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Taurine protects HK-2 cells from oxidized LDL-induced cytotoxicity via the ROS-mediated mitochondrial and p53-related apoptotic pathways

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ABSTRACT

Oxidized LDL (oxLDL) induces a pro-oxidative environment and promotes apoptosis, causing the progression of renal diseases in humans. Taurine is a semi-essential amino acid in mammals and has been shown to be a potent endogenous antioxidant. The kidney plays a pivotal role in maintaining the balance of taurine. However, the mechanisms underlying the protective effects of taurine against oxLDL-induced injury in renal epithelial cells have not been clarified. In the present study, we investigated the anti-apoptotic effects of taurine on human proximal tubular epithelial (HK-2) cells exposed to oxLDL and explored the related mechanisms. We observed that oxLDL increased the contents of ROS and of malondialdehyde (MDA), which is a lipid peroxidation by-product that acts as an indicator of the cellular oxidation status. In addition, oxLDL induced cell death and apoptosis in HK-2 cells. Pretreatment with taurine at 100 μM significantly attenuated the oxLDL-induced cytotoxicity. We determined that oxLDL triggered the phosphorylation of ERK and, in turn, the activation of p53 and other apoptosis-related events, including calcium accumulation, destabilization of the mitochondrial permeability and disruption of the balance between pro-apoptotic Bax and anti-apoptotic Bcl-2 proteins. The malfunctions induced by oxLDL were effectively blocked by taurine. Thus, our results suggested that taurine exhibits potential therapeutic activity by preventing oxLDL-induced nephrotoxicity. The inhibition of oxLDL-induced epithelial apoptosis by taurine was at least partially due to its anti-oxidant activity and its ability to modulate the ERK and p53 apoptotic pathways.

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Introduction

Reactive oxygen species (ROS) comprise both free radical (O_2^-, OH, HO_2 and RO) and non-radical (molecular) forms (H_2O_2 and $^1O_2$). ROS are produced continuously as the by-products of various metabolic pathways that are localized in different cellular compartments, such as chloroplasts, mitochondria and peroxisomes (del Río et al., 2006; Gill and Tuteja, 2010). Oxidative stress has been found to play a key role in the initiation of cardiovascular and neurological diseases and of the complications of renal failure (Foyer and Noctor, 2005; Galle et al., 1999; Klahr, 1997; Martín-Mateo et al., 1999). The kidney is highly vulnerable to damage caused by ROS due to the abundance of polyunsaturated fatty acids (PUFAs) among the renal lipids (Esterbauer et al.,

Abbreviations: SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; MDA, malondialdehyde; ROS, reactive oxygen species; O_2^-, superoxide radicals; OH, hydroxyl radical; HO_2, perhydroxy radical; RO, alkoxy radicals; H_2O_2, hydrogen peroxide; $^1O_2$, singlet oxygen; MAPK, mitogen-activated protein kinases; SAPK, stress-activated protein kinases; ERKs, extracellular signal-regulated kinases.

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1991). ROS induce DNA oxidation, protein nitration and lipid peroxidation, all of which are involved in the pathophysiological processes underlying renal dysfunction (Baliga et al., 1999; Klahr, 1997; Paller et al., 1998). Clinical and experimental evidences of the types of renal damage mediated by ROS indicated that they can be grouped into glomerular, tubulointerstitial and endothelial alterations (Baliga et al., 1999; Gwinner et al., 1998; Martín-Mateo et al., 1999). Therefore, the scavenging and/or depuration of ROS (through dietary and pharmacological antioxidants) should attenuate or prevent oxidative stress, thus mitigating the subsequent damage to renal cells.

Dietary cholesterol intake is a known risk factor for various diseases, including cardiovascular disease, atherosclerosis, hypercholesterolemia, steatohepatitis and acute or chronic renal disease (Freeman and Crapo, 1982; Irani, 2000; Klahr, 1997). The oxidative stress induced by oxidized low-density lipoproteins (oxLDLs) plays an important role in the pathogenesis of vascular-cell dysfunction (Galle et al., 1999; Irani, 2000; Ou et al., 2010). The oxLDL-induced pathophysiological signals involving ROS generation and the alteration of intracellular Ca^{2+} ion homeostasis are now considered major contributors to the development of vascular diseases (Irani, 2000; Maziere et al., 2005). Moreover, oxLDL-induced cellular oxidative stress modulates the functioning of a series of signaling pathways, including activating the cellular suicide pathway, dysregulating mitochondria and endoplasmic reticulum and promoting inflammation. Most of these pathways are potentially cytotoxic (Cominacini et al., 2000; Giovannini et al., 2002; Irani, 2000; Napoli et al., 2000; Rovin and Tan, 1993; Salvayre et al., 2002; Steinberg, 1997). Thus, oxLDL has been recognized as giving rise to renal pathogenesis. In the kidney, the process of inflammation has been associated with macrophage infiltration (Ou et al., 1999; Rovin and Phan, 1998). The proximal tubular epithelial cells are thought to mediate interstitial macrophage infiltration because of their anatomic position and their ability to produce chemotactic cytokines, chemokines and other inflammatory mediators (Agarwal et al., 1996; Massy et al., 1999; Rovin and Phan, 1998). The accumulation of macrophages within the interstitial space of the renal cortex plays a pathogenic role in the development of tubular injury and interstitial fibrosis in progressive chronic renal diseases (Massy et al., 1999; Rovin and Tan, 1993). Furthermore, it was suggested that ROS induced the expression of the inflammatory mediator genes in kidney tubular epithelial cells, resulting in the recruitment of leukocytes and the promotion of inflammation (Ou et al., 1999; Rovin and Phan, 1998). Hence, epithelial dysfunction plays a central role in the progression of renal diseases, and apoptosis is emerging as a determinant process in the progression of renal diseases.

Taurine (2-aminoethanesulfonic acid) occurs naturally in food, particularly in seafood and meat. It is a major constituent of bile and can be found in the large intestine and in the tissues of many animals, including humans. Taurine has many fundamental biological roles, such as in the conjugation of bile acids and xenobiotics, antioxidation, osmoregulation, membrane stabilization and the modulation of calcium signaling, as well as in participating in the development of skeletal muscle, retinal tissue and the central nervous system (Bouckenooghe et al., 2006; Huxtable, 1992; Marcinkiewicz and Kontny, 2012). Taurine is a semi-essential amino acid that is synthesized in the mammalian pancreas via the cysteine sulfinic acid pathway (Brosnan and Brosnan, 2006). Due to the high solubility of taurine in aqueous solutions, this amino acid has been shown to be a potent endogenous antioxidant. Moreover, supplementation with taurine prevented the oxidative stress induced by exercise (Zhang et al., 2004). Under steady-state conditions, ROS are scavenged through various renal antioxidative defense mechanisms (Gill and Tuteja, 2010; Ishikawa et al., 1994). Taurine was found to protect against renal damage by decreasing the production of oxidative stress and enhancing the activities of antioxidative enzymes in various animal models (Guz et al., 2007; Gwinner et al., 1998; Paller et al., 1998; Sener et al., 2005). Diabetic nephropathy is one major microangiopathy that occurs in diabetes mellitus, often leading to end-stage renal failure (Bryla et al., 2003). Taurine was shown to have protective or preventive effects on many types of diabetic microangiopathies, such as diabetic cardiomyopathy and polyneuropathy, via different mechanisms (Li et al., 2005). Previous studies revealed the functions of taurine; however, the detailed molecular mechanisms underlying these functions were rarely investigated. Recently, studies of taurine have focused on its apoptosis-related function (Kim and Kim, 2013; Setyarani et al., 2014). In the present study, the human proximal tubular epithelial (HK-2) cell line was used as a model to demonstrate the potential role of taurine in mitigating oxLDL-induced epithelial cell damage. The goals of this study were to investigate the cytoprotective activities of taurine and to explore the underlying molecular mechanisms. We therefore sought to determine whether the treatment of taurine attenuated the oxLDL-induced cytotoxicity involving ROS-mediated apoptotic signaling pathways in renal epithelial cells.

Materials and methods

Materials

Taurine (2-aminoethanesulfur acid), crystal violet, glutaraldehyde and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma (St. Louis, MO, USA). Acetoxymethyl ester form of bis (aminophenoxy) ethan-N,N,N′,N′-tetraacetic acid (BAPTA-AM), Dulbecco's modified Eagle's medium (DMEM)/F12 medium, 4′-6-Diamidino-2′-phenylindole (DAPI), 2′, 7′-dichlorofluorescein diacetate (DCF-DA), Fluo-4-AM, propidium iodide (PI) were purchased from Invitrogen (Auckland, NY, USA). Deoxynucleotidyl transferase (TdT) mediated dUTP nick end-labeling (TUNEL) staining kit was obtained from Roche Diagnostics (Boehringer Mannheim, Mannheim, Germany). The SOD and thiobarbituric acid-reactive substance (TBARS) assay kit were obtained from Cayman Chemical (Ann Arbor, MI, USA). The Annexin V-FITC/PI apoptosis detection kit was obtained from BD Biosciences Pharmingen (San Diego, CA, USA). The 5,58,6,68-tetraethyl-benzimidazol-carbocyanine iodide (JC-1) and Caspase 3/CRP32 colorimetric assay kits were obtained from BioVision (Mountain View, CA, USA). The primary antibodies against cytochrome *c*, COX IV and β-actin were purchased from Abcam (Cambridge, MA, USA) and antibodies against cleaved caspase 9, cleaved caspase 3, p53, phospho-p53, ERK1/2, phospho-ERK1/2 and U0126 were purchased by Cell Signaling Technology (Danvers, MA, USA). All other chemicals were of analytical grade or purer.

Cell culture and treatment

HK-2 cells, a human renal proximal tubule cell line, were obtained from the Food Industry Research and Development Institute (Hsinchu, Taiwan). Cells were subcultured every 3–4 days in 100-mm dishes and grown in DMEM/F12 medium containing 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin, pH 7.4, at 37 °C in a humidified 5% CO_2 atm incubator (RCO-3000T, Thermo scientific revco, Asheville, NC, USA). For experimental use, HK-2 cells were subcultured at 70–80% confluence and incubated at 37 °C for 16 h. The cells were harvested at the end of treatment for further analysis. According to different experiments, HK-2 cells were grown to subconfluence in normal growth medium. Then the cells were switched to medium without serum for another 16 h. Pretreatment of taurine was carried out 2 h prior to the application of oxidized LDL. Control cells were not treated with anything in this study.

Lipoprotein separation and oxidation

The methods are modified from Ou et al., (2010). Native LDL will be isolated from fresh normolipidemic human serum by sequential ultracentrifugation ($\rho = 1.019–1.063$ g/ml). Cu^{2+}-modified LDL will be prepared by the exposure of the LDL to 10 μM $CuSO_4$ for 24 h at 37 °C. The extent of oxidation will be monitored using a TBARS assay (defined as

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