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Counteraction of oxalate induced nitrosative stress by supplementation of L-arginine, a potent antilithic agent

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Abstract

Background: Our understanding of nitrosative stress in the process of urolithiasis is far from complete. Earlier studies carried out in our laboratory demonstrate the presence of nitrated THP in stone formers, L-arginine (L-arg) a precursor of nitric oxide (NO), attenuates the endothelial dysfunction caused by reactive nitrogen species. We investigated the role of L-arg in ethylene glycol (EG)-induced urolithic rat model and observed its antilithic and antioxidative properties.

Methods: Hyperoxaluria was induced using 0.75% EG in drinking water. L-arg [1.25 g/kg body weight] was given orally for a period of 28 days.

Results: EG-treated rats showed significant loss in body weight and increase in the activities of oxalate synthesizing enzymes such as glycollic acid oxidase in liver. Lactate dehydrogenase activity in liver and kidney was increased. The activity of the free radical producing enzyme xanthine oxidase, tissue oxalate and calcium levels were significantly increased in EG-treated rats. Depletion in the antioxidant enzymes, membrane bound ATPases and thiol status was observed in these rats. L-arg co-supplementation to EG-treated rats maintained the activities of the oxalate synthesizing enzymes and free radical producing enzymes with in the normal range. Tissue oxalate and calcium levels were also maintained near normal in L-arg treated hyperoxaluric rats. L-arg, by its cytoprotective effect, maintained the thiol status, thereby preserving the activities of the membrane bound ATPases and preventing proteinuria and subsequent weight loss in EG-treated rats.

Conclusion: L-arg feeding prevents the retention of calcium oxalate crystals in hyperoxaluric rats by way of protecting the renal cells from oxidative injury and also by providing a second line of defense through the normalization of the oxalate metabolism. It reduces the risk of stone formation, by curtailing free radicals and hyperoxaluria as both of them have to work in close association to form stones.

Keywords: L-arginine; Lipid peroxidation; Oxalate synthesizing enzymes; Antilithic and antioxidant properties

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1. Introduction

The precise sequence of events involved in the formation of calcium oxalate kidney stones is unclear. A number of conditions such as changes in the properties of tubular membrane and altered membrane transport have been suggested for retention of calcium oxalate crystals. Evidences are accumulating for the involvement of renal tubular injury in the pathogenesis of calcium oxalate nephrolithiasis [1,2]. A relationship between crystal deposition in renal tubules and epithelial injury has been demonstrated in experimental urolithiasis. Similarly prior damaging of urothelium by pretreatment with gentamycin [2], and cyclosporine [3] favors the binding of calcium oxalate crystals to the renal cells. Apart from oxidative stress, nitrosative stress is also considered to play an important role in stone formation. However, knowledge regarding nitrosative stress-mediated calcium oxalate crystallization is far from complete. Preliminary studies [4] indicated the presence of nitrated Tamm-Horsfall glycoprotein (THP) in stone formers urine, which shows enough evidence for the role of nitrosative stress in stone formation. So, an agent that can counteract oxidative and nitrosative stress is L-arginine (L-arg) can act as a potent antilithic agent.

L-arg is a semi-essential amino acid present in proteins. In its native form, L-arg has an important role in metabolism. The guanidino group of L-arg is a precursor of the endothelium-derived relaxing factor, nitric oxide (NO) [5], the other metabolites are creatine, agmatine and polyamines [6,7] and is an important intermediate in the urea cycle. It serves as an immunonutrient to improve the immune status in those suffering from sepsis, burns and trauma [8].

L-arg is an amino acid of choice which has shown promising results during treatment of cardiovascular diseases including atherosclerosis, hypertension, angina pectoris, hyperlipidemia, and in some kidney disorders, it is also helpful in accelerating wound healing property [9]. L-arg has been credited for its significant power of reducing lipid peroxidation in patients with diabetes mellitus [10].

The primary objective of this research work was to assess the efficacy of L-arg as an anti-urolithic and antioxidative agent to treat hyperoxaluria.

2. Materials and methods

Adult male albino rats of Wistar strain weighing 120–160 g were used in this study. Twenty four rats were divided into four groups, six rats each; group I served as controls, group II served as drug controls receiving L-arg orally (1.25 g/kg body weight) [11], group III rats were made hyperoxaluric by feeding 0.75% ethylene glycol [12], group IV rats were also induced hyperoxaluria and supplemented with L-arg at the above said concentrations. All the groups were fed with food and water ad libitum for 4 weeks. The animal experiments were conducted according to the ethics and guidelines for the care and use of laboratory animals in our university.

At the end of the experimental period (28th day), the animals were killed by cervical decapitation, kidney and liver were quickly dissected into ice-cold saline. The kidneys were trimmed free of connective tissue and finely minced. A 20% homogenate was prepared in Tris–HCl buffer (0.02 mol/l, pH 7.4) using Potter-Elvehjem homogenizer fitted with a power-driven Teflon pestle.

2.1. Biochemical assays

The total kidney homogenate was used for assaying antioxidant enzymes like superoxide dismutase (SOD) [13], catalase [14] and glutathione peroxidase (GPx) [15] which were assayed. Membrane bound ATPases [16] and activity of oxalate metabolizing enzymes, lactate dehydrogenase (LDH) [17] were assayed both in liver and kidney homogenates, whereas glycolic acid oxidase (GAO) [18] was assayed only in liver. Free radical producing enzyme system, which constitutes the activity of xanthine oxidase (XAN), was assayed both in liver and kidney [19]. Total thiol content was estimated by the method of Sedlack and Lindsay [20]. Tissue oxalate was estimated after acid extraction as described by Hodgkinson and Williams [21]. Calcium was estimated using Perkin-Elmer Flame absorption atomic spectrophotometer. The samples were processed by the method of Mustafa and Medeiros [22]. Protein content of both kidney and liver was determined using Folin's phenol reagent [23].