Tetrahydrobiopterin improves cardiac and pulmonary function after cardiopulmonary bypass

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Abstract

Objective: Tetrahydrobiopterin (BH4) is an important cofactor of endogenous nitric oxide synthesis. In the present preclinical study, we investigated the effects of BH4 on cardiac and pulmonary function during early reperfusion in an experimental model of cardioplegic arrest and extracorporeal circulation. Methods: Twelve anesthetized dogs underwent hypothermic cardiopulmonary bypass. After 60 min of hypothermic cardiac arrest, reperfusion was started after application of either saline vehicle (control, n = 6), or BH4 (n = 6). Left-ventricular end-systolic pressure volume relationship (Ees) was measured by a combined pressure–volume conductance catheter at baseline and after 60 min of reperfusion. Left anterior descending (LAD) coronary (CBF) and pulmonary blood flow (PBF), endothelium-dependent vasodilatation to acetylcholine (ACH), endothelium-independent vasodilatation to sodium nitroprusside (SNP) and alveolo-arterial O2 gradient were determined. Results: The administration of BH4 led to a significantly better recovery of Ees (given as percent of baseline: 85 ± 22 vs 46 ± 15%, p < 0.05). CBF was also significantly higher in the BH4 group (38 ± 5 vs 22 ± 5 ml min⁻¹, p < 0.05). While the vasodilatory response to SNP was similar in both groups, injection of ACH resulted in a significantly higher increase in CBF (64 ± 12 vs 25 ± 12%, p < 0.05) and PBF (49 ± 15 vs 36 ± 14%, p < 0.05) in the BH4-treated animals. Alveolo-arterial O2 gradient was significantly lower after BH4 supplementation (80 ± 15 vs 49 ± 14 mmHg, p < 0.05). Conclusions: Application of BH4 improves myocardial, endothelial and pulmonary function after cardiopulmonary bypass with hypothermic cardiac arrest. The observed protective effects indicate that BH4 could be a novel therapeutic option in the treatment of ischemia/reperfusion injury. © 2010 European Association for Cardio-Thoracic Surgery. Published by Elsevier B.V. All rights reserved.

Keywords: Cardiopulmonary bypass; Ischemia/reperfusion injury; Tetrahydrobiopterin; Endothelial function

1. Introduction

Ischemia/reperfusion injury following cardiac surgery is a common condition, which develops after cardiopulmonary bypass (CPB) operations with cardioplegic arrest. Temporary dysfunction of the heart can be observed frequently, presumably as a consequence of this phenomenon. Even if cardiac dysfunction is not always clinically remarkable, reduction of myocardial contractility may occur [1]. In addition, during CPB, there is a very little or no blood flow through the pulmonary artery, which may impair pulmonary function or lead to increase of the pulmonary vascular resistance [2]. Extracorporeal circulation is also known to induce systemic inflammatory reactions [3], with free radical release, leading to secondary organ injury, among others in the gastrointestinal tract [4].

Several studies reported that the L-arginine–nitric oxide (NO)–cyclic guanosine monophosphate (cGMP) pathway plays an important role in ischemia/reperfusion injury. The use of NO donors or L-arginine was demonstrated to be beneficial in experimental settings of cardioplegia/reperfusion [5–7]. Recently, we showed [7] that neither L-arginine nor NO levels (measured as oxidized products nitrite/nitrate) are decreased after CPB. In the same study, however, L-arginine supplementation improved the recovery of myocardial and endothelial function via enhanced NO synthesis, leading to 'supraphysiological' levels of plasma nitrite/...
nitrite. We speculated that (1) normal plasma levels of nitrite/nitrate may reflect the net effect of reduced NO synthesis by endothelial nitric oxide synthase (NOS) (‘protection’ NO in endothelial cells) and increased NO synthesis by inducible NOS (‘harmful’ NO in inflammatory reactions) and/or (2) not the level of NO precursors but endothelial NO synthesis itself is impaired.

For NO synthesis, endothelial NOS (eNOS) requires an essential pterin cofactor, tetrahydrobiopterin (BH4), which plays a pivotal role in the functional coupling of eNOS and, thus, NO production [8]. It has been recently reported that various pathophysiological conditions associated with nitro-oxidative stress lead to the oxidation and depletion of the redox-sensitive BH4, resulting in eNOS uncoupling [9—12]. In the absence of BH4, uncoupled eNOS generates reactive oxygen species (ROS) rather than NO, which aggravates oxidative damage and results in reduced NO bioavailability and endothelial dysfunction [8].

Therefore, pharmacological BH4 supplementation emerges as a novel therapeutic possibility for numerous cardiovascular diseases including hypertension, diabetes, atherosclerosis, myocardial hypertrophy or ischaemia/reperfusion [13].

In the present study, we investigated the effects of BH4 administration on NO bioavailability, as well as on cardiac, pulmonary and endothelial dysfunction after ischemia/reperfusion in a clinically relevant canine model of CPB with hypothermic cardiac arrest.

2. Material and methods

2.1. Animals and experimental groups

Twelve dogs (foxhounds) weighing 24—36 kg (28 ± 3 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. Dogs were randomly assigned to the experimental groups. Six animals received 1 mg kg⁻¹ BH4 (Sigma—Aldrich, Germany) as a short infusion starting 5 min before aortic declamping and continued during the first 25 min of reperfusion (Fig. 1) [14]. Six vehicle-treated animals served as controls.

2.2. General management and CPB

The dogs were premedicated with propionylpromazine and anesthetized with pentobarbital (15 mg kg⁻¹ initial bolus and then 0.5 mg kg⁻¹ h⁻¹ IV), paralyzed 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 (fraction of inspired oxygen (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 and to take blood samples for the analysis of blood gases, electrolytes, and pH. Basic intravenous volume substitution was carried out with Ringer 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 pericardiotomy, 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 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 HTK (histidine—tryptophan—ketoglutarate) 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, rewarming 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 restarted with 100% oxygen. All animals were weaned from CPB without inotropic support 20 min after the release of the aortic cross-clamp. Each animal underwent 90 min of CPB with 60 min of cardiac arrest (Fig. 1).

Cardiac and pulmonary functional measurements were performed at baseline (before CPB) and 60 min after starting reperfusion (after treatment and CPB, Fig. 1). After completing the experiment, all animals were euthanized.

2.3. Cardiac function

Left-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 via the apex. Stroke volume (SV) was calculated from the integrated flow signal measured by an aortic ultrasonic flow probe 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 atrium. Vena cava occlusions were performed to obtain a series of pressure—volume loops. The slope (Ees) of the left-ventricular end-systolic pressure—volume relationship and preload recruitable stroke work (PRSW) were calculated as load-independent indices of myocardial contractility. Myocardial relaxation was characterized by the relaxation time constant (Tau) of the left-ventricular pressure decay. Tau was calculated from time-expanded recordings of left-ventricular pressure. Coronary blood flow (CBF) was measured on the left anterior descending (LAD) coronary artery with a perivascular ultrasonic flow probe. Coronary endothelium-dependent vasorelaxation was assessed after intracoronary administration of a single bolus of acetylcholine (ACh, 10^-7 mol) and endothelium-independent vasodilatation after sodium nitroprusside (SNP, 10^-7 mol). The vasoresponse was expressed as percentage change of CBF.

2.4. Pulmonary function

In addition to routine blood gas analysis, blood gases were determined before and after weaning from CPB at 60 min of reperfusion at room air ventilation. Pulmonary function was characterized by the alveolar—arterial oxygen difference, which was calculated according to the standard formulas. In addition, the left-lower-lobar pulmonary artery was dissected, and blood flow was measured by a 4-mm-diameter ultrasonic flow probe. Pulmonary endothelium-dependent vasodilatation was assessed after intracoronary administration of a single bolus of acetylcholine (ACh, 10^-7 mol) and endothelium-independent vasodilatation after sodium nitroprusside (SNP, 10^-7 mol). The vasoresponse was expressed as percentage change of pulmonary blood flow (PBF).

2.5. Measurement of plasma cGMP

Plasma cGMP in aortic blood plasma samples as marker of NO bioavailability was measured by radioimmunoassay (RIA), as described previously (Immunotech SA; Marseille, France) [15].

2.6. Statistical analysis

All values were expressed as mean ± standard deviation (SD). A paired t-test was used to compare two means within a group (comparison of ‘baseline’ and ‘after CPB’ values). Means between the groups were compared by an unpaired two-sided Student’s t-test (comparison of control and BH4 groups). A p-value less than 0.05 was considered statistically significant.

3. Results

3.1. Hemodynamic parameters

Hemodynamic variables are shown in Table 1. Baseline parameters did not differ between the groups and were within the physiological range. Heart rate (HR) did not change either in the control or in the BH4 group. After 60 min of cardioplegic arrest and 60 min of reperfusion, mean arterial pressure (MAP) decreased significantly (p < 0.05) in the control group, while it remained unchanged in the BH4 group. Cardiac output (CO) did not differ significantly between the groups.

3.2. Left-ventricular systolic and diastolic function

Left-ventricular systolic function — characterized by the load-independent, sensitive contractility indices Ees and PRSW (Fig. 2) — showed a significant decrease (p < 0.05) after extracorporeal circulation and reperfusion in the control group, while it remained unchanged in the BH4-treated group. Myocardial relaxation constant Tau increased significantly (p < 0.05) in the control group at 60 min of reperfusion, but it remained at baseline level in the BH4 group (Fig. 3).

3.3. CBF and vascular function

CBF was similar in both groups before cardioplegic arrest. After 60 min of reperfusion, the control group showed significantly decreased CBF, but, in the BH4 group, CBF remained unchanged after CPB (Table 1). Endothelium-dependent vasodilatation after ACh was significantly reduced in both groups after 60 min of reperfusion in comparison to control. Improved endothelium-independent vasodilatation after SNP was significantly enhanced in both groups after 60 min of reperfusion.

Table 1. Hemodynamic variables.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>After CPB</th>
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<tbody>
<tr>
<td>Control</td>
<td>BH4</td>
<td>Control</td>
</tr>
<tr>
<td>HR (min^-1)</td>
<td>115 ± 22</td>
<td>105 ± 14</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>87 ± 21</td>
<td>91 ± 19</td>
</tr>
<tr>
<td>CO (l min^-1)</td>
<td>2.42 ± 0.72</td>
<td>2.87 ± 1.00</td>
</tr>
<tr>
<td>CBF (ml min^-1)</td>
<td>34 ± 14</td>
<td>40 ± 7</td>
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</tbody>
</table>

* p < 0.05 versus baseline. ** p < 0.05 versus control.

Fig. 2. Left-ventricular systolic function. The slope of the left-ventricular end-systolic pressure—volume relationship (Ees, left side) and preload recruitable stroke work (PRSW, right side) at baseline and after cardiopulmonary bypass (CPB) at 60 min of reperfusion. All values are given as mean ± SD.
pre-CPB values (Fig. 4). However, this decrease was significantly smaller in the BH4 group. Endothelium-independent vasodilatation after SNP showed no significant differences over time and between groups (Fig. 4).

3.4. Pulmonary function

Alveolar—arterial oxygen difference at room air ventilation was similar in both groups at baseline and increased significantly after CPB in the control group, indicating pulmonary dysfunction, while it remained unchanged in the BH4 group (Fig. 5). Vascular responses to ACh did not differ between the groups at baseline. Pulmonary vasodilatation after injection of ACh showed a decreasing tendency after CPB in the control group, and it was significantly improved in the BH4 group (Fig. 5).

3.5. Plasma cGMP levels

cGMP levels in blood plasma samples were similar in both groups at baseline, and decreased significantly in the control group after CPB. Significantly higher cGMP levels were observed in the BH4 group after CPB when compared with control (Table 2).

4. Discussion

In this study, the benefits of the application of BH4 during early reperfusion were assessed in a canine model of crystalloid cardioplegia and extracorporeal circulation. In accordance with the literature [1,5], hypothermic cardioplegic arrest and reperfusion resulted in a decline in left-ventricular, pulmonary, and endothelial function. We have shown that application of BH4 improves cardiovascular recovery after cardioplegic arrest; in addition, pulmonary function was significantly improved in terms of pulmonary endothelial function and oxygenation, after treatment with BH4.

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 ischemia 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 [5]. Due to leukocyte activation in the extracorporeal circuit and during reperfusion after global myocardial ischemia, high levels of reactive oxygen radicals and other related oxidants are produced and are central mediators of reperfusion injury. Although enhanced formation of ROS has been reported to occur in both cardiomyocytes and endothelial cells [16], leukocyte—endothelial cell interactions and increased release of ROS from leukocytes 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 CF (as demonstrated in the present experiments, Table 1) and triggers a range of problems including platelet and leukocyte adhesion and aggregation, leading to impaired cardiac performance.

Endothelium-dependent vasodilatory function is mainly derived from the endothelial production of NO by the enzyme eNOS, which converts the substrate L-arginine to L-citrulline and NO. For physiological NO synthesis, eNOS requires the substrates L-arginine, nicotinamide adenine dinucleotide phosphate reduced (NADPH) and O2 as well as the cofactor BH4. Binding of the Ca2+-calmodulin complex to eNOS is essential for its activation, and its NO-producing enzymatic activity can also be modulated by phosphorylation status, protein interactions, and by the available levels of its substrates and cofactors [17,18].

In recent years, it has been appreciated that eNOS not only synthesises NO, but also that due to a functional uncoupling, it can produce superoxide anions (O2-) and hydrogen peroxide (H2O2). Furthermore, it has been observed, that the redox-sensitive cofactor BH4 is not only essential for NO synthesis but that its depletion triggers aggregation, leading to impaired cardiac performance.

The present work, supplementation of BH4 completely prevented left-ventricular contractile dysfunction, as indicated by the assessed load-independent contractility indices; moreover, diastolic dysfunction after CPB, as characterized by the prolonged time constant of left-ventricular pressure decay (Tau), has been significantly improved.

Though numerous in vitro and in vivo studies investigated the effects of BH4 supplementation on endothelial dysfunction in various experimental models of disease [11,12,20], and also in human trials [22,23], only a little information exists about its effects on endothelial function after CPB. Stevens et al. described beneficial effects of BH4 in a porcine model of CPB; however, they investigated endothelial function in a less reliable in vitro system [14]. Correspondingly to their data, we report in the present large-animal study significant improvement of both coronary and pulmonary endothelial function assessed in vivo with direct coronary flow measurements (Figs. 4 and 5). The mechanisms, leading to an improvement of endothelial function after BH4 treatment, are discussed above and may include both the restoration of endothelial NO synthesis by recoupling of eNOS (as supported by increased cGMP levels in the present study, Table 2) and possibly also the modest direct antioxidant effects of BH4 [13].

There are only a few studies, which describe the effects of BH4 in experimental models of pulmonary diseases. Hillinger et al. reported a reduction of lung allograft ischemia/reperfusion injury after BH4 treatment in a porcine lung-transplantation model [24]. In another recent work, the pivotal role of BH4 has been observed in the context of development of hypoxia-induced pulmonary hypertension [25]. The present study is the first, which reports about a functional improvement by BH4 supplementation after lung injury in the setting of CPB.

In summary, the current results indicate that, in a clinically relevant large-animal model of CPB, pharmacological application of BH4 restores NO bioavailability and markedly attenuates early reperfusion injury, resulting in a better functional cardiac recovery, and improved pulmonary and endothelial function. Based upon our present data, BH4 supplementation may be useful to reduce cardiac and pulmonary tissue injury during cardiac surgery.

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References


