The cGMP activates the cGMP-dependent protein kinase (protein kinase G), which mediates a considerable part of the effects of cGMP. It is believed that protein kinase G phosphorilates Ca²⁺-activated potassium channels, thereby causing cell membrane hyperpolarisation and smooth muscle relaxation [18]. PDE enzymes hydrolyse the phosphodiester bond of cyclic adenosine monophosphate (cAMP) and cGMP, thereby degrading them. PDE-5 is the predominant cGMP-metabolising PDE, which is expressed in all vascular beds, platelets and other tissues such as myocardium [3].
Inhibitors of PDE-5 block the degradation of cGMP, thereby increasing its intracellular levels and evoke potent vasodilatory responses. Based on these mechanisms of action, pharmacological inhibition of PDE-5 has been first approved for the treatment of erectile dysfunction and pulmonary hypertension [1,2].

It has been recently reported that increased oxidative stress (e.g., due to ischaemia–reperfusion) decreases the expression of sGC [19] and increases the expression, phosphorylation and activity of PDE-5 [20], both leading to an imbalance between cGMP synthesis and degradation, lower cGMP levels and thus impaired regulation of all the intracellular pathways downstream of the second messenger cGMP. These observations may give explanations for the beneficial protective effects of restoration of physiological cGMP levels by pharmacological PDE-5 inhibition.

Recently, novel cardioprotective, preconditioning-like effects of the PDE-5 inhibitors sildenafil [5] and vardenafil [6] have been described in a rabbit model of myocardial ischaemia–reperfusion. The powerful protective effects of PDE-5 inhibition have been shown to be mediated by increased intracellular cGMP levels and the activation of mitochondrial ATP-sensitive potassium channels (mitoK<sub>ATP</sub>). Mitochondria are known to play a pivotal role in cell physiology by synthesis of ATP. Opening the mitoK<sub>ATP</sub> channels (possibly by the cGMP-activated protein kinase G) ultimately leads to K<sup>+</sup> influx into the mitochondrial matrix, which enables additional protons to be pumped out for the formation of H<sup>+</sup> electrochemical gradient. Enhancing the H<sup>+</sup> electrochemical gradient results in increased mitochondrial ATP synthesis, thus a better energetic state of the myocardium [6,21]. Supporting this concept, our present results demonstrate significantly increased plasma cGMP (Fig. 5) and myocardial ATP levels (Table 2) after global ischaemia–reperfusion following vardenafil pretreatment, confirming the effectiveness of PDE-5 inhibition in vivo and showing the importance of improved intracellular energy supply in the myocardial protection by PDE-5 inhibition.

Other mechanisms of the cardioprotective actions of PDE-5 inhibitors have recently been proposed, including cGMP-dependent activation of sarcoplasmic Ca<sup>2+</sup>-ATPase (SERCA) thus optimisation of myocardial Ca<sup>2+</sup> homeostasis during re-oxygenation [4] and cGMP-protein kinase G-induced over-expression of NO synthase isoforms (mainly iNOS), thereby increasing NO production [22]. The latter mechanism could not be definitely confirmed in the present study. Plasma nitrate/nitrite levels, which reflect total in vivo NO production, did not significantly differ between control and vardenafil-treated groups, only a slight tendency towards higher nitrate/nitrite levels could be documented (Fig. 6). Therefore, in our opinion, enhanced NO production by PDE-5 inhibition might be a protective mechanism only of secondary importance, overshadowed by the mechanism of opening the mitoK<sub>ATP</sub> channels and increasing ATP levels as described above.

The reported potential antioxidative effects of PDE-5 inhibition and intracellular cGMP accumulation [8,9] are in line with our results. Plasma myeloperoxidase levels in the present study show a strong tendency towards lower values in the vardenafil-treated group indicating decreased leucocyte activation and reduced oxidative stress (Fig. 6).

Although previous studies reported about improvement of myocardial dysfunction in different animal models of regional ischaemia–reperfusion by PDE-5 inhibition [5,6], this is the first in vivo study that shows the effectiveness of systemic application of the selective PDE-5 inhibitor vardenafil on cardiovascular functions in a clinically relevant large animal model of extracorporeal circulation with cardioprotective arrest. In accordance with the literature [11], CPB with cardioprotective arrest and subsequent reperfusion resulted in only a moderate decrease in some of the global haemodynamic parameters in the control group (Table 1). For a sensitive and reliable assessment of left and right ventricular myocardial contractility, we calculated the pressure-volume loop-derived, load-independent indices, E<sub>P</sub>, (slope of ESPVR) and PRSW. These parameters clearly demonstrated a significant decrease of contractile function of the left and right ventricle, which is consistent with previous studies [11]. In the present work, we show for the first time that pharmacological preconditioning with vardenafil significantly reduced biventricular contractile dysfunction as indicated by the load-independent contractility indices assessed.

Endothelial cells are known to be more sensitive to ischaemia–reperfusion injury, than cardiomyocytes [23], and a reduced endothelial responsiveness (such as impaired endothelium-dependent vasorelaxation) has been described after reperfusion [12]. In accordance, we demonstrated impaired coronary endothelial function after CPB both by in vivo coronary blood flow measurements (Fig. 3) and by in vitro vascular reactivity experiments (Fig. 4). Experimental evidence exists that the phenomenon of ischaemic and pharmacological preconditioning can be elicited at the level of the endothelium as well [24]. K<sub>ATP</sub>-channel activation [25] as well as pharmacological PDE-5 inhibition [7] has been demonstrated to protect the endothelium from ischaemia–reperfusion injury. Our present data (both in vivo and in vitro experiments) correspond to these previous works (Figs. 3 and 4); however, our present study is the first one that reports about significantly improved endothelial function by PDE-5 inhibition in a clinically relevant experimental model of CPB.

In summary, the current results demonstrate that pharmacological preconditioning with the potent selective PDE-5 inhibitor vardenafil results in higher myocardial ATP levels, better left and right ventricular functional recovery and improved endothelial function in a clinically relevant large animal model of CPB. Based on our present data, application of vardenafil may be useful to reduce myocardial and endothelial tissue injuries during cardiac surgery.

References


