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Inhibition of cystathionine gamma-lyase and the biosynthesis of endogenous hydrogen sulphide ameliorates gentamicin-induced nephrotoxicity

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ABSTRACT

Clinical use of gentamicin over prolonged periods is limited because of dose- and time-dependent nephrotoxicity. Primarily, lysosomal phospholipidosis, intracellular oxidative stress and heightened inflammation have been implicated. Hydrogen sulphide is an endogenously produced signal transduction molecule with strong anti-inflammatory, anti-apoptotic and cytoprotective properties. In several models of inflammatory disease however, tissue damage has been associated with increased activity of cystathionine gamma-lyase, biosynthesis of hydrogen sulphide and activation of leukocytes. The aim of this study was to determine effects of inhibiting hydrogen sulphide biosynthesis by DL-propargyl glycine (an irreversible inhibitor of cystathionine gamma-lyase) on inflammation, necrosis and renal function, following treatment with gentamicin in rats. Adult female Sprague–Dawley rats were divided into six groups and treated with; physiological saline, sodium hydrosulphide, DL-propargyl glycine, gentamicin, a combination of gentamicin and sodium hydrosulphide, or gentamicin and DL-propargyl glycine respectively. Gentamicin-induced histopathological changes including inflammatory cell infiltration and tubular necrosis were attenuated by co-administering gentamicin with DL-propargyl glycine ($P<0.05$ compared to saline controls and $P<0.05$ compared to gentamicin only). Similarly, DL-propargyl glycine caused a significant reduction ($P<0.05$) in lipid peroxidation, production of superoxide and the activation of tumour necrosis factor-alpha in gentamicin-treated animals. These data show that protective effects of DL-propargyl glycine might be related at least in part, to the reduced inflammatory responses observed in animals treated with both gentamicin and DL-propargyl glycine. Thus, enzyme systems involved in hydrogen sulphide biosynthesis may offer a viable therapeutic target in alleviating the nephrotoxic effects of gentamicin.

1. Introduction

Gentamicin is an efficacious and commonly used antibiotic especially against infectious Gram negative bacteria. However, clinical use of gentamicin over prolonged periods is associated with severe nephrotoxicity (Balakumar et al., 2010). Biological processes that trigger these effects have been well characterised. Primarily, lysosomal phospholipidosis, generation of intracellular reactive oxygen species, heightened inflammatory reactions; caspase-mediated apoptosis and necrosis have been implicated (Balakumar et al., 2010; Cuzzocrea et al., 2002; Lopez-Novoa et al., 2011). Collectively, these processes lead to renal damage characterised by extensive tubular epithelial cell vacuolisation and desquamation, degeneration and necrosis of tubular basement membranes, luminal congestion with loss of brush borders, enlarged Bowman's cavities, presence of intra-tubular protein casts, marked proteinuria and an increase in polymorphonuclear inflammatory cell infiltration (Ali, 1995; Martinez-Salgado et al., 1997). Accordingly, there is marked reduction in glomerular filtration rate and renal function overall.

Hydrogen sulphide is a naturally occurring water soluble gaseous molecule that is also synthesised endogenously in mammalian tissue from pyridoxal 5′-phosphate dependent metabolism of L-cysteine by either cystathionine beta-synthase predominantly expressed in the brain or cystathionine gamma-lyase expressed in the liver, kidney and cardiovascular tissue (Tripatara et al., 2009; Wang, 2002). Hydrogen sulphide has been identified as another gaseous signalling molecule with strong anti-inflammatory and anti-apoptotic, cytoprotective properties (Li and Moore, 2007; Wallace, 2007; Wang, 2003). In the heart for example, hydrogen sulphide activates cytoprotective redox sensitive, signalling pathways including extracellular regulated kinase and protein kinase B, and this contributes to preservation of mitochondrial function during ischemia–reperfusion injury (Elrod et al., 2007; Geng et al., 2004). Similarly, the role of hydrogen sulphide as a protective molecule against arterial and pulmonary hypertension, erectile dysfunction, colitis, irritable bowel syndrome and, neuronal damage-induced febrile seizures has been well documented. These studies have been reviewed elsewhere

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(Fiorucci et al., 2005; Li and Moore, 2007). By contrast however, other research efforts point to paradoxical effects of hydrogen sulphide. In several models of inflammation including ethanol-induced gastric damage, cisplatin-induced nephrotoxicity, caerulein-induced acute pancreatitis and lipopolysaccharide-induced endotoxic shock, tissue damage was associated with increased expression of cystathionine gamma-lyase and the biosynthesis of hydrogen sulphide (Chavez-Pina et al., 2010; Collin et al., 2005; Francescato et al., 2011; Li et al., 2005). Several signal transduction mechanisms have been advanced to explain the detrimental effects of hydrogen sulphide (Li et al., 2011). Perhaps more interesting is the fact that hydrogen sulphide activates leukocyte activity via the expression of neutrophil adhesion molecules in inflammatory reactions (Dal-Secco et al., 2008; Tamizhselvi et al., 2010). It has also been established that hydrogen sulphide promotes the formation of proinflammatory cytokines such as tumour necrosis factor-alpha, by upregulating nuclear factor kappa-light-chain-enhancer of activated B cells (Zhi et al., 2007). In the current study, we hypothesised that hydrogen sulphide aggravates gentamicin-induced renal toxicity. Our aim was to determine effects of the inhibition of hydrogen sulphide synthesis by DL-propargyl glycine on inflammation, necrosis and renal function, following a prolonged, 10-day administration of gentamicin in rats.

2. Materials and methods

2.1. Chemicals and bioassay kits

Chemicals and other reagents including gentamicin sulphate, sodium hydrosulphide, disodium hydrogen phosphate, potassium dihydrogen phosphate, L-cysteine, pyridoxal 5′-phosphate, zinc acetate, trichloroacetic acid, *N*,*N*-dimethyl-p-phenylenediamine sulphate, DL-propargyl glycine, hydrochloric acid, ferric chloride, bovine serum albumin, superoxide dismutase, cytochrome c and the caspase-3 colorimetric assay kit were all analytical grade reagents purchased from Sigma Chemical Company (St. Louis, MO, USA). Bioassay kits for quantification of serum creatinine (Quantichrom™ DICT-500), total blood urea nitrogen (Quantichrom™ DIUR-500) and thiobarbituric acid reactive substances (Quantichrom™ DTBA-100) were purchased from BioCore Pty, NSW, Australia. Tumour necrosis factor-alpha colorimetric assay kit was purchased from R&D Systems Inc., USA. Reagents for quantification of protein in biological samples were obtained from Bio-Rad laboratories Pty, NSW, Australia.

2.2. Experimental animals

A rodent model of gentamicin-induced nephrotoxicity was used. Adult female Sprague–Dawley rats (12 weeks, weighing 200–260 g) were obtained from the James Cook University Breeding Colony. The animals were housed at constant room temperature (22 °C) with a 12-hour light/dark cycle and allowed free access to tap water and standard rat chow in the School of Veterinary and Biomedical Sciences animal facility. All the experimental procedures were approved by James Cook University Animal Ethics Committee according to Australian guidelines for the care and use of laboratory animals (Ethics approval number A1582).

2.3. Experimental design, animal tissues and other biological samples

Adult female Sprague–Dawley rats were randomly divided into six groups of 6–8 animals and each group received different treatment as outlined below.

Group 1 Normal control, treated with 0.5 ml of normal physiological saline intraperitoneally.

Group 2 Treated intraperitoneally, with sodium hydrosulphide (a hydrogen sulphide donor) dissolved in physiological saline (50 μmol/kg/day) for 10 successive days.

Group 3 Treated intraperitoneally, with DL-propargyl glycine (an irreversible cystathionine gamma-lyase inhibitor) dissolved in physiological saline (25 mg/kg/day) for 10 successive days.

Group 4 Treated intraperitoneally, with gentamicin sulphate dissolved in deionized water (100 mg/kg/day) for 10 successive days.

Group 5 Treated intraperitoneally, with gentamicin sulphate dissolved in deionized water (100 mg/kg/day) and sodium hydrosulphide (50 μmol/kg/day) for 10 successive days.

Group 6 Treated intraperitoneally, with gentamicin sulphate dissolved in deionized water (100 mg/kg/day) and DL-propargyl glycine (25 mg/kg/day) for 10 successive days.

After 10 days of consecutive treatment, animals were humanely euthanized using carbon dioxide gas in an air-tight chamber. Whole blood samples were collected to obtain serum for the quantification of creatinine, total blood urea nitrogen and other biomarkers of oxidative stress. Approximately 2 ml of whole blood was obtained from the left ventricle via a cardiac puncture by using a 25-gauge needle. Blood samples were transferred to fresh 2 ml Eppendorf tubes without an anticoagulant and then kept at room temperature for about 20 min to allow the blood to clot. Clotted blood samples were centrifuged at 750 × *g* force, for 10 min at room temperature. Serum was extracted for immediate use or storage at −20 °C until use. The right kidneys, a piece of liver and heart tissues were rapidly excised, briefly rinsed in ice-cold physiological saline and then stored separately in cryopreservation vials at −20 °C until being utilised for biochemical analyses. Simultaneously, the left kidney, a separate piece of liver and heart tissue were also collected and stored in 10% neutral buffered formalin for histological and morphometric assessments.

2.4. Biochemical parameters

2.4.1. Quantification of serum creatinine and blood urea nitrogen

Serum creatinine (μmol/l) was quantified by using the QuantiChrom™ Creatinine assay kit (DICT-500; BioCore Pty, NSW, Australia) based on a modification of the Jaffe kinetic method (Rajs and Mayer, 1992). This method utilises picrate which forms a red complex with creatinine in alkaline media. A rate measurement at 510 nm was made over 10 min intervals to determine creatinine concentration in serum samples according to the kit manufacturer's instructions. Total blood urea nitrogen (mmol/l) was quantified by a modification of the method previously described by Jung et al. (1975). Briefly, the reaction involves condensation of urea with *o*-phthalaldehyde to form a red complex, 1,3-dihydroxyisoindoline and absorbance was read at 520 nm using a colorimetric diagnostic kit (DIUR-500; BioCore Pty, NSW, Australia).

2.4.2. Quantification of the glomerular filtration rate

Glomerular filtration rate was calculated using a method described by (Moorhead et al., 2011). Traditionally, glomerular filtration rate is calculated based on urine and serum creatinine concentrations, and total urine volume production over a 24 hour period. Due to a limitation in the availability of metabolic cages for rats, total urine formation over a 24 hour period was not determined in the current study. In light of these difficulties, Moorhead et al. (2011) demonstrated that in rats, glomerular filtration rate can be evaluated rather accurately based on serum creatinine concentration and body mass. It is argued that glomerular filtration rate is proportional to the ratio of body mass and serum creatinine with a correlation coefficient $r^2 = 0.94$. The relationship between glomerular filtration rate, serum creatinine concentration (μmol/l), and body weight (kg) is outlined in Eq. (1) below.

$$\mathrm{GFR(ml/min)} = 220 \times 10^{-3}(\mu\mathrm{mol/min/kg}) * \mathrm{Body\ weight(kg)} * [\mathrm{Cr}(\mu\mathrm{mol/l})]^{-1} \quad (1)$$