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Upregulation of PPAR-γ mediates the renoprotective effect of omega-3 PUFA and ferulic acid in gentamicin-intoxicated rats

Nahla E. El-Ashmawy, Naglaa F. Khedr, Hoda A. El-Bahrawy, Sara A. Helal*

Faculty of Pharmacy, Tanta University Tanta, El-Gharbia, 31527, Egypt

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ABSTRACT

Ferulic acid (FrA) is a natural product containing phenolic compounds. ω-3 PUFA is the major constituent of fish oil. The aim of this study was to investigate the renoprotective role of FrA and FO in gentamicin (GM)-induced nephrotoxicity in rats. Forty four male rats were divided equally into 4 groups: Control group, GM group, FrA + GM group and FO + GM group. Each of the treated groups was injected with GM (40 mg/kg) i.p. for 9 consecutive days. FrA (100 mg/kg) and FO (5 mL/kg) were given to rats orally daily for 10 days prior to GM and then concomitantly with GM for additional 9 days. Kidney function was assessed by serum BUN and creatinine, urinary albumin excretion and N-acetyl-beta-D-glucosaminidase (NAG) activity and histopathological examination. The anti-inflammatory property was evaluated by measuring renal resolvin E1 and gene expression of PPAR-γ. The antioxidant activity was indicated by renal catalase (CAT) activity. GM-induced nephrotoxicity was evidenced by the renal histopathological changes along with increased renal indices. Prior and concomitant treatment with FrA or FO ameliorated nephrotoxic effect of GM as indicated by the significant decrease of serum BUN and creatinine, urinary albumin excretion and urinary NAG activity. Both treatments significantly enhanced CAT activity and gene expression of PPAR-γ. Resolvin E1 was significantly elevated in FO but not in FrA group. FrA and FO proved anti-inflammatory and renoprotective effects, which could be through their PPAR-γ agonist activity. Because FrA and FO are natural products, they could provide a safe intervention strategy in cases of exposure to nephrotoxins.

1. Introduction

Peroxisome proliferator-activated receptor gamma (PPAR-γ) belongs to the family of nuclear receptors that act by forming a heterodimer with the retinoic-X receptor. PPAR-γ is expressed in adipose tissue, where it regulates adipocyte differentiation and regulates the metabolic responses of adipocytes, including promoting insulin sensitivity. It is also expressed in hematopoietic cells, kidney and the large intestine [1]. PPAR-γ has many important functions like glucose and lipid metabolism regulation, anti-inflammatory properties; oxidative stress inhibition and improvement of endothelial function. Previous studies indicate that PPAR-γ is involved in the normal kidney development, renal lipid metabolism, and activation of the rennin angiotensin system [2].

The interest in complementary and alternative medicine (CAM) has increased over the past decade. The argument about the effectiveness of unconventional methods has occupied a large interest among many medical professionals [3]. Many evidences suggested that various extracts of natural and dietary antioxidants have a role to protect the kidney against oxidative stress damages affecting glomerular and tubular functions [4].

Fish oil (FO) is an anti-inflammatory agent with some antioxidant properties. The ω-3 polyunsaturated fatty acids (PUFA) eicosapentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, 22:6) are abundant in FO. Fish oil exhibits significant anti-inflammatory properties possibly via suppressing some pro-inflammatory mediators [5]. EPA and DHA can act as substrate for the synthesis of a new family of lipid mediators, termed resolvins, synthesized from both EPA (E-series resolvins) and DHA (D-series resolvins) [6]. The synthesis of resolvins involves the cyclooxygenase and lipoxygenase pathways. Studies have shown potent anti-inflammatory and immunomodulatory actions of resolvins [7].

Ferulic acid (4-hydroxy-3-methoxycinnamic acid) is a ubiquitous phenolic compound found in plant tissues such as grain bran, citrus fruits, coffee, spinach and broccoli. Ferulic acid (FrA) has been approved as an additive antioxidant and food preservative in Japan since 2013 [8]. Sodium ferulate, a salt of FrA, is used in Chinese traditional medicine for treatment of cardiovascular and cerebrovascular diseases

* Corresponding author at: Department of Biochemistry, Faculty of Pharmacy, Tanta University, El-Bahr Street, Tanta, El-Gharbia, 31527, Egypt.
E-mail addresses: nahla.elashmawi@pharm.tanta.edu.eg (N.E. El-Ashmawy), naglaa.khedr@pharm.tanta.edu.eg (N.F. Khedr), hoda.elbahrawi@pharm.tanta.edu.eg (H.A. El-Bahrawy), Sara.helal@pharm.tanta.edu.eg (S.A. Helal).

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[9].

Gentamicin (GM) is an aminoglycoside antibiotic that is commonly used against Gram-negative bacteria and is an effective therapeutic agent against pseudomonas, proteus and serratia microorganisms that are insensitive to other antibiotics. The most serious limitation of its use is that it can cause ototoxicity and nephrotoxicity [10]. All aminoglycosides, including GM, are freely filtered across the glomerulus. They are partially taken up by the proximal tubular cells where they are concentrated and cause renal damage [11].

An important mechanism of GM nephrotoxicity is that increasing the production of mitochondrial reactive oxygen species (ROS) causing deficiency in intrinsic antioxidant enzymes. High levels of ROS are able to cause damage of many cellular molecules including proteins, lipids, and nucleic acids, thus impairing cell function and leading to cell death [12]. In addition, GM nephrotoxicity contribute to mesangial and vascular contraction and participate in inflammation which is characterized by infiltration of many inflammatory cells such as monocytes and macrophages. These cells release many proinflammatory cytokines such as IL-1 and TNF-α and also activate nuclear factor kappa B (NF-κB) pathway [13].

Therefore, the present study was conducted to evaluate the nephroprotective efficacy of ferulic acid and fish oil, as adjuvant therapies, against gentamicin-induced nephrotoxicity in experimental rats and to identify the underlying molecular mechanisms.

2. Materials and methods

2.1. Drugs

Gentamicin was purchased from E.I.P.I.CO. (Sharqia, Egypt). Ferulic acid was purchased from Sigma Aldrich (Germany). Ferulic acid (20 mg/mL) was freshly prepared in propylene glycol. Fish oil was purchased from The Arab Co. for Gelatin and Pharmaceutical Products for Montana Pharmaceutical (Amreya, Alexandria, Egypt).

2.2. Animals and experimental design

The experimental protocol was approved by the Local Ethical Committee of the Faculty of Pharmacy, Tanta University (Egypt). Forty four adult male Albino rats weighing 180–200 g were purchased from the animal house of Giza Institute of Ophthalmology (Cairo, Egypt). Rats were housed in wire cages for 2 weeks under identical environmental conditions for adaptation and allowed free access to balanced laboratory diet and water ad libitum.

After acclimatization period, rats were divided into four equal groups. Group1 (control group): rats received the vehicle. Group 2 (GM group): rats were given the vehicle for 10 days and then injected i.p. daily with GM 40 mg/kg [14] starting on day 11 for successive 9 days. Group 3 (FrA group): rats were given FrA 100 mg/kg [15] orally daily for 19 days and exposed to GM on day 11 as in group 2. Group 4 (FO group): rats were given FO 5 mL/kg [16] orally daily for 19 days and exposed to GM on day 11 as in group 2.

2.3. Sample collection

At the end of the experiment, each rat was kept in a metabolic cage to collect 24 h urine samples. The urine samples were then centrifuged for 10 min at 765 g to remove any debris and stored at −80 °C. After urine collection all rats were anaesthetized under light diethyl ether then blood was withdrawn via cardiac puncture and serum was collected and stored at −80 °C until analysis. Rats were killed by cervical dislocation and kidneys were removed immediately. Right kidneys were fixed in 10% buffered formalin solution at room temperature for histopathological studies. Left kidneys were divided and stored at −80 °C under liquid nitrogen for biochemical and gene expression analysis.

2.4. Determination of kidney biomarkers

The following biomarkers were assessed using commercial kits obtained from Biodiagnostic Co., (Giza, Egypt). Blood urea nitrogen (BUN) level was measured colorimetrically according to urease-Berthlot method [17]. Serum and urinary creatinine levels were determined colorimetrically according to the method described by Slot [18]. Urinary albumin was measured according to the method described by Daughaday et al. [19].

2.5. Determination of N-acetyl-??-D-glucosaminidase (NAG, E.C. 3.2.1.52) enzyme activity in urine

Urinary NAG was assayed according to the method described by Calvo et al. [20]. This method is based on the spectrophotometric determination of the enzymatically released p-nitrophenol from 4-nitrophenyl N-acetyl-β-D-glucosaminide substrate at 400 nm. The incubation mixture contained 0.1 M sodium citrate buffer (pH 4.5), 0.8 mmol p-nitrophenyl glucosaminide, and 100 µL of urine samples. The reaction was incubated for 15 min at 37 °C and stopped by the addition of 1.5 mL of cold 0.2 M sodium carbonate. One unit of enzyme activity is that catalyzes the release of 1 mmol of p-nitrophenol per min at 37 °C. The amount of p-nitrophenol was calculated using the molar extinction coefficient of $1.77 \times 10^3\,M^{-1}\,cm^{-1}$. The enzyme activity was related to urinary creatinine concentration and expressed as µmol/h/mg creatinine.

2.6. Determination of catalase (CAT, E.C. 1.11.1.6) activity in kidney tissue

CAT activity was measured according to the method described by Aebi [21] using the kit obtained from Biodiagnostic Co. (Giza, Egypt). Kidney tissue was homogenized in 5 mL cold buffer (50 mM potassium phosphate pH 7.4, 1 mM EDTA and 1mL/L Triton X-100). CAT reacts with a known quantity of H_2O_2 and the reaction is stopped after exactly one min with CAT inhibitor. In presence of horseradish peroxidase, the remaining H_2O_2 reacts with a substrate to form quinoneimine dye, which color intensity was measured at 510 nm. The enzyme activity was expressed as U/g tissue.

2.7. Determiation of resolvin E1 (RvE1) concentration in kidney tissue

RvE1 was determined in renal tissue using ELISA kit obtained from SunRed Biological Technology (China) according to the manufacturers' instructions. RvE1 concentration was expressed as pg/g tissue.

2.8. Quantitative real time polymerase chain reaction (qRT-PCR) of PPAR-γ gene in kidney tissue

Total RNA was extracted from kidney tissue using RNA-spin™ total RNA extraction kit (iNtRON Biotechnology, Korea). The purity of obtained RNA was verified spectrophotometrically at 260/280 nm. 1–5 µg total RNA was converted to cDNA by Thermoscript RNase H Reverse Transcriptase (TIAGEN RT-PCR Kit, China). The obtained cDNA was used for quantitative PCR using SYBR Green PCR master mix (iNtRON Biotechnology, Korea) as described by the manufacturer using the RT-PCR system (Thermofisher Scientific, Pikoreal 5100, Finland). Primers of PPAR-γ: 5′-TGTGGACCTCTCTGTGATGG-3′ (forward), 5′-CATTGGG TCAGCTCTTGTGA-3′ (reverse). Primers of GAPDH (Housekeeping gene): 5′-ACTCCCATTCCTCCACCTTT-3′ (forward), 5′-TTACTCCTTGG AGGCCATGT-3′- (reverse). Primers were purchased from Biosearch Technologies Co. (USA) and prepared according to Yoshida et al. [22]. The Real-time PCR instrument was adjusted to: 95 °C for 30 s and 40 cycles (95 °C for 5 s for denaturation and 53 °C for 10 s for annealing/extention). Each sample was analyzed, normalized to the level of GAPDH, and expressed as a relative copy number (RCN). Ct (threshold