Chemico-Biological Interactions 270 (2017) 33–40

Contents lists available at ScienceDirect

Chemico-Biological Interactions

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

Lansoprazole halts contrast induced nephropathy through activation of Nrf2 pathway in rats

Sahar A. Khaleel [a, *], Amany A. Alzokaky [a], Nahed A. Raslan [a], Asmaa I. Alwakeel [a], Heba G. Abd El-Aziz [b], Adel R. Abd-Allah [c]

[a] Pharmacology and Toxicology Department, Faculty of Pharmacy (Girls), Al-Azhar University, Cairo, Egypt
[b] Biochemistry Department, Faculty of Pharmacy, Al-Azhar University (Girls), Cairo, Egypt
[c] Pharmacology and Toxicology Department, Faculty of Pharmacy (Boys), Al-Azhar University, Cairo, Egypt

ARTICLE INFO

Article history:
Received 24 October 2016
Accepted 12 April 2017
Available online 13 April 2017

Keywords:
Contrast induced nephropathy
Lansoprazole
Nrf2
Oxidative stress

ABSTRACT

Contrast-induced nephropathy (CIN) is an important cause of acute kidney injury characterized by significant mortality and morbidity. To date, there is no successful protective regimen for CIN especially in poor kidney function patients. Lansoprazole has been shown to exert antioxidant action through induction of nuclear factor-erythroid 2-related factor 2 (Nrf2) pathway. The aim of the present study is to investigate the potential of lansoprazole to activate Nrf2 pathway in the kidney and consequently to protect against oxidative stress induced by iodinated contrast media. Lansoprazole, at a dose of 100 mg/kg, showed a significant induction of Nrf2 mRNA after 3 h. Administration of contrast media induced significant increase in serum creatinine and blood urea nitrogen, histological deterioration, and reduction in total antioxidant capacity. Moreover, it instigated the defensive Nrf2 gene expression and immunoreactivity. In addition, there were overexpression of HO-1, caspase 3, p53 and IL6 genes and downregulation of Bcl2 gene. Pre-treatment with lansoprazole (100 mg/kg) ameliorated the nephrotoxicity parameters and oxidative stress, improved histological lesions, and hijacked apoptotic and inflammatory markers that were provoked by contrast media. In conclusion, lansoprazole attenuates experimental CIN which might be due to activation of Nrf2 antioxidant defence pathway. These findings highlight the potential benefit of incorporating lansoprazole in the protective regimen against CIN especially for susceptible patients.

1. Introduction

Contrast-induced nephropathy (CIN) is an iatrogenic disease occurring after the intravascular injection of iodinated contrast media. It is the third leading cause of acute renal failure in hospitalized patients, contributing to prolonged hospital stay, increased medical costs, and increased mortality rates [1]. A systematic review on the economic burden of CIN in the US, covering the period between 1990 and 2007, showed that the average in-hospital expense of CIN was $10,345, while the one-year expense of treating a patient with CIN was $11,812 [2]. The significance of CIN is speculated to be increased in the future, as more patients will be referred for procedures necessitating intravascular contrast media administration [3].

A number of agents that improve renal circulation have been clinically tested for prevention of CIN, but none of them has succeeded. Prophylactic effects of antioxidants such as *N*-acetylcysteine and ascorbic acid was reported by some investigators [4,5], although the effectiveness of these compounds is still a matter of debate. At present, hydration is regarded as the only effective, albeit incomplete, prophylactic regimen for CIN [6–8].

Although the pathophysiological cellular mechanism of CIN is still not completely elucidated, several mechanisms have been suggested, namely direct toxicity of the contrast, decreased blood flow, renal oxidative stress, apoptosis, and inflammation [9]. Among these processes, reactive oxygen species (ROS)-induced oxidative stress has a crucial role [10,11], representing an important drug intervention target for the prevention of CIN in clinical practice.

Nuclear factor-erythroid 2-related factor 2 (Nrf2) is a redox-sensitive transcription factor that is crucial in regulating the basal

* Corresponding author.
E-mail addresses: sa7ar_22@yahoo.com, sakhaleel@azhar.edu.eg (S.A. Khaleel).

http://dx.doi.org/10.1016/j.cbi.2017.04.010
0009-2797/

and inducible expressions of a battery of antioxidant and cytoprotective genes, counteracting oxidative and electrophilic stress [12]. Nrf2 activity is primarily governed by the cytosolic repressor protein Kelch-like ECH-associated protein 1 (Keap1) which, under normal conditions, sequesters Nrf2 in the cytoplasm and forwards ubiquitination and proteosomal degradation [13]. Keap1 contains several reactive cysteine residues that serve as sensors of intracellular redox state, and acts as a negative regulator of Nrf2. When Keap1 cysteine residues are modified by ROS or electrophiles, Keap1 undergoes conformational changes, leading to the dissociation of Nrf2 from the Keap1/Nrf2 complex and blockade of ubiquitination/proteasomal degradation of Nrf2. As a result, Nrf2 can translocate into the nucleus to activate its target gene transcription [13,14].

Accumulating evidence has revealed that Nrf2 is a vital regulator of cellular defences against various pathological stresses in the kidney, thereby protecting against both acute and chronic kidney injury [15,16]. In fact, transgenic Nrf2 knockout mice exhibit lowered basal and inducible levels of antioxidant-encoding genes and are highly susceptible to pathologies associated with exposure to chemical toxicants such as cisplatin [17]. In addition, ablation of the Nrf2 gene causes a lupus-like autoimmune nephritis and exacerbates diabetes-induced oxidative stress, inflammation, and nephropathy in experimental animals [18,19]. These reports suggest that targeting Nrf2 might be a novel strategy for counteracting oxidative stress in the kidney.

Lansoprazole is a potent proton pump inhibitor used in the treatment and prevention of acid-related diseases such as gastroesophageal reflux disease (GERD), duodenal and gastric ulcers and non-ulcer dyspepsia. It suppresses H^+/K^+-adenosine triphosphatase, thereby reducing the secretion of gastric acid from gastric parietal cells [20]. Interestingly, lansoprazole has shown acid-independent anti-inflammatory effects in the gastrointestinal mucosa mediated via Nrf2 pathway [21]. Moreover, lansoprazole has revealed a protective effect against oxidative damage in the liver by up-regulating Nrf2 pathway [22]. Whether lansoprazole has an effect on Nrf2 pathway in the kidney is a challenging question.

We hypothesize that lansoprazole induces Nrf2 in the kidney and, as a result, protects against acute kidney injury caused by iodinated contrast media.

2. Materials and methods

2.1. Animals

Adult male Wistar rats weighing 200 ± 20 g were purchased from the National Institute for Research, Cairo, Egypt. The animals were kept at controlled environmental conditions in terms of constant temperature (23 ± 1 °C), humidity (60 ± 10%), and a 12/12 h light/dark cycle. They were acclimatized for one week before any experimental procedures and were allowed standard rat chow and water *ad libitum*. The experimental protocol used in this study was approved by the Animal Ethics Committee (No. 69/2016) of the Faculty of Pharmacy, Al-Azhar University, Egypt.

2.2. Chemicals

Lansoprazole, meglumine diatrizoate, *N*-nitro-L arginine methyl ester (L-NAME) and indomethacin were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were of highest purity and analytical grade. Lansoprazole was prepared as a suspension in carboxymethyl cellulose (0.5%).

2.3. Dose-response effect of lansoprazole on Nrf2 gene expression

To select the proper dose of lansoprazole that could induce Nrf2 gene expression significantly, dose response assessment was done as follows; Rats were administered, by gastric intubation, lansoprazole 10, 30, 100 mg/kg or vehicle; each group consisted of 6 rats. Three h after lansoprazole or vehicle administration, rats were sacrificed by cervical dislocation under light ether anesthesia [22]. The kidneys were rapidly dissected out and processed for estimation of Nrf2 and HO-1 mRNA using real time PCR.

2.4. CIN animal model

CIN was induced by a single iv injection of indomethacin (10 mg/kg), double doses of L-NAME (10 mg/kg, iv, at 15 and 30 min) and a single dose of meglumine diatrizoate 60% (6 ml/kg, iv), according to Kedrah et al. [23]. Inhibition of prostanoids and nitric oxide with indomethacin and L-NAME respectively predisposes rats to the nephrotoxic effects of contrast media [24,25].

2.5. Lansoprazole-treatment

Lansoprazole was orally administered at a dose of 100 mg/kg by oral gavage. The dose of lansoprazole was selected from the dose-response assessment test.

2.6. Animal grouping and experimental work

Twenty four rats were randomly divided into 4 groups, consisted of 6 rats in each group, as follows: Group 1; control rats administered 1 ml of 0.5% CMC 3 h before and 24 h after administration of 1 ml of normal saline iv, Group 2; lansoprazole, Group 3 CIN, Group 4 lansoprazole/CIN. The CIN model was induced 3 h post lansoprazole administration in order to synchronize between lansoprazole-induced activation of Nrf2 and contrast media-induced oxidative stress. A second boosting dose of lansoprazole was introduced 24 h after CIN induction. Forty-eight h following CIN induction, blood samples were collected by retro-orbital sinus puncture under mild ether anesthesia and serum was separated for biochemical analysis of serum creatinine and blood urea nitrogen. The samples obtained were stored at −80 °C freezer until analysis. Rats were then sacrificed by cervical dislocation and the kidneys were dissected out. The right kidneys were fixed in 10% neutral buffered formalin for histological assessments and the left kidneys were processed for associated analysis.

2.7. Real time PCR

Expression of mRNA was determined using real time-polymerase chain reaction. Primer sets for each gene are listed in Table 1. Total RNA was extracted from kidney tissues using TRIzol reagent (Invitrogen Corporation, Grand Island, NY, USA) according to the manufacturer's protocol. The purity of obtained RNA was verified spectrophotometrically at 260/280 nm. Equal amounts of RNA (2 μg) were reverse transcribed into cDNA using High-capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. PCR amplification using Step One thermal cycler was carried out in 20 μl reaction mixture consisting of 10 μl SYBR® Green PCR Master Mix (Applied Biosystems, Foster City, CA), 1 μl forward primer (nM), 1 μl reverse primer (nM), 2 μl cDNA, and 6 μl water. GAPDH was used as a reference gene and the expression of mRNA levels in each sample was normalized to GAPDH mRNA levels. The relative expression was calculated from the $2^{-\Delta\Delta CT}$ formula [26].