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Inhibition of guinea pig aldehyde oxidase activity by different flavonoid compounds: An *in vitro* study

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ABSTRACT

Aldehyde oxidase (AO), a cytosolic molybdenum-containing hydroxylase, is predominantly active in liver and other tissues of mammalian species and involved in the metabolism of extensive range of aldehydes and nitrogen-containing compounds. A wide range of natural components including polyphenols are able to interfere with AO-catalyzed reactions. Polyphenols and flavonoids are one of the extensive secondary plant metabolites ubiquitously present in plants considered an important part of the human diet. The aim of the present study was to investigate inhibitory effect of selected phenolic compounds from three subclasses of aurone, flavanone and phenolic lactone compounds on the activity of AO, spectrophotometrically. AO enzyme was partially purified from liver of guinea pig. Then, inhibitory effects of 10 flavonoid compounds including 8 derivatives of 2-benzylidenebenzofuran-3(2H)-ones, as well as naringenin and ellagic acid on the activity of aldehyde oxidase were assessed compared with the specific inhibitor of AO, menadione. Among the phenolic compounds with inhibitory effects on the enzyme, ellagic acid (IC_{50} = 14.47 μM) was the most potent agent with higher inhibitory action than menadione (IC_{50} = 31.84 μM). The mechanisms by which flavonoid compounds inhibit AO activity have been also determined. The inhibitory process of the assessed compounds occurs via either a non-competitive or mixed mode. Although flavonoid compounds extensively present in the nature, mainly in dietary regimen, aurones with promising biological properties are not widely distributed in nature, so synthesis of aurone derivatives is of great importance. Additionally, aurones seem to provide a promising scaffold in medicinal chemistry for the skeleton of new developing drugs, so the results of the current study can be valuable in order to better understanding drug–food as well as drug–drug interaction and also appears to be worthwhile in drug development strategies.

1. Introduction

Due to reduction of pharmacological efficacy and elevation of side effects, drug–drug interactions are of great concern in health practices. Consumption of natural products as alternative or complementary medicines is becoming increasingly popular. Natural preparations are commonly administered in combination with conventional drugs result in elevating the likelihood of pharmacodynamic and/or pharmacokinetic interactions causing enhancing or lessening the activity of phytochemical or chemical drugs, which in some cases are clinically important in patients [1]. The exact rate of herb–drug interactions in outpatient population has not been perfectly determined. There is restricted knowledge about interactions between chemical drugs and medicinal plants, as well as between drugs and dietary supplements. Although scientific evidences have suggested that drug–herb interactions occur less often than anticipated, it is necessary to investigate phytoconstituents of herbal therapy and also to determine interaction of the constituents with drugs and foods [1–3].

Accumulating evidences exhibited that enzymes possess a pivotal contributor in the pharmacokinetic variability of drugs as well as metabolism of xenobiotics [4]. The mechanistic understanding of

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interaction between diet-derived or complementary administrated natural compounds are limited to cytochrome P_{450}-mediated interactions. Interactions mediated by other phase-I enzymes such as aldehyde oxidase (AO) are understudied. AO (aldehyde: O_2 oxidoreductase EC1.2.3.1), a group of metalloflavoprotein enzymes, which is broadly distributed throughout the animal kingdom and need flavin adenine dinucleotide and a molybdopterin cofactor for their catalytic activity [5]. A large body of evidences has demonstrated that AO is mainly active in liver and other tissues of mammalian species and can terminate the metabolism of several aldehydes and nitrogen-containing molecules with several pharmacological and/or biological functions, resulting in particular clinical impact. It has been found that AOs participate in the metabolism of some important drugs including famciclovir, pyridoxal, allopurinol, quinine, N-methylnicotinamide, nicotine, carbazeran, zaleplon, methotrexate, azathioprine, and 6-mercaptopurine [6–10]. Likewise, AO is involved in the metabolism of endogenous agents including 2-phenylethylamine, retinal, monoamine neurotransmitters, as well as vitamins A, B3, and B6. However, several aspects of physiological and biochemical properties of this enzyme have not been reviled yet [6–8,10,11]. Moreover, AO is associated with significant pathological conditions including oxidative stress and amyotrophic lateral sclerosis seizures [11,12].

It has been found that a wide range of natural components including polyphenols (see Fig. 1) are able to interfere with AO-catalyzed reactions. Polyphenols are one of the extensive secondary plant metabolites ubiquitously present in plants considered an important part of the human diet.

Flavonoids (Fig. 2) are one of the largest groups of naturally occurring compounds which belong to polyphenolic compounds. Flavonoids extensively present in nature sources such as vegetables, fruits, grains, flowers, stems, bark, roots, as well as several remedies and tea. It is suggested that interfering with significant enzymes as well as anti-oxidative potential of flavonoids play a key role in performing their pharmacological function [4,13,14], although some reports concluded that supplemental flavonoids (as antioxidants) during chemotherapy should be discouraged [15].

Aurones, 2-benzylidenebenzofuran-3(2H)-ones, are a group of flavonoids which has the isomeric structure to the flavones. Aurones are yellow-colored naturally occurring compounds with several biological effects in plants such as protective, insect antifeedent, antiparasitic, and antifungal effect as well as their principal role in plant pigmentation. Scientific literatures showed that aurones possess various beneficial effects in human health including anti-inflammatory, anticancer, antileishmanial, and antibacterial activities [16]. Representative naturally occurring aurones are aureusidin, sulfuretin and maritimetin, possessing various hydroxylation patterns. A few natural aurones bearing methoxy substituents on either or both rings have been reported [17,18]. Fig. 3 illustrates the basic structure of 2-benzylidenebenzofuran-3(2H)-one derivatives which have been designed, synthesized and studied in the current investigation.

The spectrum of biological activity of this class of compounds has not been extensively studied. However, the existing data on the bioactivity of natural and synthetic aurones is very promising, thus these heterocyclic compounds can be considered as an attractive scaffold for drug design and development [19,20]. So far, aurones have been reported to possess anticancer, antileishmanial, and antibacterial properties, inhibitory activity against a variety of enzymes and proteins [21–26] and have been developed as potential amyloid imaging agents [27]. Surprisingly, only few studies on the antioxidant activity of aurones exist [28].

Ellagic acid is a naturally occurring phenolic lactone compound found in a variety of plant species, especially fruits. Ellagic acid is seen at high concentrations in many berries including strawberries, raspberries, cranberries and grapes. The antiproliferative and antioxidant properties of ellagic acid have prompted research into its potential health benefits. one of the main mechanisms by which ellagic acid is proposed to have anticancer benefits is by modulating the metabolism of environmental toxins and therefore preventing the initiation of carcinogenesis induced by these chemicals ([29] and references therein). Naringenin is also one of the known naturally occurring flavonoids of citrus fruits and has been pharmacologically evaluated as a potential anticancer agent and a hypolipidemic agent [30–32].

A working systematic framework in order to evaluate dietary substance–drug interaction potential consist of eliciting natural molecules from the dietary substances, screening the modulatory activity on specific drug-metabolizing enzyme, as well as evaluating probable clinical risks through dynamic and static modeling. Since previous studies have investigated the potential effects of different categories of flavonoids on AO (Fig. 1), the current study is particularly novel and significant because to the best of our knowledge, no previous similar studies on possible inhibitory potential of different aurone derivatives as well as ellagic acid and flavanone derivative, naringenin (as two important natural flavonoids) on AO enzyme have been reported. The aim of the current study was to expand this working framework through suggesting a molecular modeling component to advance the mechanistic understanding of AO-mediated polyphenols–drug interactions. AO isoform 1 (AO1 or AOX1) is the major and functionally active isoenzyme of aldehyde oxidases expressed in the human and guinea pig liver [33]. Thus, guinea pig liver was used as the source of enzyme because of high degree of overall amino acid sequence homology between the guinea pig and human hepatic aldehyde oxidases [34].

2. Materials and methods

2.1. Chemicals and reagents

Ammonium Persulfate, bromophenol Blue, Glycerol, Glycine, Methylene Bisacrylamide, SDS (Sodium Dodecyl Sulphate), TEMED (N,N,N′,N′-Tetramethylethylenediamine), HCl, ethanol, dopamine, 3-Methyle-2-benzothiazolinone, hydrazine, Cysteine, EDTA (ethylene diamine tetra-acetic acid), and ammonium sulfate were purchased from Merck. Na_2HPO_4, KH_2PO_4, Tris, NaCl, NaOH, Na_2CO_3 were purchased from Applichem. Ellagic acid, naringenin, and Bovine Serum Albumin were purchased from Sigma–Aldrich. DEAE-Sepharose Resin was purchased from Pharmacia Biotech. Acrylamide was purchased from Fluka. The structure of compounds was characterized by IR, ^{1}H NMR spectra and MS. IR spectrum was recorded on a Shimadzu 470 spectrophotometer (KBr disk). ^{1}H NMR spectrum was recorded on a Bruker FT-250 NMR spectrophotometer using DMSO-d_6 as solvent and TMS as an internal standard. The MS analysis was performed using an MS system comprising a Finnigan MAT Spectra System P4000 pump coupled with a UV6000LP diode array detector and a Finnigan AQA mass spectrometer.

2.1.1. 6-Hydroxy-2-(2′-hydroxybenzylidene)benzofuran-3(2H)-one

Yield = 72%; m.p. 250–252 °C; ^{1}H NMR (250 MHz, DMSO-d_6): δ 4.03 (s, 2H, OH*phenolic*), 6.72 (s, 1H, H_7), 6.79 (s, 1H, =CH), 6.90 (d, J = 8.2 Hz, 1H, H_4), 7.02 (d, J = 8.2 Hz, 1H, H_5), 7.12–7.27 (m, 2H, $H_{4',5'}$), 7.67 (d, J = 8.7 Hz, 1H, $H_{3'}$), 7.79 (d, J = 8.7 Hz, 1H, $H_{6'}$), ppm; IR (KBr, cm^{-1}): υ 3382, 1735, 1701, 1617, 1525, 1500, 1469, 1091, 1035, 737; MS (m/z, %): 255 (M^+, 16.5), 254 (100), 176 (75), 126 (45), 110 (60), 85 (90), 69 (20).