Biochemistry and Biophysics Reports 5 (2016) 313–327

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

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

Mangiferin attenuates oxidative stress induced renal cell damage through activation of PI3K induced Akt and Nrf-2 mediated signaling pathways

Sukanya Saha [1], Pritam Sadhukhan [1], Krishnendu Sinha, Namrata Agarwal, Parames C. Sil *

Division of Molecular Medicine, Bose Institute, P-1/12, CIT Scheme VII M, Kolkata 700054, India

ARTICLE INFO

Article history:
Received 25 September 2015
Received in revised form
28 December 2015
Accepted 12 January 2016
Available online 14 January 2016

Keywords:
tBHP
Oxidative impairment
Renal disorder
Apoptosis
Mangiferin
Antioxidant
Nephroprotection

ABSTRACT

Background: Mangiferin is a polyphenolic xanthonoid with remarkable antioxidant activity. Oxidative stress plays the key role in tert-butyl hydroperoxide (tBHP) induced renal cell damage. In this scenario, we consider mangiferin, as a safe agent in tBHP induced renal cell death and rationalize its action systematically, in normal human kidney epithelial cells (NKE).
Methods: NKE cells were exposed to 20 μM mangiferin for 2 h followed by 50 μM tBHP for 18 h. The effect on endogenous ROS production, antioxidant status (antioxidant enzymes and thiols), mitochondrial membrane potential, apoptotic signaling molecules, PI3K mediated signaling cascades and cell cycle progression were examined using various biochemical assays, FACS and immunoblot analyses.
Results: tBHP exposure damaged the NKE cells and decreased its viability. It also elevated the intracellular ROS and other oxidative stress-related biomarkers within the cells. However, mangiferin dose dependently, exhibited significant protection against this oxidative cellular damage. Mangiferin inhibited tBHP induced activation of different pro-apoptotic signals and thus protected the renal cells against mitochondrial permeabilization. Further, mangiferin enhanced the expression of cell proliferative signaling cascade molecules, Cyclin d1, NFκB and antioxidant molecules HO-1, SOD2, by PI3K/Akt dependent pathway. However, the inhibitor of PI3K abolished mangiferin's protective activity.
Conclusions: Results show Mangiferin maintains the intracellular anti-oxidant status, induces the expression of PI3K and its downstream molecules and shields NKE cells against the tBHP induced cytotoxicity.
General significance: Mangiferin can be indicated as a therapeutic agent in oxidative stress-mediated renal toxicity. This protective action of mangiferin primarily attributes to its potent antioxidant and antiapoptotic nature.

© 2016 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Nephropathy or impairment of kidney function is closely associated with renal toxicity. This toxicity may occur due to various endogenous and exogenous toxins (like environmental pollutants, drugs, metals, etc.) leading to physiological dysfunctions accompanying a wide variety of symptoms [1]. Scientific evidence suggests oxidative insult to be one of the primary phenomena leading to renal dysfunction [2,3], a very eminent problem of the modern day world. So, making a nephrotoxic *in vitro* model by intoxicating renal cells with an oxidative stress causing agent is rational. An organic peroxide, tert-butyl hydroperoxide (t-BHP) is widely used as a classic inducer of oxidative stress in many studies [4,5]. tBHP is a major environmental pollutant causing increased ROS (Reactive Oxygen Species) formation in cells and is metabolized by two independent pathways either by the formation of end products like peroxyl and alkoxyl radicals [6] or oxidized glutathione (GSSG) [7,8]. Thus, whichever may be the pathway of its metabolism, the ultimate outcome is the induction of oxidative injury. This molecule even leads to programmed cell death or apoptosis in different cells and tissues [9–11]. In addition, tBHP is also known to induce renal lesions, thus acting as a nephropathic agent also [12,13]. So, the administration of this exogenous oxidative stress inducer may simulate a situation of augmented oxidative insult and damage in normal kidney epithelial cells and helps us to understand particular mechanisms in the pathogenesis of oxidative stress induced nephropathy.

Interestingly, mammalian cells posess defense mechanisms (both enzymatic and non-enzymatic) to prevent ROS formation or

* Corresponding author.
E-mail addresses: parames@jcbose.ac.in, parames_95@yahoo.co.in (P.C. Sil).
[1] These authors have contributed equally.

http://dx.doi.org/10.1016/j.bbrep.2016.01.011
2405-5808/© 2016 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

to detoxify the already produced ROS [14]. However, these multifunctional defensive systems cannot totally counteract the deadly effects of amplified ROS, an outcome observed in different pathophysiological states [15], i.e. the physiologically available amount of antioxidant enzymes is not sufficient to counteract pro-oxidants in most cases. Thus targeting the imbalance between antioxidants and pro-oxidants in cells, i.e. oxidative stress, seems to be a logical approach for treating such disorders with possibly fruitful outcome. Therefore, external supplements, having antioxidant property that can increase the level and activities of endogenous antioxidants [16–18] can be proposed as therapeutic agents to combat ROS overproduction [19,20].

Herbal antioxidants have gained special attention in the contemporary scientific community because of the acuity about their lower toxicities in comparison to synthetic molecules [21–27]. Though quite a number of pharmaceutical molecules have originated from the phytoenvironment or been derived from tissues of plants, still they represent a comparatively unexploited source of potentially novel compounds. Polyphenols are the predominant group of natural antioxidants showing powerful antioxidant activity due to their ability of inducing gene expressions of antioxidant enzymes, and having properties like free radical scavenging, hydrogen donating, singlet oxygen quenching and so on [28–31]. In particular, mangiferin (2-C-β-D-glucopyranosyl-1,3,6,7-tetrahydroxyxanthone), derived commonly from the bark and leaves of plants belonging to Anacardiaceae and Gentianaceae families, may provide a unique and underutilized source of potential therapeutic agent [32]. This naturally occurring polyphenol is mainly isolated from the widely distributed mango tree (*Mangifera indica*) and has been in use long back even before its characterization was done, in different corners of the world, to treat disorders like melancholia, bleeding dysentery etc [33,34]. Gradually different studies revealed mangiferin's antioxidant [35,36], antitumor [32,37], antidiabetic [38,39] and immunomodulatory activities [40]. This xanthone possess the ability to scavenge ROS, a causal inducer of oxidative stress, inhibit lipid peroxidation, and increase the reduced glutathione content, thus establishing itself as a good antioxidant. Moreover, it can modulate the expression of a number of apoptosis-related genes playing very important role in regulating apoptosis.

The above-mentioned therapeutic claims made about mangiferin and some previous studies of our laboratory [38,39,41] in accordance with these claims encouraged us to evaluate its antioxidant potential in oxidative stress related nephropathy. So far, there is no study describing the protective role of mangiferin in tBHP -induced, oxidative stress mediated nephrotoxicity in normal kidney epithelial cells (NKE). In the present study, we investigate whether this polyphenol was effective in attenuating the nephrotoxicity and its mechanism of protective action, promoting better understanding of the antioxidant as well as nephroprotective properties of mangiferin. We also show that the generation of oxygen radicals by tBHP was successfully neutralized by mangiferin thereby protecting the kidney cells via PI3K/Akt pathway from oxidative stress mediated pathophysiology.

2. Materials and methods

2.1. Materials and reagents

RPMI-1640 media and other necessary chemicals like antibiotics, amino acids etc were purchased from HIMEDIA (Mumbai, India) and fetal bovine serum (FBS) from HyClone (Thermo Scientific Hy-Clone, Logan, Utah), respectively. tBHP was purchased from Sigma-Aldrich and Methylthiazolyldiphenyl-tetrazolium bromide (MTT) were purchased from Sisco Research Laboratory (Mumbai, India). Fluorescein isothiocyanate (FITC) conjugated Annexin V, Apoptosis detection kit, RNaseA, Bradford reagent, Luminol, Coumaric acid and LY294002 (a PI3K inhibitor) were also purchased from Sigma (Missouri, USA). Antibodies such as anti Caspase-3 (ab47131), anti Caspase-8 (ab25901), anti Bid (ab77815), anti Bcl2 (ab7973), anti cytochrome c (ab76237), anti Nrf2 (ab31163), anti HO1 (ab13243), anti SOD2 (ab13533), Phospho JNK (ab4821), anti Bax (ab32503), anti PI3k (ab74136), anti Akt (ab17785), Phospho Akt (ab23509), HRP (ab97051) were purchased from Abcam (Cambridge, UK). Phospho BAD (#9291), anti mTOR (#2983), anti GSK 3β (5338), anti Cyclin D1 (#2978), anti PARP (#9532), anti Caspase-9 (#9508), anti Apaf-1 (#8723), anti Bax (#2772), Anti NFκB (#3034), anti β-Actin (#4970), anti Lamin B1 (#12586) was purchased from Cell Signaling Technology (Danvers, MA 01923). Other essential chemicals used in this study were of the analytical grade.

2.2. Free radical scavenging activity of mangiferin

Mangiferin used in the present study was isolated, purified and characterized in our laboratory [38]. To evaluate the free radical scavenging potential of mangiferin and compare it to the popular antioxidant Vitamin C (Vit C), DPPH radical scavenging activity was performed in the cell-free system. The assay was performed according to the method of Blois [42]. Two ml of DPPH solution (125 μM) in methanol and 2 ml of tested samples with different concentrations (10-160) μM of mangiferin and Vit C were mixed in the tubes. The solution was incubated at room temperature for 30 min in the dark. Then the absorbance was measured at 517 nm against methanol blank using a spectrophotometer. Vit C was used as a positive control for this experiment.

2.3. 2.3. Assay of ferric reducing property

FRAP (Ferric reducing antioxidant power) assay was done according to the protocol described by Benzie et al. [43]. Briefly FRAP solution was prepared in dark at 37 °C by mixing 300 mM acetate buffer of pH = 3.6, 10 mM TPTZ in 10 mM HCl and 20 mM $FeCl_3 \cdot 6H_2O$ in a volumetric ratio of 10:1:1. Mangiferin was added to the final concentration ranging (10–200 μM) with 1 ml of FRAP solution warmed to 37 °C. The final solution was mixed well and incubated for 30 min at 37 °C. The Absorbance was measured spectrophotometrically at 595 nm. For positive control, we used Vit C in this experiment.

2.4. Cell culture

The normal kidney epithelial (NKE) cell line was derived from the uninvolved kidney tissue of a patient with renal cell carcinoma. The cells were immortalized by human telomerase subunit via transduction [44,45]. NKE cells were maintained at 37 °C in RPMI medium supplemented with 10% FBS and antibiotics in in75-cm^2 culture flasks in a humidified incubator enriched with 5% CO_2. Confluent monolayers (80%) of NKE were subjected to different treatments used in the studies undertaken.

2.5. Assessment of cell viability upon mangiferin treatment and tBHP exposure

Cell viability of NKE cells was determined by the MTT assay following the methods of Sinha et al. [46,47]. The cells were seeded at a density of 5×10^4 cells/well in a 96-well culture plates with 100 μl media/well. Mangiferin was dissolved in 0.1% DMSO and applied to cells at desired concentrations. Normal cells were treated with vehicle control. After the treatment with the desired compound for specified time, the media was discarded, and the

Download English Version:

https://daneshyari.com/en/article/1941736

Download Persian Version:

https://daneshyari.com/article/1941736

Daneshyari.com