Tetrahydrobiopterin increases myocardial blood flow in healthy volunteers: a double-blind, placebo-controlled study

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The crucial role of the vascular endothelium in vasomotor control is well recognised [1]. One important vasoactive mediator released by the endothelium, the so-called endothelium-derived relaxing factor [2], has been identified as nitric oxide (NO) [3]. Basal release of NO contributes significantly to resting macrovascular and coronary microvascular tone [4]. Furthermore, NO is critical for epicardial and microvascular vasodilatation during metabolic stimulation of the human heart [5].

Tetrahydrobiopterin (BH₄) is an obligatory cofactor for all NO synthase isoforms. The precise role of BH₄ in NO synthesis is incompletely understood, but several mechanisms are suggested: BH₄ allosterically effects NO synthases, thereby stabilizing the active state of the enzyme [6] and increasing the affinity of the substrate L-arginine [7]. Furthermore, BH₄ prevents feedback inhibition of NOS by NO itself [8]. Activation of NO synthases under suboptimal concentrations of BH₄ leads to increased formation of oxygen radicals [9] and may represent an important mechanism of oxidative vascular injury [10]. BH₄, like all reduced pteridines, is a potent antioxidant and scavenger of oxygen-derived free radicals [11]. There is increasing evidence for BH₄ vasoactivity in vivo in both the vasculature of healthy individuals and patients with risk factors for or manifest atherosclerosis [12–20], suggesting potential therapeutic implications of BH₄. However, in vivo data suggest that under physiological conditions endogenous BH₄ levels are nearly saturating and barely a limiting factor for optimal or near optimal vascular NOS activity. For example, intraarterial infusion of BH₄ in a dose of 500 µg/min over 10 minutes influenced neither mean arterial blood pressure nor basal or stimulated forearm blood flow in healthy volunteers [13]. Similarly, inhalation of 500 mg BH₄ did not affect systemic haemodynamics and ECGs remained unaffected in both groups. BH₄ was very well tolerated.

Conclusion: Systemically administered BH₄ is safe and effectively increases resting MBF in healthy volunteers.

Keywords: atherosclerosis; endothelium; heart diseases; myocardium; nitric oxide; tetrahydrobiopterin

Introduction

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rapid vasodilatation of precontracted hand veins in healthy volunteers [20]. It is not yet known whether differences in susceptibility to exogenous BH4 or local differences in BH4 concentrations exist in different parts of the vasculature.

The coronary circulation is a major target for atherosclerotic vessel disease, resulting in high morbidity and mortality. Although the influence of NO on cardiac vessels has been elucidated, the influence of BH4 on myocardial blood flow (MBF) in health and disease is at present poorly characterised. In explanted vessels from patients with coronary atherosclerosis, sepiapterin, a BH4 precursor, significantly improved endothelium-dependent vasodilatation [22]. Intracoronary administration of BH4 restored abnormal endothelium-dependent coronary vasomotion in response to acetylcholine in patients with coronary artery disease [23]. Likewise, preliminary data indicate the potential of intracoronary BH4 for improving endothelium-dependent vasodilatation and decreasing free radical generation in coronary arteries of patients with one or more cardiovascular risk factors but no significant coronary artery disease, and for enhancing acetylcholine-induced vasodilatation in patients with atypical chest pain and angiographically normal coronary arteries [17].

However, for practical reasons the therapeutic impact of locally administered BH4 is somewhat restricted. Thus, the present double-blind, placebo-controlled study using positron emission tomography (PET) for MBF quantification was designed to investigate the short-term effect of intravenously administered BH4 on MBF in healthy volunteers.

Methods

Study population

15 healthy volunteers (one woman and 14 men; age range 22.7 to 31.3 years; mean 25.9 years) were included in the study. None of the subjects had a history of cardiovascular disease or coronary risk factors (except for smoking). Entry criteria included normal heart rate, blood pressure, and electrocardiogram, as well as low clinical probability of coronary artery disease [24]. Cholesterol screening confirmed a normal lipid profile in all participants. All refrained from ingesting caffeinated beverages for 24 hours and alcohol for 12 hours before the study. The local Ethics Committee approved the study, and written informed consent was obtained from all participants.

Study protocol

The study protocol is summarised in Fig. 1. MBF was assessed on a GE advanced positron emission tomograph (GE Medical Systems, Milwaukee, Wis, USA) and [13N]ammonia. After injection of 700 MBq [13N]ammonia, acquisition of the serial transaxial tomographic images of the heart was started (baseline scan). After allowing for physical decay of [13N]ammonia, this was followed by infusion of either BH4 or placebo over 30 minutes. A second bolus of 700 MBq [13N]ammonia was injected 8 minutes after starting BH4 administration. After the end of the infusion, a 20-minute transmission scan for correction of photon attenuation was performed. After the transmission scan, a third bolus of 700 MBq [13N]ammonia was injected. ECGs were monitored continuously. Heart rate and blood pressure were recorded every 5 minutes.

Tetrahydrobiopterin (BH4) and placebo preparation

A sodium bicarbonate-buffered solution of 10 mg/kg of (6R)-5, 6, 7, 8-tetrahydro-L-biopterin-dihydrochloride (BH4, Dr. B. Schircks Laboratories, Jona, Switzerland) was prepared as previously described [21] in a total volume of 10 ml immediately before use and diluted with 0.9% NaCl to a total volume of 50 ml. The steriley filtered, clear, colourless solution was infused into a peripheral vein of the forearm using a perfusor at a constant flow of 99.9 ml/h. Placebo resembling exactly the chemical background of the drug was prepared immediately before use from equivalent amounts of sodium bicarbonate powder and HCl solution in a total volume of 10 ml, diluted in 50 ml 0.9% NaCl and infused as described above. Both volunteers and physicians administering the substance were blinded for the treatment group.

Estimation of myocardial blood flow (MBF)

PET scan analysis was performed blinded for randomisation. Three consecutive representative midventricular slices were chosen for quantitative analysis. Segmental regions of interest (ROIs) were placed over the septal, anterior and lateral wall. A spherical ROI was placed into the blood pool of the left ventricle. MBF was estimated by model fitting of the blood pool and myocardial time-activity curves using a three-compartment model [25]. The correction for partial volume and spill over was performed using the method described and validated by Hutchins and co-workers [26]. Briefly, the region of interest (ROI) is chosen to contain only myocardial tissue and blood, and thus the relation between the measured PET counts in a region (CPET) and the true counts in myocardium (Cm) and arterial blood (Ca) is modelled as follows: \( CPET(t) = F_r C_m(t) + (1-F_r)C_a(t) \). \( F_r \) is the fractional contribution of the blood pool to measured PET counts in a region and is dependent on the placement of the region, camera resolution and movement of the myocardium. Since the contribution of myocardium to total regional counts decreases with increasing blood pool fraction, \( C_m \) is multiplied by \((1-F_r)\). \( F_r \) is estimated together with the other kinetic tissue parameters using least squares fitting.

Statistical analysis

Results are presented as means ± (SEM). Paired and unpaired Student’s t-tests were performed as appropriate using GraphPad InStat 3.00 (San Diego, CA, USA). P<0.05 was considered significant.
MBF Mean myocardial blood flow (MBF) was assessed before (scan 1), during (scan 2), and after (scan 3) the administration of either BH₄ (10 mg/kg) or placebo (see Fig. 1). Due to technical problems, scan 2 could not be acquired in 2 volunteers (one from each group), and therefore this scan was not included in the final analysis. As shown in Fig. 2, the 10 healthy volunteers receiving BH₄ showed a significant increase in mean MBF from 0.91±0.09 (scan 1) to 1.18±0.10 ml/min/g tissue (scan 3), corresponding to a mean percentage increase of 37.5±9.5% (p = 0.0042). In contrast, in the group receiving placebo mean MBF remained unchanged (non-significant decrease from 0.97±0.19 to 0.84±0.11 ml/min/g; p = 0.36). Using an unpaired t-test, a p = 0.93 was found for intergroup comparison of scan 1, whereas p = 0.054 was found for intergroup comparison of scan 3.

MBF changes in individuals from whom all scans could be acquired were as follows: scan 1: 0.932+/–0.295; scan 2: 1.006+/–0.350; scan 3: 1.158+/–0.335, ANOVA for repeated measurements: p = 0.0028, scan 1 vs. scan 3 p<0.01; scan 2 vs. scan 3: p<0.05, in the BH₄ group (n = 9), and scan 1: 0.968+/–0.386; scan 2: 0.925+/–0.230; scan 3: 0.840+/–0.225, ANOVA for repeated measurements: p = 0.3577, in the placebo group (n = 4) respectively.

Haemodynamics and ECG
There were no significant changes in haemodynamics during or after BH₄ infusion. ECGs remained unchanged throughout the study (not shown).

Side effects
Only 3 subjects felt a slight burning sensation at the site of administration shortly after the start of BH₄ infusion, while some reported increased diuresis 4-6 hours after BH₄ administration. Otherwise BH₄ was very well tolerated.

Discussion
The present placebo-controlled study demonstrates for the first time that intravenous administration of BH₄ results in increased blood flow in the myocardium. Infusion of 10 mg/kg BH₄ was chosen because this dose proved safe and effective in loading tests on newborns and children for differential diagnosis of inborn BH₄ deficiencies [27], and is locally effective without systemic side effects in a swine model of reperfusion injury [28], in a diabetic rat model [29] and in forearms of healthy volunteers [12]. In our experimental setting, BH₄-induced increases in MBF were not accompanied by any sign of systemic vasodilatation. Although non-invasive haemodynamic monitoring of arterial blood pressures may not be the most sensitive method for detection of subtle peripheral vasodilatation, the data presented here are compatible with the hypothesis that BH₄ at a dose of 10 mg/kg has no systemic vasodilating effect when administered intravenously. These data thus suggest either greater susceptibility of the coronary circulation to BH₄ compared to the systemic vascular bed, or lower functional BH₄ concentrations in endothelial cells of blood vessels of the heart. In contrast to the BH₄-treated group, mean MBF remained stable after placebo administration in 5 subjects, corroborating earlier findings of highly reproducible results on PET scans [30]. PET scans were acquired and blood flows estimated using standardised protocols, and baseline MBF values of all subjects were well within the range of previously published studies [31]. Interestingly, the vasodilating effect of BH₄ lasted longer than could have been assumed from forearm experiments [12, 13], not being significant shortly after starting BH₄ administration but still maximal at the end of our measurements. Radiotracer doses limited further MBF measurements to extend follow-up of MBF, and further studies will be required to assess the exact time-course of the BH₄-induced increase in MBF. However, this would be very helpful in guiding BH₄ dosing intervals in future clinical trials. Similarly, the optimal dose of BH₄ remains to be clarified.

Impaired endothelium-dependent vasodilatation has been observed in several diseases predisposing to atherosclerosis [32], and a dysfunctional endothelium may promote the development of atherosclerosis [33]. Recent studies showed that exogenous BH₄ restores impaired endothelium-dependent NO production, suggesting that availability or production of endogenous BH₄ is reduced in these pathologies and that a local BH₄ “deficiency” significantly contributes to impaired
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