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Cilostazol attenuates gentamicin-induced nephrotoxicity in rats

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ABSTRACT

Introduction: Gentamycin is a widely used antibiotic. The nephrotoxic adverse effects of the drug may limit its use. Cilostazol, a phosphodiesterase III inhibitor, was reported to protect from renal oxidative stress.

This work aimed to investigate the possible protective effect of cilostazol on gentamicin-induced nephrotoxicity and the possible underlying mechanisms.

Materials and methods: 40 male albino rats were divided into 4 equal groups: (1) Control; (2) Cilostazol, 10 mg/kg, p.o.; (3) Gentamicin, 80 mg/kg, i.p.; (4) Gentamicin 80 mg/kg, i.p. along with cilostazol 10 mg/kg, p.o. All drugs were administered once daily for 8days. On 9th day blood samples were collected for the estimation of creatinine, urea and uric acid in serum. Then the rats were sacrificed and kidneys were removed for light and electron microscope studies. Moreover, reduced glutathione (GSH) and malondialdehyde (MDA) levels as well as catalase (CAT) and superoxide dismutase (SOD) activities were determined in renal tissues.

Results: Gentamicin elevated the serum levels of creatinine, urea and uric acid as well as the MDA level in the renal tissue, while it decreased CAT, SOD activities and GSH levels as well as produced degenerative changes in glomeruli and tubules associated with increased expression of apoptotic markers and decreased expression of anti-apoptotic markers. Administration of cilostazol decreased urea, creatinine, uric acid and MDA levels while increased CAT and SOD activities and GSH levels as well as ameliorated the histopathological changes in relation to gentamicin group.

Conclusion: Cilostazol protected rats from gentamicin-induced nephrotoxicity possibly, in part through its antioxidant and anti-apoptotic activity.

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1. Introduction

Gentamicin is a widely used aminoglycoside antibiotic either alone or in combination with a cell wall-active drug in management of severe and life-threatening infections caused by Gram-positive and Gram-negative aerobes (Choi et al., 2011). The ototoxic and nephrotoxic adverse effects of the drug may limit its use. In some situations, these adverse effects are so severe that the treatment must be discontinued (Ali, 2011).

The pathogenesis of gentamicin nephrotoxicity involves multiple pathways, including oxidative stress, inflammation, reduced renal blood flow, and increased nitric oxide (NO) level (Balakumar et al., 2010; Christo et al., 2011). Several agents have been used, with various degrees of success, to ameliorate or prevent gentamicin nephrotoxicity (Otunctemur et al., 2013; Rodrigues et al., 2014).

Cilostazol, a selective phosphodiesterase III inhibitor, has potent antiplatelet and vasodilator effects. The drug is approved for treatment of intermittent claudication in patients with peripheral vascular diseases (Chen et al., 2015). Cilostazol was generally well tolerated. Adverse events reported are headache, palpitation and tachycardia with mild to moderate intensity and rarely required treatment withdrawal (Kim et al., 2015). Several investigation in different cells and tissues have indicated to inhibitory effect of cilostazol on reactive oxygen species and superoxide generation as well as hydroxyl radicals scavenging action (Kim et al., 2002; Lee et al., 2010).

The aim of the present study is to assess the possible protective effect of cilostazol on gentamicin-induced nephrotoxicity and the possible underlying mechanisms.

2. Materials and methods

2.1. Materials

Gentamicin sulfate (Memphis Pharm., & Chemical Ind., Cairo, Egypt); Cilostazol powder, (Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan).

2.2. Animals

Male albino rats weighing 150–180 gm were used after one week for proper acclimatization to the standard housing conditions (25 ± 2 °C temperature and 12 h light/dark cycle) and were supplied with standard rodent chow and tap water ad libitum. All experimental protocols were approved by the Ethics Committee of Zagazig University.

2.3. Experimental design

40 male albino rats were randomly divided into 4 equal groups: Group (1) non-treated control; Group (2) was treated with cilostazol 10 mg/kg, p.o. dissolved in saline (Gokce et al., 2012) and served as cilostazol-treated control. Group (3) was injected with gentamicin 80 mg/kg, i.p. (Reddy et al., 2011) for induction of experimental nephrotoxicity; Group (4) was given cilostazol 10 mg/kg, p.o. 1 h before gentamicin 80 mg/kg, i.p. All drugs were administered daily for 8 consecutive days. On the 9th day blood samples were collected, and serum was separated by centrifugation at $3000 \times g$ for 10 min for estimation of creatinine, uric acid and urea levels. A longitudinal section from the left kidney was excised from each animal for histological examination. The renal cortex of the rest of the kidneys was stored at −80 °C and subsequently homogenized in ice-cold phosphate buffer (0.05 M, pH 7.4) for biochemical analysis.

2.3.1. Biochemical analysis

2.3.1.1. Serum analysis. Creatinine, uric acid and urea levels were estimated using kits supplied by spinreact (Girona, Spain).

2.3.1.2. Estimation of the reduced glutathione (GSH). The GSH level in the kidney was estimated using the method described by Ellman (1959). Briefly, the renal homogenate was mixed with 10% w/v trichloroacetic acid in ratio of 1:1 and centrifuged at 40 °C for 10 min at 5000 rpm. The supernatant obtained (0.5 ml) was mixed with 2 ml of 0.3 M disodium hydrogen phosphate buffer (pH 8.4) and 0.4 ml of distilled water. Then 0.25 ml of 0.001 M freshly prepared DTNB (5,51-dithiobis (2-nitrobenzoic acid)) dissolved in 1% w/v sodium citrate) was added. The reaction mixture was incubated for 10 min and absorbence of yellow colored complex was noted spectrophotometrically at 412 nm. A standard curve was plotted using reduced form of glutathione.

2.3.1.3. Determination of lipid peroxidation. Lipid peroxide was estimated by measurement of malondialdehyde (MDA) levels spectrophotometrically in kidney homogenate whereas kidney samples were homogenized in ice-cold 50 mM potassium phosphate buffer (pH 7.5), centrifuged at 4 °C 12,000 × g for 15 min and then the supernatant was collected. MDA in the supernatant can react with freshly prepared thiobarbituric acid (TBA) to form a colored complex which has maximum absorbance at 535 nm (Buege and Aust, 1978). The nmol MDA/g wet tissue was calculated from the plotted standard curve prepared from 1,1,3,3-tetraethoxypropane.

2.3.1.4. Determination of catalase (CAT). It was determined using colorimetric assay kits supplied from Biodiagnostic Company for diagnostic reagents: Dokki, Giza, Egypt. The resulting quinoneimine dye is measured at 520 nm (Aebi, 1984).

2.3.1.5. Determination of superoxide dismutase activity (SOD). Determination of total (Cu, Zn and Mn) SOD activity was determined kinetically (Sun et al., 1988) using kits supplied from Biodiagnostic Company for diagnostic reagents: Dokki, Giza, Egypt.

2.3.2. Histopathological studies

At the time of sacrifice, the animals were anesthetized by intraperitoneal injection of sodium pentobarbital at a dose of 50 mg/kg. The kidneys were rapidly removed and opened to be processed. For light microscopy, specimens were fixed in 10% formalin solution and processed to prepare 5 μm thick paraffin sections and stained with hematoxylin and eosin (H&E) (Bancroft and Gamble, 2002).

For immunohistochemical study, the deparaffinized sections on charged slides were used for detection of Bax (apoptosis inducers) and BCL2 (antiapoptotic marker). Immunohistochemical reaction was carried using avidin biotin peroxidase system. The primary antibody used was a rabbit polyclonal antibody (Sigma Laboratories, Saint Louis, USA). The primary antibody used for Bax and BCL2 were rabbit polyclonal antibody, sigma laboratories (Cat No ≠ anti Bax: B3428, anti BCL2 sc-492). Universal kit used avidin biotin peroxidase system produced by NovaCastra Laboratories Ltd., UK. The same method was applied to prepare negative control sections but the primary antibody was not added. Mayer's hematoxilin was added as counter stain. The cells that displayed brown precipitation were considered positive for Bax and BCL2 expression (Albamonte et al., 2013).

Specimens for electron microscope examination were cut into 1 mm³ pieces and fixed in 2.5% glutraldehyde buffered with 0.1 mol/L phosphate buffer at pH 7.4 for 2 h. They were post fixed in 1% osmium tetroxide for 1 h, dehydrated through graded alcohol series, and embedded in epoxy resin. Ultrathin sections (50 nm thick) were collected on copper grids and stained with uranyl acetate and lead citrate (Glauret and Lewis, 1998).

2.3.3. Quantitative morphometric measurements

Area percentage of the positive Bax & Bcl2 immune reactive cells lining the renal tubules was estimated by using "Leica Quin 500C" image analyzer computer system (Leica Imaging System Ltd., Cambridge, England). The measuring frame of a standard area

Table 1
Effect of cilostazol, gentamicin alone and in combination on serum urea, creatinine and uric acid in albino rats (n = 10).

Groups	Control	Cilostazol	Gentamicin	Gentamicin + cilostazol
Urea (mg/dl)	35 ± 2.33	38 ± 2.47	109 ± 11.77*	66.83 ± 5.73#
Creatinine (mg/dl)	0.75 ± 0.06	0.81 ± 0.09	2.15 ± 0.13*	1.54 ± 0.21#
Uric acid (mg/dl)	1.22 ± 0.21	1.36 ± 0.19	5.83 ± 0.33*	2.98 ± 0.23#

Data are presented as means ± SEM; n = number of rats in each group. Statistical analysis was done using one-way ANOVA followed by Tukey post-hoc test for multiple comparisons. *, $P < 0.05$ versus control and cilostazol groups; #, $P < 0.05$ versus control, cilostazol, and gentamicin groups.