Increased Expression of Endothelin-1 and CYP11B2 in Gentamicin-Induced Nephropathy in Rat Kidney

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Purpose: An altered activity of vasoactive hormones as well as aldosterone synthase (CYP11B2) in the kidney may involve the pathogenesis of gentamicin-induced nephropathy. The present study was designed to investigate whether there are changes of local renin-angiotensin-aldosterone system (RAAS) and endothelin (ET) in the kidney of gentamicin-induced nephropathy in rats.

Methods: Male Sprague-Dawley rats (180-200 g) were intramuscularly injected with gentamicin (100 mg/kg per day) for 5 days. Vehicle was given for the control rats. The mRNA expression of local renin-angiotensin system, aldosterone synthase (CYP11B2), ET system and transforming growth factor-β 1 (TGF-β 1) was determined in the kidney by real-time polymerase chain reaction. The protein expression of TGF-β in the kidney was determined by immunoblotting and immunohistochemistry.

Results: Following the gentamicin treatment, a renal failure was noted as evidenced by increased serum concentrations of creatinine along with a decrease of its clearance. TGF-β 1 expression was significantly increased in the kidney in gentamicin treated rats compared with that in controls. The abundance of ET-1 mRNA was significantly increased. The endothelin type A receptor expression was decreased while endothelin type B receptor was not changed. The expression of angiotensin converting enzyme 1 (ACE1) and ACE2 was decreased, whereas renin expression was not changed. The CYP11B2 expression was significantly increased in gentamicin treated rats, while mineralocorticoid receptor expression was not changed.

Conclusion: The expression of ET-1 and CYP11B2 was up-regulated which may play a role in the pathogenesis of gentamicin-induced nephropathy.

Key Words: Gentamicin, Endothelin, Aldosterone synthase, Transforming growth factor-β 1

Introduction

Aminoglycoside antibiotics including gentamicin are widely used in the treatment of gram negative infections. One of its main side-effects is nephrotoxicity, manifested by nonoliguric renal failure with a progressive increase of serum creatinine levels. The specificity of gentamicin for renal toxicity is apparently related to its preferential accumulation in the renal proximal convoluted tubules1,2. The animal models of aminoglycoside nephrotoxicity show acute tubular necrosis, interstitial fibrosis in the renal cortex as well as glomerulosclerosis3-5. However, its underlying molecular mechanisms remain to be further elucidated.

Progressive kidney disease is characterized by the
accumulation of fibrotic molecules in the kidney. Angiotensin II (Ang II) plays a crucial role in mediating the up-regulation of fibrogenic factors, like transforming growth factor-β1 (TGF-β1), in various kidney diseases. Although most previous works have focused on the role of Ang II in the development of progressive renal injury, the classical view of the renin–angiotensin–aldosterone system (RAAS) has been challenged by the discovery of angiotensin converting enzyme 2 (ACE2) and aldosterone synthase (CYP11B2). ACE2 enzymatic activities include the degradation of both angiotensin I and Ang II, with the subsequent formation of Ang-(1–7), which is known to have biological effects opposite to those of Ang II.

CYP11B2 is the enzyme responsible for aldosterone synthesis mainly in the adrenal gland, but recently it is reported that CYP11B2 is expressed in kidney and it can produce aldosterone locally. Importantly aldosterone also stimulates cellular hypertrophy, matrix formation and fibrosis, hence it may play an important role in renal fibrosis. However, the role of locally produced aldosterone in the kidney is not clear in gentamicin induced nephropathy and whether the activated local aldosterone production may contribute to the progressive renal injury remains to be further elucidated.

Gentamicin–induced acute renal failure is characterized by a decrease in renal plasma flow and creatinine clearance. This effect seems to be mediated by vasoactive substances. The endothelin (ET) family of peptides are potent vasoconstrictor and vasopressor agents which may play a role in impaired renal function. Experimental studies demonstrated that endothelin gene and/or protein expression is increased during nephropathy and colocalized with fibrotic lesions. Inversely, pharmacological antagonism of endothelin receptors delayed the evolution of progressive renal disease. These effects, together with the capability of ET to induce contraction and proliferation of mesangial cells as well as accumulation of mesangial matrix proteins, have suggested that ET may participate in the renal events that lead to renal disease progression.

In this context, an altered regulation of vasoactive hormones as well as CYP11B2 in the kidney may be involved in the pathogenesis of GM–induced nephropathy. The present study was aimed to determine whether there is an altered regulation of ET and RAAS systems in the kidney of GM–treated rats.

Materials and Methods

1. Animals

Male Sprague–Dawley rats weighing 180 to 200 g were used. They (n=6) were treated with gentamicin (GM, 100 mg/kg per day, intramuscularly) for 5 days. Control rats (n=5) were injected with saline. They were maintained in metabolic cages to allow urine collections. On the experimental day, the rats were decapitated under a conscious state. The kidneys were taken and kept at -70°C until assayed for the immunoblotting and real–time PCR. The experimental procedure confirmed to the Institutional Guidelines for Experimental Animal Care and Use.

Another set of animal experiment had been done for the immunohistochemistry. Rats were anesthetized with ketamine and a large laparotomy was made. The kidneys were fixed by retrograde perfusion as described below.

2. Isolation of total RNA

Cortex was homogenized in Trizol reagent (Invitrogen, Carlsbad, CA, USA). RNA was extracted with chloroform, precipitated with isopropanol, washed with 75% ethanol, and then redissolved in distilled water. The RNA concentration was determined by the absorbance read at 260 nm (Ultraspec 2000; Pharmacia Biotech, Cambridge, UK).

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