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Sodium Nitrite-Induced Hypoxic Nephrotoxicity in Adult Rats

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Abstract

Kidney hypoxia plays an important role in the pathogenesis of acute and chronic kidney disease and has pronounced effects on renal function. Ischemic-induced renal tissue hypoxia is thought to be a major component in the development of acute renal failure. The objective of this study was to explore the effects of chemically-induced hypoxia on renal structure.

Methodology: Adult male albino rats, weighing 180 gm to 200 gm were used in this study. The animals were fasted for three hours prior to subcutaneous injection of sodium nitrite (75 mg/kg). One hour after drug administration, rats were decapitated. The kidneys were removed and placed overnight in fixative containing 10% formalin. Paraffin-embedded kidney tissue blocks were cut serially into coronal slices of 5 μ thickness and stained with Hematoxylin and Eosin (H and E) staining. Morphological assessment of injury was determined in the cortex by counting the evidence of glomerular degeneration, tubular dilatation & necrosis, loss of brush border and renal congestion, from 10 high-power (X40) fields/rat.

Results: Numerous kidney glomeruli showed atrophy with dilatation of Bowman's space. Many of the proximal convoluted tubules were dilated and showed epithelial desquamation, degeneration and necrosis. The distal convoluted tubules showed no changes.

Conclusion: Sodium nitrite induced hypoxia results in necrotic changes in the glomeruli and the proximal convoluted tubules in adult rats.

Keywords: Hypoxia; Kidney; Sodium nitrite; Proximal convoluted tubules; Glomerulus; Tubular necrosis

Introduction

Kidney hypoxia plays an important role in the pathogenesis of acute and chronic kidney disease and has pronounced effects on renal function. The kidneys are particularly susceptible to ischemic injury in many clinical conditions such as renal transplantation [1], treatment of suprarenal aneurysms [2], renal artery reconstructions, contrast agent–induced nephropathy [3], cardiac arrest, and shock. Aging [4], diabetes [5], hypertension [6], chronic salt and volume depletion [7], and urinary outflow obstruction are also associated with intensified renal hypoxia. Hypoxia has a significant regulatory impact on cellular functions & gene expression, and is a strong stimulus for angiogenesis and fibrogenesis [8-10], and for in vitro prostaglandin production [11]. Systemic hypoxia causes the release of adenosine from many tissues including the kidney and increases renal sympathetic activity [12,13].

It is recognized that systemic hypoxia can have pronounced effects on renal function. In normal human volunteers and experimental animals, induction of hypoxia has been reported to result in increased [14], decreased [15], and unchanged urine flow [16]. Sodium nitrite is an inorganic salt with wide spread application in food industry as color fixative and preservative for meats and fish [17]. It is consumed in manufacturing of Azo dyes, Nitroso compounds and other organic compounds [18]. Sodium nitrite has many medical applications; it is used as a vasodilator, a bronchial dilator, an intestinal relaxant [19], in post-hemorrhagic cerebral vasospasm and in myocardial infarction [20,21].

The toxic effects of nitrates and nitrites are well documented in mammalians including impairment of reproductive functions [22], hepatotoxicity [23], dysregulation of inflammatory responses and tissue injury [24], growth retardation and endocrine disturbance [25,26]. It inhibits a number of anti-tumor cytotoxic effector cell types as free natural killer cells against pathogens and

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Table 1: Counts of incidence of histological changes in kidney of adult rats after administration of sodium nitrite, expressed as mean numbers ± S. E. M.

Glomerular changes	Tubular dilatation & necrosis	Loss of brush border	Casts in lumen	Congestion
22.67 ± 5.9	76.17 ± 18.32	65.4 ± 10.18	45.83 ± 16.47	27.33 ± 8.18

tumor cells [27].

Sodium nitrite ($NaNO_2$) is commonly used for induction of hypoxia in experimental animal models [28]. It induces chemical hypoxia by reducing oxygen-carrying capacity of the blood with converting hemoglobin to methemoglobin which unlike ferrous form of hemoglobin does not bind oxygen strongly, thus leading to hemic hypoxia. These effects were attributed to excessive free radicals generation and impairment of oxidant/antioxidant balance [28].

In the present study we explored the effects of sodium nitrite-induced hypoxia on the kidney structure in adult rats.

Materials and Methods

Chemicals

All drugs and chemicals used in the present study were of high analytical grade and were obtained from Sigma-Aldrich Co. Sodium nitrite was dissolved in normal saline.

Animals

Adult male albino rats, weighing 180 gm to 200 gm were used in this study. They were fed with a standard laboratory diet and tap water ad libitum and housed in cages. All animals were kept at standardized laboratory conditions (25°C ± 5°C, 55% ± 5% humidity, and a 12 h light/dark cycle). One week after acclimatization, the animals were fasted for three hours prior to administration of sodium nitrite subcutaneously. All experiments were carried out according to recommendations of Experimental Animals Ethics for handling of experimental animals. The dose of sodium nitrite used in the current study matched with those in the literature [28].

Kidney tissue preparation

Animals were divided into two groups and were treated as follows: Group I (n=2 rats): Served as control and received normal saline, Group II (n=6 rats) served as hypoxic rats and received sodium nitrite (75 mg/kg) subcutaneously. One hour after sodium nitrite injection, rats were decapitated. The kidneys were removed and placed overnight in fixative containing 10% formalin. Paraffin-embedded kidney tissue blocks were cut serially into coronal slices of 5 µ thickness and stained with Hematoxylin and Eosin (H and E) staining.

Morphological assessment of injury was determined in the cortex by counting the evidence of glomerular degeneration, tubular dilatation & necrosis, loss of brush border and renal congestion, from 10 high-power (X40) fields/rat. For the diagnosis of tubular necrosis, identification of necrotic nuclear and cytoplasmic debris within tubular lumens was required. Counts of histological changes were expressed as mean numbers ± S. E. M.

Results

Significant degenerative changes were observed in kidney tissues from the rats that received sodium nitrite (Figure 1 and 2). Numerous glomeruli showed reduction of glomerular tuft and dilated Bowman's space. Many of the proximal convoluted tubules were dilated and showed epithelial desquamation, degeneration and necrosis. Their lumen often contained granular eosinophilic material and exfoliated epithelial cells. At places denuded basement membrane was seen.

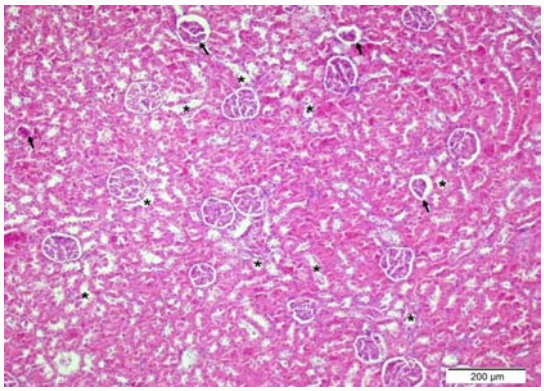

Figure 1: Hypoxic kidney showing necrotic glomeruli (arrows) and dilated proximal convoluted tubule (astrix).

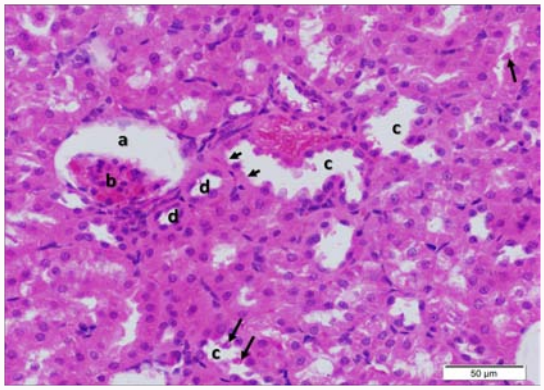

Figure 2: Hypoxic kidney showing a: Dilated Bowman's space. b: Shrunken glomerulus. c: Dilated proximal convoluted tubule showing uncrossed epithelial cells with loss of brush border and denuded basement membrane (short arrows). Long arrows indicate epithelial casts in their lumen. d: Distal convoluted tubules.

Severe congestion was also observed in renal interstitium. The distal convoluted tubules remained unaffected. In the control kidneys none of the above reported changes were seen.

Discussion

Results of this study showed that sodium nitrite induces hypoxia results in severe damage to glomeruli and the proximal convoluted tubules (Table 1). The adverse effects of nitrite on kidney might be due to Nitric Oxide (NO) formations which causes kidney dysfunctions or could be attributed to oxidation of important iron containing enzymes such as the cytochromes responsible for cellular respiration and other oxidation reduction processes where oxidation of hemoglobin to methemoglobin induced cell hypoxia and cell injury [29-31].

The kidneys are second only to the heart in terms of O_2 consumption. Relative to other organs, the kidneys receive a very high blood flow and oxygen extraction, but are particularly susceptible to hypoxic injury. Hypoxia indicates a state in which oxygenation is reduced below the critical PO2, the level of oxygen required for complete oxidation of cytochrome C. This value for

proximal tubules is 10 mmHg to 17 mmHg [32,33]. Failure of cells to maintain ATP levels is the hallmark of ischemia and underlies most of the changes in cellular cation balance and metabolism which characterize that state. Oxygen deprivation most directly limits mitochondrial ATP production, although subsequent changes of cell pH can ultimately reduce glycolysis as well [34]. Thus, cells with the greatest dependence on mitochondrial ATP production, such as the proximal tubule [35,36], will generally be more susceptible to oxygen deprivation-induced ATP depletion. A very early event during *in vivo* experimental ischemic acute renal failure is a rapid decrease in ATP to <10% to 15% of control values [37,38]. Proximal tubules have little capacity for glycolysis, as evidenced by their failure to produce lactate either under control conditions or in the presence of blockade of oxidative phosphorylation with antimycin A [36]. Structurally, ischemic injury leads to extensive disruption of the apical surface of proximal tubule cells [39,40]. It has been shown in earlier studies that the predominant site of ischemic renal damage in humans, experimental animals and isolated kidneys perfused with an erythrocyte-containing medium, is the proximal tubule [41,42]. In experimental models of unilateral or bilateral renal ischemia, induced either by transient, complete cessation of renal blood flow or partial reduction of blood flow [41,43-45], cell injury and necrosis has been uniformly shown to be more severe in proximal than distal tubules.

In anoxic cell injury, certain common pathways leading to cell death have been proposed, including calcium influx, lipid peroxidation, and energy depletion [46]. Cell types, however, differ in their response to O_2 deprivations. It is generally assumed that these variations are related to differences in cellular metabolic rate. In the kidney, different segments of the nephron exhibit different metabolic rates as well as different types of metabolism [35]. Hypoxic injury evaluated morphologically in isolated rat kidneys perfused for 90 min without O_2 or with various metabolic inhibitors showed that the proximal tubule and the thick ascending limb have markedly different responses to cellular energy depletion, suggesting disparate mechanisms for hypoxic injury along the nephron [47]. It has been shown that the initial segment of the rat proximal tubule is highly vulnerable to ischemic damage [48].

Moreover renal ischemia triggers the activation of multiple mechanisms leading to (micro) vascular dysfunction. These mechanisms include functional endothelial dysfunction. The endothelial cells differ in structure and function at different sites of the vascular tree and demonstrate diverse responses to hypoxic stimuli [49]. Because of this endothelial heterogeneity and the broad range of tissue O_2 pressures throughout the kidney, endothelial cells in different regions of the kidney will likely show different levels of susceptibility to ischemic or hypoxic injury [50].

Conclusion

The glomeruli and the proximal convoluted tubules of rat kidney show a high degree of vulnerability towards sodium nitrite-induced hypoxic damage.

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