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Protective effect of allicin against gentamicin-induced nephrotoxicity in rats

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ABSTRACT

In this study, the modulator effect of allicin on the oxidative nephrotoxicity of gentamicin in the kidneys of rats was investigated by determining indices of lipid peroxidation and the activities of antioxidant enzymes, as well as by histological analyses. Furthermore, the effect of allicin on gentamicin induced hypersensitivity of urinary bladder rings to ACh was estimated. Twenty-four male Wistar albino rats were randomly divided into three groups, control, gentamicin (100 mg/kg, i.p.) and gentamicin + allicin (50 mg/kg, orally). At the end of the study, all rats were sacrificed and then urine, blood samples and kidneys were taken. Gentamicin administration caused a severe nephrotoxicity as evidenced by an elevated kidney/body weight ratio, serum creatinine, blood urea nitrogen (BUN), serum lactate dehydrogenase (LDH) and proteinuria with a reduction in serum albumin and creatinine clearance as compared with control group. In addition, a significant increase in renal contents of malondialdehyde (MDA), myeloperoxidase (MPO), nitric oxide (NOx) and tumor necrosis factor-alpha (TNF-α) concomitantly with a significant decrease in renal reduced glutathione (GSH) and superoxide dismutase (SOD) activities was detected upon gentamicin injection. Exposure to gentamicin increased the sensitivity of isolated urinary bladder rings to ACh and induced acute renal tubular epithelial cells necrosis. Administration of allicin significantly decreased kidney/body weight ratio, serum creatinine, LDH, renal MDA, MPO, NOx and TNF-α while it significantly increased creatinine clearance, renal GSH content and renal SOD activity when compared to gentamicin-treated group. Additionally, allicin significantly reduced the responses of isolated bladder rings to ACh and ameliorated tissue morphology as evidenced by histological evaluation. Our study indicates that allicin exerted protection against structural and functional damage induced by gentamicin possibly due to its antioxidant, anti-inflammatory and immunomodulatory properties in addition to its ability to retaining nitric oxide level.

1. Introduction

Gentamicin (GNT) is commonly applied in human clinical practices for treatment of life-threatening Gram-negative infections [1,2]. However, the usefulness of GNT is limited by the development of nephrotoxicity. In some cases, this side effect is so severe that the use of the drug must be discontinued. In spite of the introduction of newer and less toxic antibiotics, GNT is still used clinically because of its rapid bactericidal action, broad-spectrum activity, chemical stability, and low cost [3,4]. GNT-induced nephrotoxicity is characterized by direct tubular necrosis, without morphological changes in glomerular structures [1]. The mechanism of gentamicin-induced nephrotoxicity is not completely known. However, studies have implicated reactive oxygen species particularly superoxide anion radical in the pathophysiology of gentamicin nephropathy [5]. It has been demonstrated that gentamicin administration increases renal cortical lipid peroxidation, nitric oxide generation and mitochondria hydrogen peroxide production [1,6,7,8]. Abnormal production of such molecules may damage macromolecules, induce cellular injury and necrosis via several mechanisms including peroxidation, protein denaturation and DNA damage [6,9]. The alteration in kidney functions induced by lipid peroxidation is a proximal event in the injury cascade of gentamicin mediated nephrotoxicity [1]. Accordingly, the administration of compounds with antioxidant activity has been successfully used to prevent or ameliorate gentamicin-induced nephrotoxicity [1,5].

In the past few years, much interest has been laid on the role of naturally occurring dietary substances for the control and management of various chronic diseases [10]. Allicin (diallyl thiosulfinate), the major pharmacological component of garlic [11], has attracted attention of the international medical field gradually due to its potential for disease prevention and treatment. It is formed by the action of the enzyme alliinase on alliin in crushed fresh garlic cloves. It possesses antioxidant activity and is shown to cause a variety of actions potentially useful for human health. Allicin exhibits hypolipidemic, antiplatelet, antibacterial, and antifungal effects. It has been reported that allicin inhibits various cancer cells demonstrating anticancer and chemopreventive activities [12,13]. The present study aimed at studying the renoprotective properties of allicin against gentamicin-induced nephrotoxicity.

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2. Materials and methods

2.1. Chemicals

Allicin was obtained as pharmaceutical drug (Allimax capsule containing 100% allicin powder, Allisure ® AC-23) obtained from (Allimax Nutriceuticals, US). Gentamicin was purchased as a pharmaceutical preparation (Gentamicin ampoules 80 mg, Alexandria Chemical Co., Egypt). All other chemicals and reagents used were of the highest analytical grade commercially available.

2.2. Animals

Twenty-four adult male Wistar rats weighing 160–200 g were used for the experimental procedures. Rats were allowed 1 week to adapt to the surroundings before beginning any experimentation. Animals were housed in individual plastic cages with bedding. Standard rat food and tap water were available ad libitum for the duration of the experiments unless otherwise noted. Temperature was maintained at 25 °C with 12/12-h light/dark cycle. All animal experiments described in this study comply with the ethical principles and guidelines for the care and use of laboratory animals adopted by "Research Ethics Committee, Faculty of Pharmacy, Mansoura University".

2.3. Experimental design

Gentamicin-induced acute kidney injury was established in male Wistar rats. The animals were randomly divided into three groups containing eight rats in each group.

Group (1): Control group, rats did not receive any solvent or drug during the experiment and were on a usual diet.

Group (2): Gentamicin (GNT) group, rats were injected with GNT (100 mg/kg, i.p.) for 7 consecutive days [14].

Group (3): Gentamicin/Allicin (GNT/AL) group, rats were injected with GNT (100 mg/kg, i.p.) and received AL (50 mg/kg, orally) [15] starting seven days before GNT injection till the end of experimental study. Allicin treatment schedule and dose level were chosen after pilot experiments.

After the last dose, all control and experimental animals were immediately kept in individual metabolic cages for collection of 24 h urine samples. These samples were centrifuged for 15 min at 3000 rpm, and kept frozen until analyzed. Blood samples were obtained from overnight fasted animals through retro-orbital sinus, under diethyl ether anesthesia, into non-heparinized tubes. The collected blood samples were allowed to clot for 30 min at 25 °C. After clotting, they were centrifuged at 1000 ×*g*, 4 °C for 15 min. using cooling centrifuge (Damon/IEC Division, Model: CRU-5000, Needham, Mass., USA). Sera were collected and stored frozen for the determination of levels of Cr, BUN, albumin and LDH. The animals were sacrificed after anesthesia by cervical dislocation, then the lower abdomen was opened and the contractile response of the isolated urinary bladder rings towards ACh was tested. Fatty adherents from the kidneys were removed and the kidneys were weighed using a digital balance to calculate the kidney body weight ratio. The left kidneys were excised immediately, rinsed in ice-cold normal saline (0.9% w/v), homogenized in 0.1 M phosphate buffer (pH 7.4) to yield 10% w/v tissue homogenates that were then used for biochemical assays. The right kidneys were harvested for histopathological examination.

2.4. Determination of serum creatinine

Creatinine was measured in rat serum as described by Bartels et al. [16]. Creatinine in alkaline solution reacts with picric acid to form a colored complex that was measured spectrophotometrically at 550 nm. The amount of the complex formed is directly proportional to the creatinine concentration. A kit from Biodiagnostics Co., Egypt was used.

2.5. Determination of blood urea nitrogen (BUN)

Urea was measured enzymatically in rat serum according to Fawcett and Scott [17]. Urea in the sample was hydrolyzed by urease enzyme to yield ammonia and carbon dioxide. In a modified Berthelot reaction, the blue dye indophenol product reaction absorbs light proportional to initial urea concentration and was measured spectrophotometrically at 550 nm. A kit from Biodiagnostics Co. was used.

2.6. Determination of creatinine clearance (Ccr)

Glomerular filtration was assessed by creatinine clearance based on serum and urine creatinine levels, with values expressed in ml/min, computed with the formula:

Ccr = urine creatinine (mg/dl) × urine flow (ml/min)/Serum creatinine (mg/dl).

Urine flow was calculated dividing 24 h of urine volume by 1440, which corresponds to the number of minutes in 24 h (60 min × 24 h = 1440): urine flow (ml/min) = value of urine volume (24 h) / 1440.

2.7. Determination of protein in urine

Protein in urine was determined by Folin–Lowry colorimetric method; two reactions are involved: (a) an initial interaction of protein and Cu^{+2} in alkali (related to biuret reaction); (b) a reduction of the phosphotungstic and phosphomolybdic acids to molybdenum blue and tungsten blue both by the Cu–protein complex and by the tyrosine and tryptophan of the protein. The latter two give color in the absence of Cu^{+2} but the rest of the protein gives no color without Cu^{+2}. About 75% of the color is dependent on the Cu^{+2}. The maximum absorption of the colored products is at 750 nm [18].

2.8. Determination of serum albumin

Albumin, in the presence of bromocresol green at a slightly acid pH, produces a color change in the indicator from yellow-green to green-blue. The intensity of the color formed is proportional to the albumin concentration in the sample [19].

2.9. Determination of serum lactate dehydrogenase

Lactate dehydrogenase (LDH) activity was assessed according to the method described by Henry [20]. The method depends on the conversion of pyruvate to lactate by LDH consuming NADH+, which absorbs at 340 nm. Its consumption is directly proportional to serum LDH concentration. LDH activity was calculated as units/l (U/l).

2.10. Determination of lipid peroxidation

Lipid peroxidation was determined by the method of Ohkawa et al. [21]. The principle of this method being that malondialdehyde (MDA), an end product of lipid peroxidation, reacts with thiobarbituric acid (TBA) to form a pink chromogen. For this assay, 0.2 ml of 8.1% sodium dodecyl sulfate (SDS), 1.5 ml of 20% acetic acid (pH 3.5) and 1.5 ml of 0.8% thiobarbituric acid aqueous solution were added in succession in a reaction tube. To this reaction mixture, 0.2 ml of the kidney homogenate was added, and the mixture was then heated in boiling water for 60 min. After cooling to room temperature, 5 ml of butanol: pyridine (15:1, v/v) solution was added. The mixture was then centrifuged at 2236 ×*g* for 15 min following which the upper layer was separated, and the intensity of the resulting pink color was read at 532 nm.

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