Food Chemistry 141 (2013) 1147–1157

Contents lists available at SciVerse ScienceDirect

Food Chemistry

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

Tyrosol exerts a protective effect against dopaminergic neuronal cell death in *in vitro* model of Parkinson's disease

Pradeep Dewapriya [a], S.W.A. Himaya [b], Yong-Xin Li [b], Se-Kwon Kim [a,b,*]

[a] Marine Biochemistry Laboratory, Department of Chemistry, Pukyong National University, Busan 608-737, Republic of Korea
[b] Marine Bioprocess Research Center, Pukyong National University, Busan 608-737, Republic of Korea

ARTICLE INFO

Article history:
Received 15 December 2012
Received in revised form 1 April 2013
Accepted 4 April 2013
Available online 13 April 2013

Keywords:
Tyrosol
Antioxidant
Neuroprotection
Parkinson's disease
Oxidative stress

ABSTRACT

Experimental evidence suggests that tyrosol [2-(4-hydroxyphenyl)ethanol] exhibits potent protective activities against several pathogeneses. In this study, we evaluated the protective effect of tyrosol against 1-methyl-4-phenylpyridinium (MPP^+)-induced CATH.a neuron cell death. Tyrosol dose-dependently protected CATH.a cells from MPP^+-induced cell death and the protection was more apparent after prolong incubation (48 h). The data showed that tyrosol treatment suppressed the reduction of phospho-tyrosine hydroxylase level in CATH.a cells. Further, the compound repressed MPP^+-induced depletion of mitochondrial membrane potential ($\Delta\psi_m$) and thereby maintained intracellular ATP production in the cell. The cellular signalling pathway studies revealed that tyrosol protected CATH.a cells from MPP^+-induced apoptotic signalling, most likely *via* activation of PI3K/Akt signalling pathway along with up-regulation of anti-oxidative enzymes (SOD-1 and SOD-2) and DJ-1 protein in the cell. Collectively, present study demonstrates that tyrosol significantly protects dopaminergic neurons from MPP^+-induced degradation, and reveals potential neuroprotective mechanism of tyrosol.

1. Introduction

Dietary antioxidant intake plays a pivotal role in prevention of chronic diseases due to its ability to neutralise cellular oxidative stress, which leads to oxidation of cellular macromolecules (Leopoldini, Russo, & Toscano, 2011; Serafini, 2006). Tyrosol, 4-(2-hydroxyethyl)phenol (Fig. 1A), is a biologically active molecule, which comes under the category of natural phenolic antioxidants, and has gained increasing attention as a dietary antioxidant. The principle source of tyrosol in the human diet is olive oil, but many other food sources of tyrosol have been reported (Mazzotti et al., 2012; Vissers, Zock, & Katan, 2004). Several *in vitro* chemical studies have pointed out that tyrosol shows a weaker anti-oxidative strength than its hydroxyl form, hydroxytyrosol [(2-(3′,4′-dihydroxyphenyl)ethanol), due to the absence of the *ortho*-diphenolic group in its chemical structure (Mateos, Domínguez, Espartero, & Cert, 2003; Rietjens, Bast, Vente, & Haenen, 2007). Therefore, many attempts have been made to evaluate the biological importance of hydroxytyrosol while giving lesser attention to tyrosol (Fernández-Mar, Mateos, García-Parrilla, Puertas, & Cantos-Villar, 2012).

However, in several cellular experimental paradigms, it has been observed that tyrosol shows potent biological activities regardless of its weak anti-oxidative strength (Giovannini et al., 1999; Samuel, Thirunavukkarasu, Penumathsa, Paul, & Maulik, 2008; Sun et al., 2012). Moreover, many foods, such as virgin olive oil, wine and natural extracts containing tyrosol as a major constituent have shown great protection against many pathological conditions (Bertelli et al., 2002; Palumbo, Occhiuto, Spadaro, & Circosta 2012). Interestingly, Cañuelo et al. (2012) reported that tyrosol increases lifespan and stress resistance in the nematode *Caenorhabditis elegans*. This finding could be related to the high longevity associated with the Mediterranean diet, more specifically, olive oil and wine. In biological systems, tyrosol is readily absorbed and retained for a prolonged period. Hydroxytyrosol is also absorbed dose-dependently, but quickly excreted with urine and disappears after few hours (Benedetto et al., 2007; Miró-Casas et al., 2003). This has been postulated as one of the reasons why tyrosol is more active than hydroxytyrosol for certain biological processes in a cellular system. Vauzour, Corona, and Spencer (2010) reported that tyrosol protects 5-*S*-cysteinyl-dopamine induced neurotoxicity to a similar extent seen in flavonoids. An *in vivo* analysis of the protective activity of tyrosol against ischaemic brain injury has shown that rats treated with 30 mg/kg of tyrosol exhibited a significant protective effect against sensory motor dysfunction (Bu et al., 2007).

Even though the protective effect of tyrosol against neuronal degradation has been reported, molecular level detailed investigations

* Corresponding author at: Marine Biochemistry Laboratory, Department of Chemistry, Pukyong National University, Busan 608-737, Republic of Korea. Tel.: +82 51 629 7094; fax: +82 51 629 7099.
E-mail address: sknkim@pknu.ac.kr (S.-K. Kim).

0308-8146/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2013.04.004

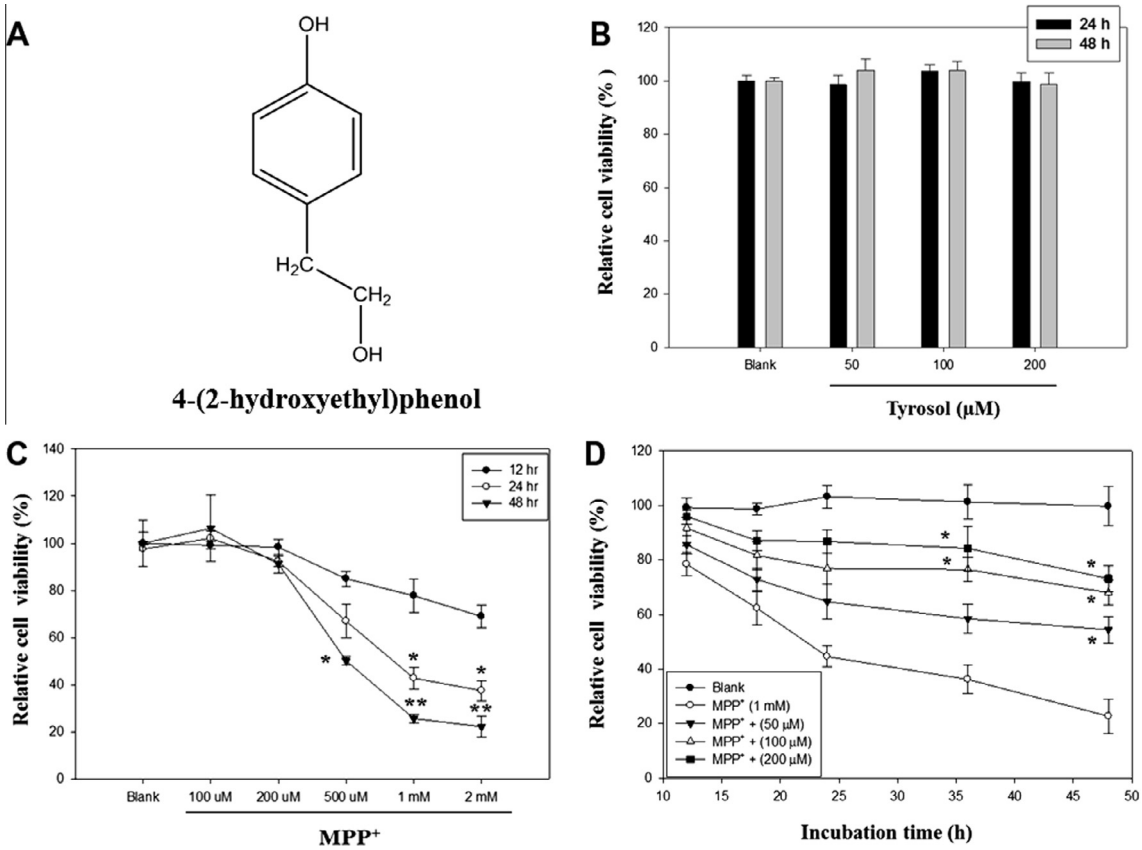

Fig. 1. Effect of tyrosol on MPP^+-induced cytotoxicity in CATH.a cells. (A) The chemical structure of tyrosol. (B) Cytocompatibility of working concentrations of tyrosol evaluated at 24 h and 48 h of incubation. (C) Time bound effect of MPP^+ concentrations (100 μm, 200 μm, 500 μm, 1 mM, and 2 mM) on viability of CATH.a cells assessed using MTT assay. (D) Effect of tyrosol on MPP^+-induced CATH.a cell death. Cell viability were compared and presented as a percentage ± SD of the blank group. $^*(p < 0.05)$ and $^{**}(p < 0.01)$ indicate that treatments were significantly different.

on mechanism of the protection remain unexplored. In the present study we aimed to further investigate the potential neuroprotective ability of tyrosol and the molecular mechanism of the protection, with reference to Parkinson's disease (PD). The depigmentation of the substantia nigra pars compacta (SNpc) caused by the selective and progressive loss of dopaminergic (DA) neurons and presence of intraneuronal proteinaceous inclusions known as Lewy bodies (LBs) within the surviving neurons of the SNpc and other brain regions have been identified as characteristic features of PD. The most common treatments available for Parkinson's disease only control disease progression, but do not reverse the disease. Therefore, disease preventive approaches are of great interest. Here, we tried to evaluate the potential of tyrosol as a preventive agent of PD using an *in vitro* model. Natural preventive medicines that can prevent the development of PD and protect the neurons from progressive loss have great value over synthetic chemicals (Goldenberg, 2008). The oxidative and nitrosative stress developed in dopaminergic neurons due to mitochondrial dysfunction have been identified as one of the main reasons behind the neuron loss in PD (Zhao, 2009). In order to evaluate the protective effect of tyrosol against oxidative and nitrosative stress in dopaminergic neurones, the parkinsonian toxin 1-methyl-4-phenylpyridinium (MPP^+) was used as the inducer of neuronal death. This mitochondrial toxin selectively enters into dopamine-producing neurones through dopamine transporters and blocks the mitochondrial electron transporter chain and, thereby, produces oxidative radicals in neurons, which ultimately cause neuronal death (Schapira, 2008). Further, blockage of the respiratory chain by MPP^+ drastically reduces intracellular ATP levels, which have been observed as a unique cause of mid-brain neuronal death (Double, Reyes, Werry, & Halliday, 2010). In this study, experiments were performed to investigate the protective effect of tyrosol against oxidative and nitrosative stress in catecholaminergic neuron cell CATH.a (ATCC; CRL-11179).

2. Materials and methods

2.1. Materials

Mouse brain-derived catecholaminergic neuron cells, CATH.a (ATCC; CRL-11179), were purchased from ATCC (American Type Culture Collection, Manassas, VA). Tyrosol was purchased from Sigma–Aldrich Inc (St Louis, MO). RPMI 1640 cell culture medium, foetal bovine serum (FBS), horse serum (HS), penicillin, streptomycin, trypsin, phosphate-buffered saline (PBS), and supplements were obtained from Gibco® Life Technologies (Heidelberg, Germany). Primary and secondary antibodies used for western blot analysis were purchased from Cell Signaling Technology Inc. (Danvers, MA) and Santa Cruz Biotechnology Inc. (Santa Cruz, CA). Dimethyl sulfoxide (DMSO), MPP^+, 2′,7′-dichlorofluorescin diacetate (DCFH-DA), Griess reagent, MTT reagent [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide], 4′,6-diamidino-2-phenylindole (DAPI), paraformaldehyde, Tween 20, secondary antibody conjugated with Alexa Fluor® 488, and fluoroshield mounting media were purchased from Sigma–Aldrich Inc. Annexin V fluorescein isothiocyanate (FITC) apoptosis detection kit was from BD Bioscience (Franklin Lakes, NJ). BCA Protein Assay Kit used for western blot analysis was from Thermo Scientific (Waltham, MA). MitoCapture™ apoptosis detection kit, ATP colorimetric/fluorometric assay kit, and mitochondria/cytosol