Chemico-Biological Interactions 234 (2015) 96–104

Contents lists available at ScienceDirect

Chemico-Biological Interactions

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

Protective effects of ferulic acid and related polyphenols against glyoxal- or methylglyoxal-induced cytotoxicity and oxidative stress in isolated rat hepatocytes

Abdullah Al Maruf [1], HoYin Lip [1], Horace Wong, Peter J. O'Brien *

Graduate Department of Pharmaceutical Sciences, Leslie L. Dan Faculty of Pharmacy, University of Toronto, Canada

ARTICLE INFO

Article history:
Available online 18 November 2014

Keywords:
Glyoxal
Methylglyoxal
Oxidative stress
Polyphenols
Ferulic acid
Isolated rat hepatocytes

ABSTRACT

Glyoxal (GO) and methylglyoxal (MGO) cause protein and nucleic acid carbonylation and oxidative stress by forming reactive oxygen and carbonyl species which have been associated with toxic effects that may contribute to cardiovascular disease, complications associated with diabetes mellitus, Alzheimer's and Parkinson's disease. GO and MGO can be formed through oxidation of commonly used reducing sugars e.g., fructose under chronic hyperglycemic conditions. GO and MGO form advanced glycation end products which lead to an increased potential for developing inflammatory diseases. In the current study, we have investigated the protective effects of ferulic acid and related polyphenols e.g., caffeic acid, *p*-coumaric acid, methyl ferulate, ethyl ferulate, and ferulaldehyde on GO- or MGO-induced cytotoxicity and oxidative stress (ROS formation, protein carbonylation and mitochondrial membrane potential maintenance) in freshly isolated rat hepatocytes. To investigate and compare the protective effects of ferulic acid and related polyphenols against GO- or MGO-induced toxicity, five hepatocyte models were used: (a) control hepatocytes, (b) GSH-depleted hepatocytes, (c) catalase-inhibited hepatocytes, (d) aldehyde dehydrogenase (ALDH2)-inhibited hepatocytes, and (e) hepatocyte inflammation system (a non-toxic H_2O_2-generating system). All of the polyphenols tested significantly decreased GO- or MGO-induced cytotoxicity, ROS formation and improved mitochondrial membrane potential in these models. The rank order of their effectiveness was caffeic acid ~ ferulaldehyde > ferulic acid > ethyl ferulate > methyl ferulate > *p*-coumaric acid. Ferulic acid was found to decrease protein carbonylation in GSH-depleted hepatocytes. This study suggests that ferulic acid and related polyphenols can be used therapeutically to inhibit or decrease GO- or MGO-induced hepatotoxicity.

1. Introduction

Glyoxal (GO) and methylglyoxal (MGO) cause protein and nucleic acid carbonylation and oxidative stress by forming reactive oxygen (ROS) and carbonyl species (RCS). RCS has also been associated with toxic effects that may contribute to cardiovascular disease, complications associated with diabetes mellitus, Alzheimer's and Parkinson's disease [1,2]. These reactive dicarbonyls (GO and MGO) can be formed through the oxidation of commonly used reducing sugars e.g., fructose under chronic hyperglycemic conditions. Moreover, endogenous MGO can also be formed from the triose phosphate intermediates of glycolysis [3,4]. Abnormal glucose metabolism often leads to glucose intolerance and hyperglycemia in diabetes mellitus patients. These conditions overwhelm the endogenous antioxidant and detoxifying systems and result in oxidative stress.

GO and MGO (structures are presented in Fig. 1) covalently bind to free amino and thiol groups of biomolecules and form reversible Schiff bases [2]. The Schiff bases form irreversible Amadori products and advanced glycation end products (AGEs) which can lead to an increased potential for developing inflammatory diseases [5]. AGEs have been implicated in the pathogenesis of many diabetic complications such as atherogenesis, nephropathy, and cataractogenesis [6,7]. Agents e.g., metformin, aminoguanidine,

Abbreviations: ALDH2, aldehyde dehydrogenase; BSA, bovine serum albumin; DNPH, 2,4-dinitrophenylhydrazine; GO, glyoxal; GSH, reduced glutathione; MGO, methyl glyoxal; MMP, mitochondrial membrane potential; ROS, reactive oxygen species.

* Corresponding author at: Leslie L. Dan Faculty of Pharmacy, University of Toronto, Toronto, Ontario M5S 3M2, Canada. Tel.: +1 (416) 978 2716; fax: +1 (416) 978 8511.

E-mail address: peter.obrien@utoronto.ca (P.J. O'Brien).

[1] Co-first author.

http://dx.doi.org/10.1016/j.cbi.2014.11.007
0009-2797/

N-acetyl cysteine etc. for decreasing AGE formation have largely focused on inhibiting Schiff base adduct formation by preventing RCS [8–11].

GO or MGO increased cytotoxicity, ROS formation, DNA oxidation, protein carbonylation, and decreased mitochondrial membrane potential (MMP) in a concentration and time dependent manner in isolated rat hepatocytes [1,3,8]. Glyoxalase I was found to be the main detoxification enzyme for MGO while it played a very minor role in detoxifying GO [12]. Moreover, GO and MGO can also be metabolized by aldehyde dehydrogenase (ALDH2). GO metabolism relied on ALDH2 whereas glyoxalase I played a more important role than ALDH2 in MGO's detoxification [3,13,14]. Additional hydrogen peroxide (H_2O_2) from either endogenous catalase inhibition or from an exogenous source such as glucose/glucose oxidase (a non-toxic H_2O_2 generating system) increased GO formation from glycolaldehyde and also promoted radical formation from GO and possibly from MGO [3]. Our laboratory previously demonstrated that fructose-induced hepatotoxicity increased more than 100-fold in the presence of a non-toxic concentration of H_2O_2 so as to mimic H_2O_2 levels formed by NADPH oxidase (NOX) released by activated immune cells such as neutrophils, eosinophils, and macrophages during an acute episode of inflammation [15,16]. The increased hepatotoxicity was mainly due to the enhanced conversion of fructose and fructose metabolites to form GO which could produce reactive radicals leading to increased ROS formation [1]. Increased H_2O_2 cellular concentrations may also impair ALDH2 by oxidizing a cysteine residue (C302) at the binding pocket and increase GO levels thereby increasing cytotoxicity [3,17].

Polyphenols have been studied extensively for their therapeutic benefits. Polyphenols are a group of secondary plant metabolites with at least one aromatic ring structure containing one or more hydroxyl groups [18] and are found in a variety of foods such as fruits, vegetables, cereals, and beverages etc. [19]. Polyphenols in foods or drinks are readily metabolized to phenolic acids and aldehydes by the gut microflora. This raised the possibility that these metabolites rather than the parent compounds were responsible for their anti-inflammatory properties [20]. Ferulic acid (also known as coniferic acid), one of the antioxidants in pre-germinated brown rice, is a polyphenolic compound that has been reported previously to decrease protein glycation in forebrains of glutathione (GSH)-depleted mice and attenuated Aβ-induced learning and memory deficits in mice [21]. It has also been reported to have a neuroprotective effect, radioprotective effect, pulmonary protective effect, and anti-atherogenic effect [22]. Ferulic acid has also been found to decrease the level of some inflammatory mediators such as prostaglandin E2 and TNF-α [23]. Due to its phenolic nucleus and an extended side chain conjugation, it readily forms a resonance stabilized phenoxy radical which accounts for its potent antioxidant potential [24]. Ferulic acid can also be formed in liver from methylation of caffeic acid, an antioxidant found in wine [25]. *p*-Coumaric acid (also known as hydroxycinnamic acid) is reported to reduce the risk of stomach cancer by reducing the formation of carcinogenic nitrosamines [26]. The scavenging ability of *p*-coumaric acid and its derivatives against DPPH (1,1-diphenyl-2-picrylhydrazyl) radical was dependent on the number of hydroxyl groups on the benzene ring and ortho substitution with the electron donor methoxy group which increases the stability of the phenoxy radical [27]. Methyl and ethyl derivatives of ferulic acid were also reported to have potent antioxidant properties [28]. Ferulaldehyde (also known as coniferaldehyde) is a water-soluble degradation end product of dietary flavonoids. High concentrations of ferulaldehyde were found in human urine after consumption of red wine and chocolate [20,29]. Ferulaldehyde has also been reported to inhibit the lipopolysaccharide-induced inflammatory response in mice [30]. Differences in the antioxidant properties and structure activity relationships of ferulic acid and its related polyphenols have been studied using different physical systems [28] as well as by computational methods [31].

Previously our lab reported protective effects of several B vitamins (B1 and B6) [32], carbonyl scavenging drugs (e.g., penicillamine, cysteine, *N*-acetylcysteine, aminoguanidine, pyridoxamine, and metformin) [8], almond skin extracts or catechins [33,34], and polyphenolic compounds (e.g., gallic acid, methyl gallate, ethyl gallate, propyl gallate, rutin, and curcumin) [35] against GO- or MGO-induced cytotoxicity, ROS formation, and protein carbonylation in cell (isolated rat hepatocytes) and cell-free system (bovine serum albumin). In the present study, we investigated the protective effects of ferulic acid and related polyphenols (structures are presented in Fig. 1) on GO- or MGO-induced cytotoxicity and

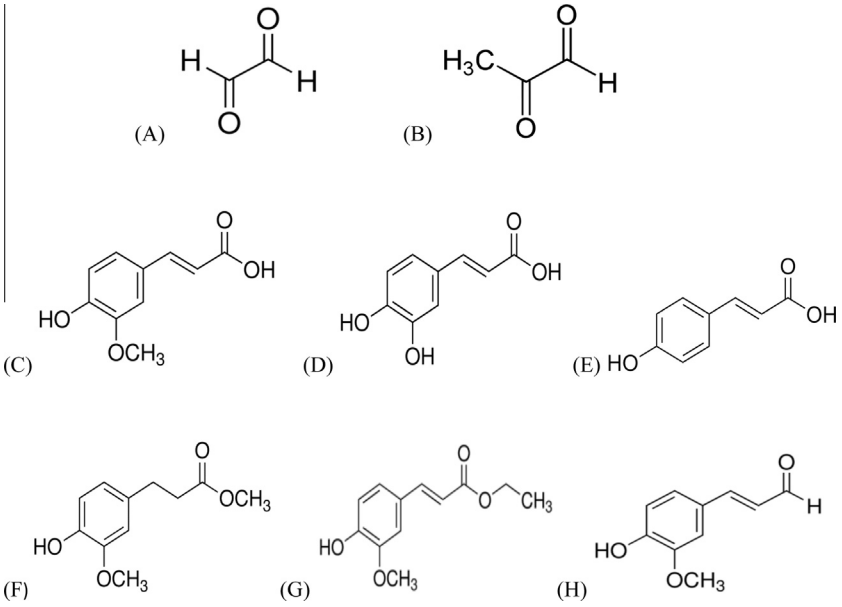

Fig. 1. Chemical structures of the reactive carbonyl species and the polyphenols investigated. (A) Glyoxal; (B) methylglyoxal; (C) ferulic acid; (D) caffeic acid; (E) *p*-coumaric acid; (F) methyl ferulate; (G) ethyl ferulate; (H) ferulaldehyde.