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

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Summary

Objectives: Tetrahydrobiopterin (BH_4) is a regulatory cofactor for the activity of nitric oxide synthases. Vasodilating properties of BH_4 have been reported *in vitro* and *in vivo*. The influence of BH_4 on myocardial blood flow (MBF), however, is largely unknown. We therefore performed a double-blind, placebo-controlled study to investigate the effect of intravenous BH_4 on MBF in healthy volunteers.

Methods and Results: Resting MBF was assessed in 15 subjects receiving either intravenous BH_4 (10 mg/kg) or placebo using positron emission tomography (PET) and [^{13}N]ammonia. From a mean baseline MBF of 0.91 ± 0.09 ml/min/g, MBF increased to 1.18 ± 0.10 ml/min/g after BH_4 (n = 10; 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; n = 5; p = 0.36). Systemic haemodynamics and ECGs remained unaffected in both groups. BH_4 was very well tolerated.

Conclusion: Systemically administered BH_4 is safe and effectively increases resting MBF in healthy volunteers.

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

Introduction

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 (BH_4) is an obligatory cofactor for all NO synthase isoforms. The precise role of BH_4 in NO synthesis is incompletely understood, but several mechanisms are suggested: BH_4 allosterically effects NO synthases, thereby stabilizing the active state of the enzyme [6] and increasing the affinity of the substrate L-arginine [7]. Furthermore, BH_4 prevents feedback inhibition of NOS by NO itself [8]. Activation of NO synthases under suboptimal concentrations of BH_4 leads to increased formation of oxygen radicals [9] and may represent an important mechanism of oxidative vascular injury [10]. BH_4, like all reduced pteridines, is a potent antioxidant and scavenger of oxygen-derived free radicals [11]. There is increasing evidence for BH_4 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 BH_4. However, *in vivo* data suggest that under physiological conditions endogenous BH_4 levels are nearly saturating and barely a limiting factor for optimal or near optimal vascular NOS activity. For example, intraarterial infusion of BH_4 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 BH_4 did not affect systemic haemodynamics in healthy volunteers despite elevated systemic arterial and venous BH_4 levels [21]. In contrast, high doses of BH_4 (8–32 mg/min) in the brachial artery induced marked local vasodilatation in the perfused limb of healthy subjects, whereas blood flow in the non-perfused limb, systemic blood pressure, and heart rate remained unchanged [12], and BH_4 (250 nmol/min for 20 min) also caused

rapid vasodilatation of precontracted hand veins in healthy volunteers [20]. It is not yet known whether differences in susceptibility to exogenous BH_4 or local differences in BH_4 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 BH_4 on myocardial blood flow (MBF) in health and disease is at present poorly characterised. In explanted vessels from patients with coronary atherosclerosis, sepiapterin, a BH_4 precursor, significantly improved endothelium-dependent vasodilatation [22]. Intracoronary administration of BH_4 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 BH_4 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 BH_4 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 BH_4 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 [^{13}N]ammonia. After injection of 700 MBq [^{13}N]ammonia, acquisition of the serial transaxial tomographic images of the heart was started (baseline scan). After allowing for physical decay of [^{13}N]ammonia, this was followed by infusion of either BH_4 or placebo over 30 minutes. A second bolus of 700 MBq [^{13}N]ammonia was injected 8 minutes after starting BH_4 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 [^{13}N]ammonia was injected. ECGs were monitored continuously. Heart rate and blood pressure were recorded every 5 minutes.

Tetrahydrobiopterin (BH_4) and placebo preparation

A sodium bicarbonate-buffered solution of 10 mg/kg of (6R)-5, 6, 7, 8-tetrahydro-L-biopterin-dihydrochloride (BH_4, 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 sterilely 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.

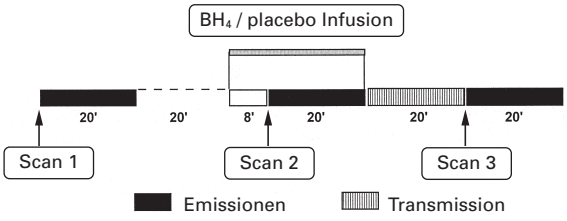

Figure 1
Study protocol.

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 (C_{PET}) and the true counts in myocardium (C_m) and arterial blood (C_a) is modelled as follows: $C_{PET}(t) = F_aC_a(t) + (1–F_a)C_m(t)$. F_a 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 $(1–F_a)$. F_a 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.

Results

MBF

Mean myocardial blood flow (MBF) was assessed before (scan 1), during (scan 2), and after (scan 3) the administration of either BH_4 (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 BH_4 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 BH_4 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.

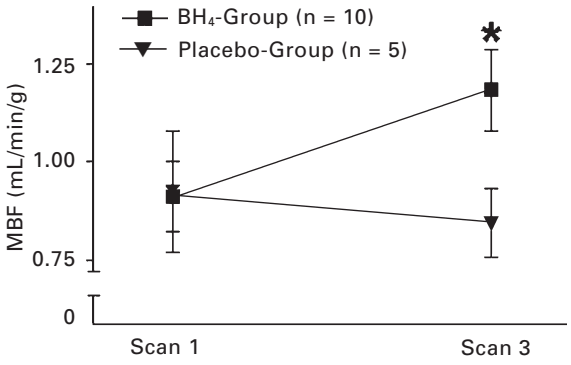

Figure 2

Effect of intravenous BH_4 (10 mg/kg; n = 10) or placebo (n = 5) on MBF in healthy volunteers. Means ± (SEM). *p = 0.0042 as compared to scan 1.

Haemodynamics and ECG

There were no significant changes in haemodynamics during or after BH_4 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 BH_4 infusion, while some reported increased diuresis 4-6 hours after BH_4 administration. Otherwise BH_4 was very well tolerated.

Discussion

The present placebo-controlled study demonstrates for the first time that intravenous administration of BH_4 results in increased blood flow in the myocardium. Infusion of 10 mg/kg BH_4 was chosen because this dose proved safe and effective in loading tests on newborns and children for differential diagnosis of inborn BH_4 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, BH_4-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 BH_4 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 BH_4 compared to the systemic vascular bed, or lower functional BH_4 concentrations in endothelial cells of blood vessels of the heart. In contrast to the BH_4-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 BH_4 lasted longer than could have been assumed from forearm experiments [12, 13], not being significant shortly after starting BH_4 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 BH_4-induced increase in MBF. However, this would be very helpful in guiding BH_4 dosing intervals in future clinical trials. Similarly, the optimal dose of BH_4 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 BH_4 restores impaired endothelium-dependent NO production, suggesting that availability or production of endogenous BH_4 is reduced in these pathologies and that a local BH_4 "deficiency" significantly contributes to impaired

NO production [13–15, 34–36]. The potential of locally administered BH_4 for restoring impaired endothelium-dependent vasodilatation has also been demonstrated in the coronary circulation [17, 18, 22, 23]. Our results suggest that intravenous BH_4 increases MBF in healthy young subjects, establishing the basis for a further study to investigate the influence of peripherally administered BH_4 in coronary heart disease.

In conclusion, systemically administered BH_4 significantly increases resting MBF in healthy volunteers. Further work is needed to clarify the role of BH_4 as a novel approach in the treatment of endothelial dysfunction and in preventing progression of atherosclerosis in patients with cardiovascular disease.

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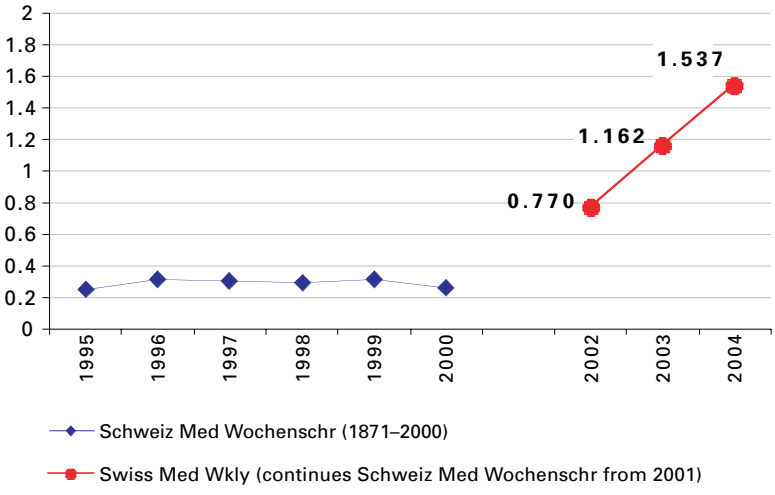

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