Protective Effects of Rosmarinic Acid on Kim-1 and HO-1 in Vancomycin Induced Nephrotoxicity in Wistar Rats

Subramanian Sathishkumar and Perumal Subramanian*,
Department of Biochemistry and Biotechnology, Faculty of Science, Annamalai University, Annamalai Nagar – 608 002, Tamil Nadu, India.

Abstract
The aim of this study was to examine the influence of rosmarinic acid (RA) against vancomycin (VMC) induced renal toxicity in rats. Rats were divided into four groups (n=6 in each group) - the first group was used as control, the second group was treated with VMC (200 mg/kg/b.w ip) for 7 days, third group was orally treated with RA (100 mg/kg/b.w/day) for 21 days and fourth group was treated with RA+VMC (RA was administered for 21 days; VMC was administered from 15th day to 21st day). We examined whether RA is able to down-regulate Kim-1 and HO-1 expressions. The western blot analysis of Kim-1 and HO-1 performed in kidney tissues of control and experimental groups of rats. VMC induced group displayed an increased expression of Kim-1 and HO-1 when compared to control group. RA treatment to VMC induced group resulted in a decreased expressions of Kim-1 and HO-1. RA alone treated group exhibited a similar expression as seen in control group. The results of the present study have clearly indicated the supplemental role of RA, a phytophenolic compound, against the VMC-induced nephrotoxicity by decreasing the expressions of Kim-1 and HO-1 by maintaining the cellular integrity of kidney without any side effects.

Article History
Received: 05.04.2017; Revised: 20.04.2017
Accepted: 30.04.2017

Key words: Rosmarinic acid, Vancomycin and Nephrotoxicity.

1. Introduction
Vancomycin (VMC) is a glycopeptide antibiotic and has been extensively used for the treatment of serious gram positive infections involving methicillin resistant Staphylococcus aureus for approximately 50 years (Beauchamp et al., 1990). The dose and duration of administration of VMC could cause its deposition on proximal tubules and lead to renal dysfunction (Hodoshima et al., 2007). Kidney injury molecule-1 (Kim-1) is a more sensitive biomarker to detect early kidney injury than other routinely used biomarkers such as plasma creatinine, blood urea nitrogen, urinary NAG, glycosuria and proteinuria (Vaidya et al., 2008). Kim-1 is a member of a gene family encoding T-cell immunoglobulin mucin (Tim) proteins, and is involved in immune regulation as well as renal tubular regeneration after ischemic- or nephrotoxicant-induced injury (Han et al., 2002; Ichimura et al., 1998). It has been suggested to serve as a sensitive biomarker for renal proximal tubule injury in preclinical and clinical studies of drug safety evaluation, chemical related renal injury, and the monitoring of renal disease states (Han et al., 2002; Vaidya and Bonventre, 2006; Zhou et al., 2008). Kim-1 functions as a regulator of cell–cell adhesion and endocytosis at a time when the dedifferentiated regenerating cells...
of the injured proximal tubules reform a continuous epithelial layer (Bailly et al., 2002; Ichimura et al., 2008).

Heme oxygenase-1 (HO-1), is recognized as a protective gene in the kidney and involved in the production of anti-inflammatory, antioxidant, and antiapoptotic metabolites (Abraham, 2009). Previous studies indicated a protective role for HO-1 in heme and non-heme-mediated models of acute renal injury using chemical inducers and inhibitors of HO-1 (Sahin et al., 2010).

Protective effects of RA against VMC-induced nephrotoxicity have not been studied earlier. In the present study, we have investigated the therapeutic activity of RA against oxidative stress and injury in VMC induced rats.

2. Materials and Methods

Chemicals

RA was obtained from Sigma Chemical Company (St Louis, MO, USA) and VMC from Ranbaxy Laboratories Ltd (Mumbai, India). Antibodies such as Kim-1 and HO-1 were purchased from Santa Cruz Biotechnology, India. All other biochemicals and chemicals used in the study were of analytical grade and purchased from Himedia Laboratories and Sisco Research Laboratories (Mumbai, India).

Experimental Animals

All the experiments were carried out in male Wistar albino rats (180-200 g), obtained from the Central Animal House at the Rajah Muthiah Institute of Health Sciences (Annamalai University, Tamil Nadu, India). Rats were housed six per cage and maintained with 12 hours a day and night cycle at room temperature of 22 °C and relative humidity was 70%. The animals were fed with commercial pellet diet (Hindustan Lever Ltd, Bangalore, India) and water ad libitum. The experiments using animals were conducted according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, and approved by the Animal Ethical Committee of Annamalai University (160/1999/ CPCSEA)(Approval No: 1007 dated: 02.05.2013).

Experimental design

VMC was injected intraperitoneally (i.p.) to animals (200 mg/kg (b.w.) which is the normal dose to induce nephrotoxicity (Ahmida, 2012). RA was dissolved in water and administered orally at a dose of 100 mg/kg (body weight) (Tavafi and Ahmadvand, 2011). Rats were divided into four groups; the first group rats were used as control, the second group rats were treated with VMC (200 mg/kg/b.w ip) for 7 days, third group rats were orally treated with RA (100 mg/kg/b.w/day) for 21 days and fourth group rats were treated with RA+VMC (RA (100 mg/kg/b.w/day) for 21 days; VMC (200 mg/kg/b.w ip) was administered from 15th day to 21st day).

Western Blot Analysis

The expression of Kim-1 and HO-1 by western blotting. The kidney tissue sections were homogenized in chilled RIPA buffer (Triton (1 %), SDS (0.1 %), 0.5 % deoxycholate (0.5 %), EDTA (1 mmol), Tris (20 mM, pH 7.6), NaCl (150 mmol), NaF (10 mmol), and 0.1 mmol/L phenyl methyl sulfonyl fluoride (PMSF, 0.1 mmol)) and centrifuged at 13,000 rpm for 15 min at 5°C. The total protein was estimated (Lowry et al., 1951). Samples encompassing 50 μg of proteins were electrophoresed on a 10.5 % sodium dodecyl sulfate–polyacrylamide gel. The resolved proteins were blot transferred on to PVDF membrane (Millipore). The membranes were kept in blocking buffer with BSA (5 %) for 2.5 h to diminish non-specific binding. The membranes were then incubated with primary antibodies (in Tris-buffered saline and Tween-20 (0.04 %, TBST)), recognizing Kim-1, HO-1 and β-actin (1:1000 dilution in a quaking platform overnight at 5 °C. Then, the membranes were incubated with the related secondary antibodies (anti-rat IgG conjugated to horseradish peroxidase) for 2 h. The membranes were washed thrice with TBST for 20 min. The protein bands were visualized by an enhanced chemiluminescence method using an ECL kit (GenScript ECL kit, USA), scanned and analysed by Image J software (Bethesda, USA).
Statistical analysis

The data were expressed as mean ± standard error of the mean (SEM) and were analysed using one way analysis of variance (ANOVA) followed by Duncan’s Multiple Range Test (DMRT) (Duncan, 1957). A p < 0.05 was considered significant. Statistical analyses were performed using SPSS 12.0 software package, Tokyo, Japan.

3. Results and Discussion

The Western Blot analysis of Kim-1 and HO-1 performed in kidney tissues of control and experimental groups of rats. VMC induced group displayed a increased expression of Kim-1 and HO-1 when compared to control group. RA treatment to VMC-induced group resulted in an decreased expression of Kim-1 and HO-1. RA alone treated group exhibited a similar expression as seen in control group. (Fig.1)

Kim-1 is found in toxic injury of the renal proximal tubule (Han et al., 2002). Several investigators have proposed Kim-1 as an early biomarker of renal diseases involving acute injury of the proximal tubule epithelium (Bailly et al., 2002; Han et al., 2002)in both animal models and humans. Kim-1 expression is not present in the normal kidney and its marked upregulation and its insertion into the apical membrane of the renal proximal tubular cells after injury, its persistence in the renal epithelial tubular cell until the cell has recovered and the rapid cleavage of the ectodomain (Bonventre, 2009). It has been suggested that Kim-1 plays an important role in regenerating damaged epithelial cells and in removal of dead cells from the tubular lumen by phagocytosis.(Ichimura et al., 2008 ; Bonventre, 2009). Transcript levels for the gene that encodes Kim-1 are strongly up regulated in dedifferentiated proximal tubule epithelial cells in kidney after ischemic or toxic injury (Zhou et al., 2008). Our study results showed increased expression of Kim-1 in VMC-induced rats is mainly due to tubulointerstitial damage (Han et al., 2002; Vaidya and Bonventre, 2006; Zhou et al., 2008). However, RA co-administration markedly suppressed its expression in kidney might be due to its protective effect on the renal tubular cells, thus consistent with earlier reports (Zhou et al., 2008).

HO-1, the rate-limiting enzyme in heme catabolism, is important for regulation of the adaptive tissue protective mechanisms against oxidative stress and inflammation. HO-1 is also called as stress-responsive enzyme, responsible for the breakdown of heme to biliverdin, free iron and carbon monoxide and yield the antioxidants to scavenge free radicals (Bataille and Manautou, 2012; Surh and Na, 2008; Maines and Gibbs, 2005). It is induced by a variety of cellular stresses including heme, hyperoxia, hypoxia, and electrophiles (Surh and Na, 2008; Surhet al., 2008). In a model of acute renal damage in rats, bio active compound was found to improve markers of oxidative stress by increased expression of the antioxidant and detoxification enzyme HO-1(Mayo et al., 2003). HO-1 is induced by oxidant stress, and its robust expression provides protection against oxidative insults. The heme molecule is frequently found to be associated with proteins but might be found to be dissociated in different pathological situations. Free or intracellular heme destabilizes membranes and can catalyze the generation of free radicals by reacting with organic hydroperoxides (Gallucci and Malzinger, 2001). Heme degradations products, carbon monoxide, biliverdin/ bilirubin, and iron/ferritin possess potent antioxidant and antiapoptotic properties (Abraham et al., 2009; Agarwal and Nick, 2000; Baranano et al., 2002; Ryter and Choi, 2010). Additionally, biliverdin and bilirubin are involved in HO-1-induced protection against cellular stress by reducing the generation of ROS through their antioxidant activity(Ollinger et al., 2007). Recent reports have indicated that activation of the HO-1 suppresses different inflammatory events including macrophage infiltration (Zager et al., 2007; Petry et al., 2007). Previous report stated that the use of ROS scavengers prevents HO-1 exerted a remarkable protective effect against the oxidative cell damage induced by PMB (Fonseca et al., 2012). In our study also revealed that increased expressions of HO-1 in VMC group, and their expression was significantly decreased.
expressions of HO-1 with RA treatment along with VMC.

The strong antioxidant capacity of RA to decreased expression of HO-1 in this study. Further, the expression pattern was nearly similar in control and RA only treated rats.

![Western Blot Analysis](image)

**Figure - 1: Western Blot analysis of Kim-1 and HO-1**

RA - rosmarinic acid; VMC- vancomycin. Values are mean ± standard error of the mean (SEM) of six rats from each group. 

*Significant as compared to control (p<0.05; ANOVA followed by DMRT). *Significant as compared to VMC (p<0.05; ANOVA followed by DMRT).

5. Conclusion

Our experimental results added evidences that RA protected VMC-induced nephrotoxicity possibly by enhancing protective role of HO-1 in renal antioxidant system and reduce injury due to down regulation of Kim-1. The findings in this study, suggest the potential therapeutic use of RA against VMC-induced nephrotoxicity.

Acknowledgement

This research work is supported by the Indian Council of Medical Research ICMR 45/40/2013-PHA/BMS, New Delhi, India, in the form of Senior Research Fellow to S. Sathishkumar gratefully acknowledged.
Conflict of Interest
The authors declare that there is no conflict of interest

6. References