Nitric Oxide 64 (2017) 7–15

Contents lists available at ScienceDirect

Nitric Oxide

journal homepage: www.elsevier.com/locate/yniox

Dietary nitrate load lowers blood pressure and renal resistive index in patients with chronic kidney disease: A pilot study

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ARTICLE INFO

Article history:
Received 17 November 2016
Received in revised form
16 January 2017
Accepted 25 January 2017
Available online 28 January 2017

Keywords:
Chronic kidney disease
Dietary nitrate
Beetroot
Renal resistive index
Blood pressure
Cardiovascular disease

ABSTRACT

Beetroot has a high concentration of inorganic nitrate, which can serially reduced to form nitrite and nitric oxide (NO) after oral ingestion. Increased renal resistive index (RRI) measured by Doppler ultrasonography is associated with higher cardiovascular mortality in hypertensive patients with reduced renal function over time defined as chronic kidney disease (CKD). Our aim was to investigate whether the supplementation of dietary nitrate by administration of beetroot juice is able to reduce blood pressure and renal resistive index (RRI) as prognostic markers for cardiovascular mortality in CKD patients.

In a cross-over study design, 17 CKD patients were randomized to either a dietary nitrate load (300 mg) by highly concentrated beetroot juice (BJ) or placebo (water). Hemodynamic parameters as well as plasma nitrate concentration and RRI were measured before and 4 h after treatment.

In this cohort, CKD was mainly caused by hypertensive or diabetic nephropathy. The mean eGFR was 41.6 ± 12.0 ml/min/m^2. Plasma nitrate concentrations were significantly increased after ingestion of BJ compared to control. Peripheral systolic and diastolic blood pressure as well as mean arterial pressure (MAP) were significantly reduced secondary to the dietary nitrate load compared to control (e.g. $\Delta MAP_{BJ} = -8.2 \pm 7.6$ mmHg vs. $\Delta MAP_{control} = -2.2 \pm 6.0$ mmHg, $p = 0.012$). BJ also led to significantly reduced RRI values ($\Delta RRI_{BJ} = -0.03 \pm 0.04$ versus $\Delta RRI_{control} = 0.01 \pm 0.04$; $p = 0.017$). Serum potassium levels were not altered secondary to the treatment.

In this study, administration of the nitrate donor BJ led to significantly reduced RRI values and peripheral blood pressure which might be explained by release of the vasodilatator NO after oral intake. Whether supplementation of dietary nitrate in addition to routine pharmacologic therapy is able to decelerate progression of cardiovascular and renal disease in CKD, remains to be investigated.

1. Introduction

Patients with a reduced glomerular filtration rate (<90 ml/min/m^2) over a period of month or years hereinafter defined as chronic kidney disease (CKD) display a high prevalence of hypertension. Cardiovascular disease is the leading cause for morbidity and mortality in these patients [1,2]. In comparison to healthy controls, patients at all stages of CKD are considered the “highest risk group” for developing cardiovascular diseases [3,4].

Hypertension can be both causal for the development of CKD and a result of the disease [5]. Hypertension and diabetes are the two major causes of CKD and subsequent end stage renal disease [6]. Several other factors are involved in CKD progression, including age, obesity, lower glomerular filtration rate, and albuminuria [6–10]. All these factors also influence the renal resistive index (RRI) [11,12], which reflects changes in intrarenal perfusion/hemodynamics and predicts progression of renal disease in hypertensive as well as diabetic nephropathy [11,13]. The mean reference value for normal RRI in adults is about 0.60 ± 0.10 and values above 0.70 predict poor renal outcome and higher risk for cardiovascular

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http://dx.doi.org/10.1016/j.niox.2017.01.011
1089-8603/

events in hypertensive patients with CKD [14,15]. Furthermore RRI correlates with renal function and renal histopathology such as renal atherosclerosis and tubulointerstitial damage [16–18]. A recently published study in 1962 CKD patients without renal artery stenosis showed that a RRI≥0.70 is associated with a higher mortality in these patients [19].

In healthy subjects a vegetable rich nutrition has been shown to protect from cardiovascular disease and hypertension [20–24]. The strongest reduction of coronary heart disease risk was observed with diets rich in leafy vegetables [20], which have a high content of inorganic nitrate (NO_3^-) [25,26].

After oral ingestion and absorption in the upper intestine, inorganic nitrate is concentrated and secreted into the oral cavity via salivary glands and reduced to nitrite (NO_2^-) by facultative anaerobic bacteria on the dorsal surface of the tongue [27]. After swallowing the nitrite-rich saliva, nitrite is absorbed in the stomach into the circulation where it can be converted to nitric oxide (NO) by different enzymatic systems (various nitric oxide reductase pathways) [28,29]. NO acts as a potent vasodilator by activating guanylyl cyclases (GC) and thereby increasing production of the second messenger cGMP, which among other effects leads to relaxation of vascular smooth muscle cells [30]. The blood pressure lowering effect of nitrate or nitrite from dietary sources can be abolished by an antiseptic mouthwash or as recently found by a proton pump inhibitor [31–33]. This suggests that the bioactivation of nitrite and the metabolization to NO and other bioactive nitrogen species is dependent on an acidic ventricle [33]. Several preclinical studies in murine and rat models revealed protective effects of dietary nitrate and nitrite derived NO in ischemia-reperfusion injury models of brain, heart, kidney, and liver as well as improvement of revascularisation and endothelial dysfunction in chronic ischemia [34–40]. Furthermore, dietary nitrate supplementation prevented proteinuria as well as renal fibrosis and tubular atrophy apart from attenuation of hypertension and cardiac hypertrophy in a rat model of unilateral nephrectomy and chronic high-salt diet. In this study, dietary nitrate also reduced levels of oxidative stress markers [41]. In line with these findings, it was shown that the nitrate- and nitrite-mediated antihypertensive effects were primarily associated with reduction of oxidative stress and vasodilatation of afferent arterioles in a model of renal hypertension. Hereby, NADPH oxidase could be revealed as primary target for the blood pressure lowering effects of inorganic nitrate and nitrite [42].

Larsen et al. demonstrated a lowering effect of diastolic and mean arterial pressure in healthy volunteers with a 3-day dietary supplementation of sodium nitrate compared to placebo [43]. Further clinical investigations by *Webb* et al. [44] and *Kapil* et al. [45,46] demonstrated blood pressure reduction and improvement of platelet and endothelial function in healthy and hypertensive volunteers by elevating nitrite and cGMP levels via dietary nitrate supplementation with beetroot juice (BJ), which is a source of concentrated inorganic nitrate.

Interventional studies investigating dietary nitrate supplementation in CKD patients which have a high prevalence of hypertension and a high risk to develop cardiovascular disease are thus far lacking. In this pilot study we sought to investigate whether the treatment with a single dietary nitrate application via BJ is able to reduce blood pressure and RRI, both prognostic markers for cardiovascular mortality and progression of renal disease in CKD patients.

2. Methods

2.1. Volunteers

The ethics committee of the Technical University of Munich approved the study protocol. The study was performed in concordance with the Declaration of Helsinki. All subjects were recruited from the Nephrology Clinic Weissenburg. Written informed consent was obtained from each patient prior to inclusion. Inclusion criteria were estimated glomerular filtration rate (eGFR) between 15 and 89 ml/min/1.73 m^2 (CKD stage II-IV according to the K/DOQI (kidney outcomes quality initiative) guidelines [47]), independence of dialysis, and hypertension, treated with at least one antihypertensive drug. Patients with renal artery stenosis diagnosed by duplex sonography were not included.

2.2. Study design

Using a randomized open-label cross-over design, 17 patients with CKD due to hypertensive or diabetic nephropathy were examined over a period of 4 h after an ingestion of BJ or placebo. To ensure the availability of equal numbers of individuals in each treatment group, treatments were assigned in an alternate fashion. Randomization was achieved by random assignment of examination time. Nine patients started with intervention, 8 patients with control. 30 g of beetroot powder (Schoenenberger®, Magstadt, Germany) were dispended in 200 ml tap water to achieve a defined nitrate load of around 300 mg. The nitrate content in the beetroot powder was measured by an official analytical laboratory (Institut Kuhlmann, Ludwigshafen) and provided by the manufacturer (Schoenenberger®, Magstadt, Germany). The average content of used batches was 10,125 mg/kg, which equates to an average content of 304 mg nitrate in one glass of BJ. 200 ml of tap water were administered as control. Every patient was examined twice on two different days. At one time point the patient received BJ, at the other time point water as control. The two days of the intervention were separated by a washout period of at least two days. The cross over study design is illustrated in Fig. 1. Blood sampling and measurements of hemodynamic parameters as well as RRI values were performed before and 4 h after intake of dietary nitrate load or water. The duration of 4 h was chosen according to the oral bioavailability of cooked beetroot with a mean maximum plasma concentration at 1.7 h and a plasma half-life of 6.1 h [48]. During the observation time, blood pressure was measured every 15 min. During the observation time patients were allowed to walk, but instructed not to exercise. The degree of activity was not different between the treatment regimes. Participants were allowed to take their routine antihypertensive medication before the examination but not after intake of BJ/control until termination of measurements and sampling of blood after 4 h. Further, patients were held to continue their normal diet at the day of intervention, but instructed to avoid coffee or tea during the 4 h observation timeframe after intervention. Patients were not instructed to avoid food with a moderate or high nitrate content.

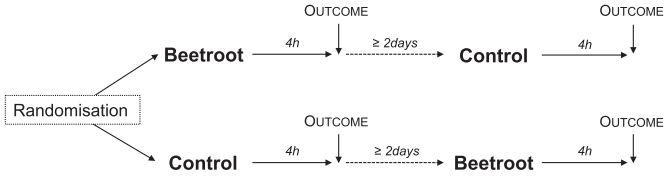

Fig. 1. Study design.