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Diallyl sulfide enhances antioxidants and inhibits inflammation through the activation of Nrf2 against gentamicin-induced nephrotoxicity in Wistar rats

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ABSTRACT

The protective role of diallyl sulfide (DAS) in attenuating gentamicin-induced nephrotoxicity has been reported earlier. However, the mechanism of induction of antioxidants by DAS in nephrotoxicity remains elusive. This study is aimed to elucidate the role of a transcription factor, Nuclear factor E2-related factor 2 (Nrf2) in inducing antioxidants and phase II enzymes during gentamicin toxicity in Wistar rats. DAS was administered intraperitoneally at a dosage of 150 mg/kg body weight once daily for 6 days. Gentamicin was administered intraperitoneally at a dosage of 100 mg/kg body weight, once daily for 6 days. Gentamicin-induced rats showed a significant increase in the levels of kidney markers and the activities of urinary marker enzymes, which was reversed upon treatment with DAS. A significant increase in kidney myeloperoxidase (MPO) and lipid peroxidation (LPO) levels was observed in gentamicin-induced rats, which was reduced upon treatment with DAS. Gentamicin-induced rats also showed a significant decrease in the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST) and quinone reductase (QR) in rat kidney, which was increased upon treatment with DAS. Immunohistochemical studies in gentamicin-induced rats demonstrated a marked increase in the immunoreactivity of inducible nitric oxide synthase (iNOS), nuclear transcription factor (NF-κB) and tumor necrosis factor alpha (TNF-α) that were reduced after treatment with DAS. Further, the involvement of Nrf2 in antioxidant induction was analyzed by Western blot and immunofluorescence. To conclude, DAS enhances antioxidants and suppresses inflammatory cytokines through the activation of Nrf2, thereby protecting the cell against oxidative stress induced by gentamicin.

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1. Introduction

Gentamicin, an aminoglycosidic antibiotic is widely used to treat various gram negative infections (Appel, 1990). However, treatment schedule induces nephrotoxicity, which accounts for 10–20% cases of acute renal failure (Erdem et al., 2000). Gentamicin administration into rats provides an excellent model of acute renal failure for studying the therapeutic potential of different drugs (Ali, 1995). In gentamicin-induced nephrotoxicity, reactive oxygen species (ROS) is believed to play a pivotal role in cellular damage and necrosis via several complex mechanisms including peroxidation of membrane lipids, protein denaturation and DNA damage (Parlakpinar et al., 2005). It was reported that gentamicin acts as an iron chelator, and that the iron–gentamicin complex is a potent catalyst of free radical formation (Yanagida et al., 2004). Accordingly, the administration of several compounds with antioxidant activity has been successfully used to prevent or ameliorate gentamicin-induced nephrotoxicity (Karahan et al., 2005; Cuzzocrea et al., 2002).

The transcription factor NF-κB regulates the expression of genes which are involved in inflammation, cell proliferation and apoptosis (Martindale and Holbrook, 2002). It is known that NF-κB is present in an inactive form, in the cytoplasm of all cells, regardless of cell type (Guijarro and Egido, 2001). Upon stimulation, NF-κB is released from an inhibitory subunit (IκB) and translocates into the nucleus, where it promotes the transcriptional activation of target genes (Terquankar, 2006). TNF-α is a potent proinflammatory cytokine, which is produced by many cell types including macrophages, renal mesangial and epithelial cells (Baud and Ardaillou 1994). TNF-α can control progression of diseases and reduce the production of other cytokines mediating inflammation.

Phase II enzymes such as glutathione-S-transferase and quinone reductase play important roles in protecting cells against oxidative stress imposed by reactive oxygen species and toxins. The induction of these enzymes is widely accepted as an efficient strategy for reducing the risk of diseases related to exposure of toxins, mutagens and carcinogens (Talalay, 2000; Zhang and Gordon, 2004). Nuclear

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factor E2-related factor 2 (Nrf2) is a member of the cap "n" collar basic region-leucine zipper (CNC bZip) transcription factors that defend against a range of toxicants. Nrf2 plays a critical role by binding to the antioxidant response element in the promoter region of a number of genes, encoding for antioxidative and phase II enzymes in several types of cells and tissues (Nguyen et al., 2003; Lee and Johnson, 2004). The importance of Nrf2, as a multiorgan protector has been extensively reviewed (Lee et al., 2005). Under the basal condition, Nrf2-dependent transcription is repressed by a negative regulator Keap1. When cells are exposed to oxidative stress, electrophiles, or chemopreventive agents, Nrf2 escapes Keap1-mediated repression and activates antioxidant responsive element (ARE)-dependent gene expression to maintain cellular redox homeostasis (Zhang, 2006).

Diallyl sulfide (DAS) is an organo sulfur component of garlic, very well known for its antioxidant property (Grudzinski et al., 2001; Yin et al., 2002). Recently, we have reported the anti-proliferative, anti-inflammatory and anti-fibrotic effects of DAS in various experimental systems (Sriram et al., 2008; Kalayarasan et al., 2008a,b). There are also few reports on the ameliorating effect of DAS against gentamicin-induced nephrotoxicity (Pedraza-Chaverri et al., 2003; Ali, 2003). However, the mechanism of the induction of antioxidant enzymes mediated by Nrf2 during gentamicin-induced renal damage is obscure. To our understanding, the role of garlic or its derivative, DAS in influencing Nrf2 to enhance antioxidants in nephrotoxicity is not known. In this study, we present evidence for the first time that Nrf2 protects cells against oxidative stress induced by gentamicin through co-ordinatively controlling the induction of antioxidant and phase II enzymes.

2. Materials and methods

2.1. Chemicals

DAS was obtained from Sigma chemical Co. (St. Louis, MO, USA). Gentamicin was procured from Ranbaxy laboratories, Mumbai, India. Rabbit polyclonal NF-κB p65, iNOS, and goat polyclonal TNF-α used were from Santa Cruz Biotech, USA. Rabbit polyclonal Nrf2 was a gift from Dr. Xiaolan Zhang, Ohio State University Medical Center, USA. Secondary antibodies were purchased from Bangalore Genei, India. All other reagents used were of analytical grade.

2.2. Animals

Wistar male albino rats weighing between 150 g and 200 g were housed in animal cages with food and water *ad libitum*. The animals were housed 6 rats per cage, and maintained on a 12 h day and night cycle. The animals were fed with commercial pellet diet (Hindustan lever Ltd., Bangalore, India). The experiments involved with animals were conducted according to the ethical norms approved by the Ministry of social justices and empowerment, Government of India and Institutional animal ethics committee guidelines (Approval no. 360/01/a/CPCSEA).

2.3. Experimental design

The animals were randomly divided into four groups containing six rats in each group. Gentamicin was injected to animals intraperitoneally at a dose of 100 mg/kg body weight, for six consecutive days, which is well known to cause significant nephrotoxicity in rats (Cuzzocrea et al., 2002). DAS was suspended in corn oil and administered to animals intraperitoneally at 150 mg kg^{-1} body weight (based on effective dosage fixation studies).

Group I control animals received corn oil (2 ml/kg body weight) as a vehicle by intraperitoneal (*i.p.*) injection for 6 days. Group II animals received gentamicin (100 mg/kg body weight) for a period of 6 successive days. Group III animals received DAS (150 mg/kg body weight, *i.p.*) for 6 days concomitant with gentamicin administration as in group II. Group IV animals received DAS alone at a concentration of 150 mg/kg body weight for 6 days.

2.4. Preparation of kidney tissue

After the last dose of gentamicin, all the animals were immediately kept in individual metabolic cages to collect urine for the estimation of renal function. The animals were sacrificed by decapitation and the blood samples were drawn by cardiac puncture and centrifuged to harvest the serum with which the renal function assessment parameters were analyzed. Then kidney tissues were excised immediately and rinsed in ice-cold physiological saline and homogenized in 0.1 M Tris–HCl buffer (pH 7.4). The resultant tissue homogenate was used for biochemical measurements. A section of the kidney was set aside for histological studies, immunohistochemical and immunofluorescent studies.

2.5. Antioxidant enzymes

Antioxidant enzymes such as superoxide dismutase (Misra and Fridovich, 1972), Catalase (Takahara et al., 1960), glutathione peroxidase (Rotruck et al., 1973) and glutathione reductase (Staal et al., 1969) were estimated using tissue homogenate. Total protein content in the tissue homogenate was measured by the method of Lowry et al. (1951). The kidney tissue homogenate was also used to assay the following parameters such as reduced glutathione (Ellman, 1959), Vitamin C (Omaye et al., 1979) and Vitamin E (Desai, 1984).

2.6. Kidney tissue myeloperoxidase, lipid peroxidation and reactive oxygen species

Kidney tissue myeloperoxidase activity was assayed using 4-aminoantipyrine/phenol solution as the substrate for myeloperoxidase-mediated oxidation by H_2O_2, and changes in absorbance at 510 nm were recorded (Wei and Frenkel, 1993). The tissue homogenate was used for the lipid peroxidation estimation, which was done by measuring the formation of thiobarbituric reactive substances (TBARS) according to the method of Ohkawa et al. (1979). Hydroperoxides generation was assessed by the spectrophotometric method of Pick and Keisari (1981). HOP production was quantified by the method of Puntarulo and Cederbaum (1988).

2.7. Renal function assessment

The levels of urea, uric acid and creatinine were assessed in the serum of control and experimental animals. Urea was estimated by the method of Natelson et al. (1951). The method of Caraway (1963) was followed for the estimation of uric acid. Creatinine content was determined by the method of Owen et al. (1954). γ-glutamyl transpeptidase (γ-GT) activity was determined by the method of Orlowski and Meister (1965). N-acetyl-β-D-glucosaminidase (NAG) was estimated by the method of Maruhn (1976) using p-Nitrophenyl β-D-glucosaminide as the substrate. β-glucuronidase was estimated by the method of Kawai and Anno (1971).

2.8. Phase II enzymes

The activity of glutathione-S-transferase was determined by using 1-chloro-2,4, dinitrobenzene (CDNB) as the substrate. The reaction mixture contained 1 mM of CDNB, 1 mM reduced glutathione in 0.1 M phosphate buffer (pH 6.5). The formation of the reduced glutathione–CDNB conjugate was measured spectrophotometrically at 340 nm with CDNB as substrate according to Habig et al. (1974). Quinone reductase (QR) activity was determined as described by Benson et al. (1980).

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