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Review

Microbiota and the nitrogen cycle: Implications in the development and progression of CVD and CKD

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ABSTRACT

Chronic kidney disease (CKD) is associated with an increased risk of death from cardiovascular disease (CVD). One factor involved in CVD development is nitric oxide (NO), which acts as a powerful vasodilator. NO is produced via the nitrogen cycle, through the reduction of nitrate to nitrite with the process mainly occurring in the mouth by commensal microbiota. People with CKD have compromised microbiota (dysbiosis) with an increased abundance of potentially pathogenic and pro-inflammatory bacteria capable of producing uremic toxins that contribute to CKD development and reduce enzymatic NO production. However, to date, few studies have comprehensively documented the gut or saliva microbiota in the CKD population or investigated the role of NO in people with CKD. This review will discuss NO pathways that are linked to the progression of CKD and CVD and therapeutic options for targeting these pathways.

Contents

1. Introduction

People with chronic kidney disease (CKD) have a 2–3-fold greater risk of CVD-related mortality [1,2], and are 20–30 times more likely to die from CVD than experience end-stage kidney failure [3]. The increased CVD risk is only partially explained by traditional cardiovascular risk factors, and there is increasing evidence that non-traditional risk factors (e.g. inflammation, oxidative stress, and endothelial dysfunction) play a key role [4–6].

An important molecule associated with the development and

Abbreviations: CKD, chronic kidney disease; CVD, cardiovascular disease; NO, nitric oxide; eNOS, endothelial nitric oxide synthase; ADMA, asymmetric dimethylarginine; GIT, gastrointestinal tract; TLR, toll-like receptor; NADPH, nicotinamide adenine dinucleotide phosphate; NF, nuclear factor; bNOS, bacterial NOS; DRNA, dissimilatory nitrate reduction to ammonium; N_2O, nitrous oxide; AOB, ammonia-oxidizing bacteria; NOB, nitrite-oxidizing bacteria.

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progression of CVD is nitric oxide (NO). NO is a free radical with important cellular signalling properties involved in many physiological and pathological processes [7,8]. Acting as a powerful vasodilator, NO regulates regional blood flow and relaxation of smooth muscle [8,9]. The primary source of NO is L-arginine, which is converted to NO by endothelial nitric oxide synthase (eNOS) [10]. Under certain circumstances, L-arginine-derived NO production can be inhibited (e.g. by asymmetric dimethylarginine or ADMA), resulting in reduced vasodilation and increased risk of CVD [11]. Fortunately, under these circumstances, there are alternative pathways capable of producing NO. Perhaps the most important of these alternative NO-producing pathways is the nitrogen cycle in which NO is produced during the conversion of nitrate to urea [12] (Fig. 1).

The aim of this review is to provide an overview of the nitrogen cycle as it relates to CVD development in CKD and discuss therapeutic treatments that target the nitrogen cycle. Specifically, this review will discuss the production of NO from nitrite, as well as the associated pathways that have been linked to the progression of CKD and CVD (Fig. 2).

2. Nitrate and nitrite

Both nitrate and nitrite play important roles in the production of NO and are important mediators in the development of CVD. Specifically, low levels of nitrate and nitrite have a strong correlation with increased endothelial dysfunction leading to CVD [13,14]. As it pertains to renal health, this is noteworthy as patients with CKD have a high prevalence of endothelial dysfunction and CVD [2,15,16]. Reduced bioavailability of nitrate and nitrite not only leads to endothelial dysfunction in patients with CKD, but also has been linked to the progression of CKD [17]. Supplementation with nitrite has been shown to increase NO production and reduce endothelial dysfunction in some disease models [18,19]. However, to date studies that have examined the bioavailability or specific role of nitrate and nitrite in patients with CKD are limited.

Nitrite and nitrate act as important products in an NO production pathway, via their ability to be reduced. This may proceed via a bacteria-mediated enzymatic reduction of nitrate (a one-electron reduction of nitrite produces NO) in the mouth [20–22]. This occurs when plasma bound nitrate entering the salivary glands via blood vessels is extracted and commensal bacteria, residing in the mouth, enzymatically reduce nitrate to nitrite (Fig. 3). When the reduced nitrite, residing in the saliva, enters the stomach it rapidly undergoes protonation to form nitrous acid. This then decomposes to form NO and other nitrogen oxides [23,24]. In humans, nitrate levels are generally more than 200 times higher in plasma than saliva (plasma = 20–50 μM; saliva = 10 mM) and nitrite levels more than 1,000 times higher in saliva than plasma (plasma = 50–300 nM; saliva = 1–2 mM) [22,25–27].

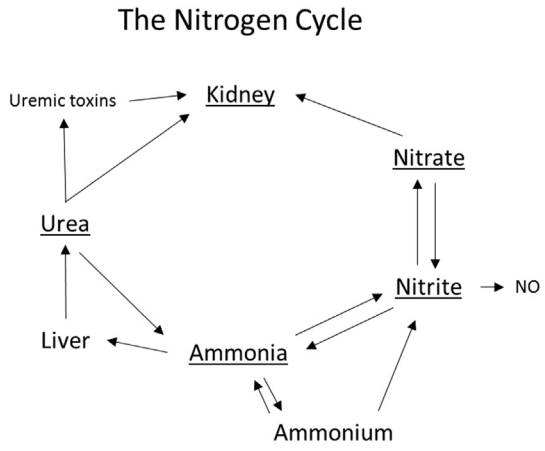

Fig. 1. Illustrates the core of the nitrogen cycle with the pathway consisting of the conversion of nitrate to nitrite (produces NO) to ammonia to urea (via the liver) and the urea converted to uremic toxins or excretion from the kidneys. Under certain circumstances, the whole cycle, or parts of the cycle, can reverse. The direction the pathway and individual compounds take depends on a number of factors including: concentrations, pH, oxygen availability and bacteria. NO – nitric oxide.

Nitrite can be reduced directly to NO and ammonia. Nitrite may also be oxidized to nitrate, at which point nitrate will either be removed or reduced back to nitrite [12,28]. Which pathway nitrate and nitrite follow depend on the physiological conditions of the areas in which they are located. Under low pH and/or anaerobic conditions, the potency of nitrite is increased and preferentially reduced to produce NO which then acts as a vasodilator [29]. Striking a balance between oxidation and reduction is crucial for homeostasis of the system. If the nitrogen cycle indiscriminately favours reduction, a large amount of ammonia may be produced and little nitrite will be available for reduction to NO. If the nitrogen cycle indiscriminately favours oxidation, nitrate may be preferentially removed, leaving low concentrations available for reduction back to nitrite (and eventually to NO). During these unbalanced conditions an important vasodilator, NO, becomes scarce and, consequently, diseases such as CVD may be encouraged to develop and/or progress.

Supplementation with nitrite has been shown to partially compensate for a reduction in NO formation via NOS pathways. Studies have estimated concentrations of NO and haemoglobin-bound NO (HbNO) to be between 1 and 100 μM [30–33] with a maximum formation rate of 100 nM/s [30]. The addition of nitrite to haemocytes has been shown to increase NO formation by 20–30 nM/min [34]. However, there is variability in reported concentrations, largely due to the difficulty in measuring NO and the majority of reported values coming from in-vitro studies. Other factors affecting concentrations are: type of sample (blood, urine, organ tissue), pH, temperature, and method for measurement. Yoshida and colleagues found NO levels to be highest in blood (including serum and red blood cells) and urine, and lowest in the lung, liver, muscle, and kidney [35]. This is likely due to the organ tissue being a site of high NO use. In-vivo studies have shown that supplementation with nitrite increases HbNO concentrations in a dose-dependant manner. For instance, one study showed that rats supplemented with 1, 3, or 10 mg/kg of nitrite all experienced increases in HbNO concentrations. However, the HbNO concentration remained considerably higher, even at the end of the 60-min study period, for the 3 mg/kg (HbNO concentration peaked at 5 min; 10.58 ± 0.40 μM) and 10 mg/kg treatments (HbNO concentration peaked at 15 min; 38.27 ± 9.23 μM), compared to the 1 mg/kg treatment (HbNO concentration peaked at 5 min; 4.93 ± 0.52) [36]. That said, Poderoso and colleagues showed NO concentrations in myocardial tissue are constant at 0.1–0.3 μM [37]. These conflicting results highlight the difficulty in comparing and measuring NO. Nevertheless, both studies support the notion that nitrate/nitrite supplementation can increase NO formation and have a tissue specific effect.

3. Ammonia

Ammonia is continually produced as the body digests protein. Generally, ammonia is converted to the less toxic compound, urea, in the liver and it is removed by the kidneys. When the liver or kidneys are not functioning properly, ammonia or urea can build up leading to disease development [38–40]. Elevated concentrations of ammonia may have further damaging effects on the body by