Life Sciences 208 (2018) 64–71

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Nephroprotective effect of saxagliptin against gentamicin-induced nephrotoxicity, emphasis on anti-oxidant, anti-inflammatory and anti-apoptic effects

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ARTICLE INFO

Keywords:
Gentamicin
Saxagliptin
Nephro-protection
TNFα
VCAM-1
Caspase-3

ABSTRACT

Nephrotoxicity is a serious adverse effect frequently encountered with aminoglycosides administration. Given the value of aminoglycosides in management of serious infections, nephro-protection is highly recommended. The current study investigated the nephro-protective effect of saxagliptin (SAXA) (12.5 mg/kg, I.P.) against gentamicin (GEN)-induced nephrotoxicity in rats. SAXA administration for 14 days conferred significant nephro-protection against GEN-induced nephrotoxicity manifested in decreased kidney/somatic index, enhanced cytoprotection and significant decrease in serum LDH activity together with functional renal improvement; significant increase in creatinine clearance with significant reduction in serum creatinine, BUN, proteinuria and albuminuria. Oxidant/antioxidants hemostasis was significantly improved with SAXA treatment with significant reduction in kidney MDA content and enhancement of GSH concentration and catalase activity. Moreover, kidney content of NO significantly declined with significant decline in kidney tumor necrosis factorα (TNFα), vascular adhesion molecule-1 (VCAM-1) and caspase-3 content. Ultimately, SAXA administration was associated with significant attenuation of GEN-induced necrotic and inflammatory changes. In conclusion; the modulatory effect of SAXA on inflammatory cytokines, its anti-apoptic properties, ameliorative impact on oxidative load and positive impact on host antioxidant defenses accounts for the observed nephro-protective impact.

1. Introduction

Aminoglycosides have long been associated with one of the most commonly encountered problems in the field of clinical practice; drug-induced nephrotoxicity. Several approaches have been adopted to attenuate the aminoglycosides-associated nephrotoxicity including; clear identification of patient- and treatment-related risk factors together with once-a-day administration regimen and strict monitoring procedures. These approaches have significantly improved the clinical outcome of aminoglycosides use compared to the early 1980s [1].

Gentamicin (GEN) is a member of the aminoglycosides whose nephrotoxicity presents clinically as non-oliguric renal failure. Serum creatinine slowly rises and a hypo-osmolar urinary output develops within several days of treatment initiation. Approximately 5% of the administered dose is retained in the epithelial cells lining S1 and S2 segments of the proximal tubules after glomerular filtration. The highest concentrations are mainly localized within endosomal and lysosomal vacuoles and the Golgi complex. Such localization elicits an array of functional and morphological alterations of increasing severity of nephrotoxicity [1].

GEN-induced cytotoxicity occurs in those cell types in which the drug mainly accumulates; the epithelial cells of the renal cortex and the proximal tubule and in the distal and collecting ducts [2, 3]. GEN binds to membrane phospholipids, altering their turnover and metabolism and drives a condition referred to as phospholipidosis [3, 4].

A central aspect of GEN-induced nephrotoxicity is its tubular cytotoxicity. Both apoptosis [5] and necrosis [6] of tubular epithelial cells have been reported to be observed in renal specimen of experimental animals treated with GEN. Moreover, GEN-induced nephrotoxicity has been reported to be associated with induction of several inflammatory mediators, reactive oxygen species (ROS) production and enhanced oxidative stress [3, 7].

Given the effectiveness of aminoglycosides and their importance for treatment of severe infections, the maintenance and even development of efforts to improve their therapeutic indices and associated nephrotoxic adverse effects is highly recommended.

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https://doi.org/10.1016/j.lfs.2018.07.021
Received 9 April 2018; Received in revised form 8 July 2018; Accepted 12 July 2018

Dipeptidyl peptidase-4 (DPP-4) inhibitors are a class of drugs used for management of type II diabetes mellitus. They function mainly by inhibiting the enzyme responsible for the breakdown of glucagon like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP), prolonging the half-life of endogenous GLP-1 and GIP, with enhanced glucose-dependent insulin secretion and decreased glucose-dependent glucagon secretion [8, 9]. Saxagliptin (SAXA) is a member of the DDP-4 inhibitor, together with its active metabolite; they prevent the inactivation of the incretin hormones GLP-1 and GIP. This increases GLP-1 levels, stimulates insulin secretion, and reduces postprandial glucagon and glucose levels [10].

The action of various members of DPP-4 inhibitors on the kidney varies according to the compound. Saxagliptin exerted reno-protective effects through its tight covalent binding and long-lasting inhibition of renal membrane-bound DPP-4 compared to other members of DDP-4 inhibitors [11]. SAXA treatment in diabetic patients at high renal risk was associated with reduction in albuminuria and enhanced glomerular filtration stability [12]. Nevertheless, SAXA demonstrated potent reno-protective effect in Dahl salt-sensitive hypertensive rats. The reno-protective effect was independent on SAXA's glucose-lowering actions and was superior to that of sitagliptin [13, 14]. Interestingly, SAXA has been reported to be well tolerated in patients with chronic kidney diseases [15].

The current study protocol was designed to evaluate the therapeutic value of SAXA as a nephro-protective agent against GEN-induced nephrotoxicity in rats with particular emphasis on the three main checkpoints in the pathogenesis of GEN-induced nephrotoxic effects; oxidative, inflammatory and apoptic pathways.

2. Materials and methods

2.1. Animals

Twenty-four adult male Sprague-Dawley rats, (180–230 g) were bought from “Urology and Nephrology Center”, Mansoura, Egypt. The rats were housed under controlled environmental conditions and Nutrional conditions throughout the experimentation period. The experimental protocol complied with the ethical guidelines of research ethics and experimental research adopted by “Research Ethics Committee”, Faculty of Pharmacy, Mansoura University, Egypt.

2.2. Drugs and chemicals

Saxagleptin (SAXA) as, Onglyza; 5 mg tablets, (Bristol-Mayers, Pennington, NJ, USA), it was dissolved in 0.9%, w/v Nacl for intraperitoneal (I.P.) injection. Tablets were completely soluble in normal saline which ensured uniform sampling and dosing to all rats. Gentamicin (GEN) as; Epigent ampoules (80 mg/2 ml); obtained from Egyptian Pharmaceutical International Industries Co. EPICO, (10th of Ramadan City, Egypt).

2.3. Experimental protocol

2.3.1. Evaluation of the nephro-protective effect of SAXA (12.5 mg/kg) orally for 14 days against GEN (100 mg/kg)-induced nephrotoxicity in rats

Nephrotoxicity was induced by daily injection of GEN (100 mg/kg, I.P.) as described by Chashmi et al., (2017) [16]. Rats were randomly allocated to three experimental groups, 6 rats/group; **normal control (CTRL)**; rats received I.P. 0.9%, w/v Nacl for 14 days; **SAXA control (SAXA CTRL)**; rats received daily I.P. SAXA (12.5 mg/kg) [13] for 14 days, **GEN control**; rats received daily I.P. GEN (100 mg/kg) for 7 days starting from the 8th day of the experiment and **SAXA-treated group (GEN + SAXA)**; rats received daily I.P. SAXA (12.5 mg/kg) [13] for 7 days prior to I.P. GEN (100 mg/kg) which began from the 8th day of the experiment and for further 7 days along with SAXA administration. Collectively, SAXA (12.5 mg/kg) was administered for 14 days; duration of the experimental protocol.

Following the last GEN and SAXA doses, rats were individually housed in metabolic cages for 24 h for urine samples collection and assessment of proteinuria and albuminuria. After which, rats were weighed and sacrificed by an overdose of thiopental sodium (40 mg/kg, I.P.). Blood samples were collected from the heart by cardiac puncture and sera were used immediately for biochemical assessments.

After exsanguination, rats were placed on their dorsal side, the abdominal cavity was opened, right and left kidneys were excised, rinsed in ice-cold saline and weighed for calculation or renal/somatic index. The left kidneys from the different experimental groups were slit lengthwise and fixed in neutral 10% formalin solution for histopathological evaluation.

The right kidneys were used for preparation of 10% w/v kidney homogenate as described by Bueg and Aust (1978) [17] and Said et al., (2015) [18] for quantification of kidney contents of oxidants/antioxidants stress biomarkers; Malondialdehyde (MDA) and nitric oxide (NO) contents, reduced glutathione (GSH) concentration and catalase activity. Tumor necrosis factor α (TNF-α) vascular adhesion molecule-1 (VCAM-1) and caspase-3 were also quantified in kidney homogenate.

2.3.2. Assessment of renal functions; serum creatinine, creatinine clearance, blood urea nitrogen (BUN), albuminuria and proteinuria

Serum samples were used for assessment of creatinine, creatinine clearance and BUN using commercially available Biomed assay kits (Badr City, Egypt) according to the manufacturer's instruction. Urine albumin and protein contents were assessed using Biomed assay kits (Badr City, Egypt) and Spinreact assay kits (Girona, Spain) respectively according to the manufacturer's instructions.

2.3.3. Assessments of renal malondialdehyde (MDA) and nitric oxide (NO) contents, reduced glutathione (GSH) concentration and catalase activity

The kidney homogenate was used for colorimetric assessment of MDA and NO contents, reduced GSH concentration and catalase activity using Biodiagnostic kits (Giza, Egypt) as instructed by the manufacturer.

2.3.4. Assessments of renal tumor necrosis factor α (TNF-α), vascular adhesion molecule-1 (VCAM-1) and caspase-3 contents

The kidney homogenate was used for quantification of kidney contents of VCAM-1, csapase-3 using Enzyme linked Immunosorbent assay (ELISA) kits, Cloud-Clone Co., (Houston, USA) and TNF-α contents using platinum ELISA assay kits Bender Med. systems Gmbh, (Vienna, Austria) as instructed by the manufacturers.

2.4. Histopathology

The left kidneys from the different experimental groups were slit lengthwise, fixed in 10% neutral buffered formalin, embedded in paraffin wax blocks, sectioned into 5 μm thick slides and stained with hematoxylin and eosin (H&E) stain for histopathological examination and assessment of tissue changes due to GEN-induced nephrotoxicity and impact of SAXA administration.

2.5. Statistical analysis

Data are presented as mean ± SEM, significance was accepted at ($p < 0.05$). Linear regression analysis and one way analysis of variance (ANOVA) followed by Tukey-Kramer's multiple comparisons test for analysis of the best fitting line of all the standard points and statistical comparison between means of parametric data respectively. Statistical calculations were carried out using Instat-3 computer program (Graph Pad Software Inc. V2. 04, San Diego, CA, USA).