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Vanillin as a new modulator candidate for renal injury induced by cisplatin in experimental rats

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ABSTRACT

Cisplatin is commonly prescribed for the treatment of various solid tumors but its use is limited due to certain side effects and renal injury is a true example. Oxidative stress and inflammation may contribute to the cisplatin induced nephrotoxicity. Accordingly, we evaluated the effect of oral vanillin intake (100 mg/kg body weight) daily for 4 weeks to combat this hazard. The present results have demonstrated significant attenuation of oxidative stress and renal injury where reduced glutathione (GSH) showed significant increase along with malondialdehyde (MDA) decrease. Fibrotic markers like fibroblast growth factor-23 (FGF-23), transforming growth factor-β1 (TGF-β1), inflammatory mediators such as nuclear factor-κB (NF-κB) and tumor necrosis factor-α (TNF-α) showed also significant decrease in vanillin treated rats as compared with the control group.

Renal function showed also significant improvement where urea and creatinine demonstrated significant decrease and the histopathological study presented a good support to the biochemical markers results. Our conclusion that vanillin is a potent antioxidant, anti-inflammatory and anti-fibrotic agent. Additionally, it is a good modulator candidate for the renal injury induced by cisplatin intake.

1. Introduction

Cisplatin is a chemotherapeutic agent for the treatment of various tumors [1,2]. Its therapeutic use however, is limited due to its multiorgans toxicity. Oxidative stress and inflammation have been observed during its intake as probable causes for cisplatin nephrotoxicity [3,4]. Additionally, its preferential accumulation in renal tubules is another causal factor [2]. Irreversible damage of parenchymal tissues and the replacement of highly differentiated cells by scarring connective tissue may constitute the common pathogenic mechanism for chronic renal failure [5–8].

Tissues fibrosis represent the last step observed in non-treated chronic renal injuries as manifested by excessive deposition of the matrix components leading to a decline in the function of the organs. This is mostly attributed to the replacement of normal tissues with fibrotic ones along with common inflammation which may be observed in certain tissues like the lungs, the liver and the kidneys [9,10].

In the kidney, fibrosis is the final common pathway of glomerular, vascular or interstitial inflammation leading to the end stage renal failure (ESRF) [10,11]. Chronic injurious stimuli like that observed in most progressive renal diseases may lead to glomerular sclerosis, tubular atrophy, interstitial fibrosis of tubules and glomerular refraction in addition to the peritubular capillaries [12].

Fibrotic changes may also lead to the disruption of glomerular and tubular architecture. Accordingly, major inhibition of the mediators responsible for matrix accumulation may slow or block the fibrosis progression [13]. Trials to inhibit or modulate factors that promote fibrosis like fibroblast growth factor (FGF) and connective tissue growth factor (CTGF) may be of benefit here [14,15]. Enhancing factors that attenuate or modulate fibrosis like bone morphogenetic protein-7 (BMP-7) and hepatocyte growth factor (HGF) to improve the renal architecture and function are additionally requested [16].

Vanillin is a natural phenolic compound, showing an extensive variety of helpful biochemical and pharmacological activities. It is generally utilized in food flavorings, drinks, drugs, perfumes [17]. Moreover, it has many chemo-protective properties like antioxidant activity[18], sickle cell frailty treatment [19], mutagenesis inhibition [20], anticarcinogenic impact [21], enhancing the response of tumor cells to chemotherapy [22] and restraint of the cells intrusion and migration [23,24]. Vanillin can also inhibit peroxynitrite–mediate reactions which are involved in the pathogenesis of many neurodegenerative diseases like Alzheimer's and Parkinson's diseases [25,26]. It can control also cognitive decrease, oxidative stress, and neurodegeneration in experimental model of Huntington's illness [26,27].

The present work therefore was undertaken to demonstrate vanillin

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potential as antioxidant anti-inflammatory and anti-fibrotic agent to ameliorate inflammation, nephrotoxicity and renal fibrosis induced by cisplatin in experimental rat model.

2. Materials and methods

2.1. Animals

Male albino rats weighing 160 ± 20 g (3 months old) as supplied from the Egyptian Organization for serum and Vaccines (Cairo, Egypt) were utilized in the present study. Rats were housed in stainless steel cages under controlled conditions and left for one week for acclimatization at room temperature with a 12 h light/dim cycle. Rats were fed chow diet (El-Nasr Pharmaceuticals and chemicals enterprises, Egypt) and were permitted free access of drinking water. The experimental work was done according to the Animal Welfare Act and the Guide for Care and Use of Laboratory Animals built up by Zagazig University, Zagazig, Egypt.

2.2. Experimental design

Renal injury was initiated through intraperitoneal (IP) injection of cisplatin (4 mg/kg body weight, single dose). The extent of renal injury was monitored through periodical determination of serum creatinine and urea after cisplatin intake. The rats were classified later into two groups (8 rats/each) the first one received drug free vehicle only and kept as control (DC) while the second group received oral vanillin for treatment (Van group) in a dose level 100 mg/kg daily for 4 weeks. Other normal rats (received vehicle only) were included (NC group) and expressed as normal group (n = 8 rats).

2.3. Blood sampling

Blood samples were collected after 4 weeks of vanillin administration and were centrifuged directly for serum separation. Samples were later directed for creatinine, urea, fibroblast growth factor-23 (FGF-23), insulin like growth factor-1 (IGF-1), HGF and NF-kB determinations.

2.4. Tissue collection

Following blood gathering, rats were sacrificed and kidneys were expelled in a flash, flushed with frosty typical saline and dried with filter paper. The first kidney was immediately frozen in liquid nitrogen (−196 °C) and kept at −80 °C for later determination of MDA, GSH in addition to the gene expression of TGF-β1 and TNF-α. The other one was fixed in formalin:saline (1:9) solution at 4 °C for one week then was processed to histopathological examination.

2.5. Analytical methods

Creatinine and urea were determined using commercial packs provided by Diamond Diagnostics, Cairo, Egypt. Serum FGF-23 (detection range was 15.6–1000 ng/ml), IGF-I (detection range was 25–2000 pg/ml), HGF (detection range was 12–4000 pg/ml) and NF-kB (detection range was 0.156–10 ng/ml) were determined using commercial ELISA packs supplied by AMS Biotechnology (Europe) Ltd (Abingdon, UK), Novozymes Biopharma AU Ltd (Adelaide, Australia), Quantikine R and D systems Inc (Minneapolis USA) and EIAab Science Co., Ltd (Wuhan, China) respectively. Kidney MDA [28] and GSH contents [29] were determined calorimetrically using Bio-Diagnostics Kits, (Bio-diagnostics Co. Giza, Egypt) and we have followed exactly the recommendations of the manufacturers.

2.5.1. TNF-α and TGF-β1 gene expression analysis using RT-PCR

RNA was extracted from kidney samples byQiagen tissue extraction kit (Qiagen, USA), kidney tissue was placed in lysis Buffer and the lysate was homogenized by tissue homogenizer and centrifuged for 3 min. The supernatant was carefully removed and transferred into a new micro centrifuge tube, reverse transcribed to cDNA using high capacity cDNA reverse transcription pack (Fermentas, USA) and amplified by RT-PCR pack (Stratagene, USA). qPCR amplification and analysis were carrying out by Applied Biosystem with software version 3.1 (StepOne™, USA). qPCR assay with the primer sets were optimized at the annealing temperature.

Reactions were performed in a 25 µl volume (12.5 µl SYBR Green Mix (2×), 5 µl cDNA, 2 µl primer pair mix, 5.5 µl H_2O). qPCR reaction was: 50 °C for 2 min (1 cycle) and 40 cycles of 95 °C for 15 s, 60 °C for 1 min and 72 °C for 1 min. Relative gene expression was calculated using 2-ΔΔCt method. PCR primers were planned with Gene Runner Software (Hasting Software, Inc., Hasting, NY) utilizing RNA arrangements from GenBank. The successions of forward and invert primers were as per the following: TNF-α (forward: 5′-CATTGAGGTGTATTT CACGG-3′ and reverse: 5′- GGCAAGTGGCCATTGTGTTC-3′), TGF-β1 (forward: 5′- CCTTGCCTCTAAGCCTTTGC-3′ and reverse: 5′- GCCCTC CAGAAGTGGTCATT-3′) and GAPDH (forward: 5′- GATGCTGGTGCT GAGTATGTCG-3′ and reverse: 5′-GTGGTGCAGGATGCATTGCTGA-3′).

2.6. Histopathologic examination

The kidney samples were dried out with a progression of rising evaluation ethanol from 75% to 100%. Tissues were set from there on in xylon and embedded in paraffin. Cross areas of around 2 µm thickness were cut utilizing a microtome (Leica RM 2155, England), stained with H & E and Masson's trichrome stains [30] for microscopic examination.

2.7. Statistics

The statistics were made by GraphPad Prism 6, CA, USA. The results were expressed as mean ± SD and statistical differences were done by one-way analysis of variance (ANOVA test) taken $p < 0.05$ as confident interval.

3. Results

3.1. Metabolic parameters

The induction of renal injury resulted in a significant rise in serum creatinine and urea. Renal content of MDA demonstrated a significant increase along with GSH decrease as compared with Normal (NC) group.

The administration of Vanillin significantly decreased serum creatinine, urea and renal MDA content as compared with the control group (DC) while renal GSH content showed a significant increase (Table 1).

3.2. Inflammatory and fibrotic parameters

The induction of renal injury resulted in a significant increase in serum NF-κB (Fig. 1a), IGF-1 (Fig. 1c), FGF-23 (Fig. 1d) and a significant decrease in HGF (Fig. 1f) as compared with Normal group.

Table 1
Kidney functions' parameters.

Parameter	NC	DC	Van
Serum creatinine (mg/dl)	0.28 ± 0.04	1.94 ± 0.03*	0.47 ± 0.08#
Serum urea (mg/dl)	11.60 ± 0.40	54.12 ± 0.78*	27.29 ± 0.89#
Renal MDA (nmol/g.tissue)	7.70 ± 0.49	53.73 ± 0.50*	18.59 ± 1.98#
Renal GSH (mmol/g.tissue)	6.08 ± 0.32	1.50 ± 0.25*	3.28 ± 0.07#

Results were expressed as mean ± SD, n = 8. *Significant from NC and #Significant from DC, $p < 0.05$.