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Investigation of cytochrome P450 inhibitory properties of maslinic acid, a bioactive compound from *Olea europaea L.*, and its structure–activity relationship

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ABSTRACT

Maslinic acid (MA), the main pentacyclic triterpene of *Olea europaea L.* fruit, possesses a variety of pharmacological actions, including hypoglycemic, antioxidant, cardioprotective and antitumoral activities. Despite its importance, little is known about its effects on the cytochrome P450 (CYP) activity in both humans and animals. Therefore, the aim of this study was to investigate the effects of MA on the CYP 1A2, 2C9/11, 2D1/6, 2E1 and 3A2/4 activities by human and rat liver microsomes and specific CYP isoforms. In humans, MA only weakly inhibited CYP3A4 activity in human liver microsomes and specific CYP3A4 isoform with IC_{50} value at 46.1 and 62.3 μM, respectively. In rats, MA also exhibited weak inhibition on CYP2C11, CYP2E1 and CYP3A2 activities with IC_{50} values more than 100 μM. Enzyme kinetic studies showed that the MA was not only a competitive inhibitor of CYP3A4 in humans, but also a competitive inhibitor of CYP2C11 and 3A2 in rats, with K_i of 18.4, 98.7 and 66.3 μM, respectively. Moreover, the presence of hydroxyl group at C-2 position of triterpenic acid in MA compared with oleanolic acid could magnify its competitive inhibition on human CYP3A4 activity. The relatively high K_i values of MA would have a low potential to cause the possible toxicity and drug interactions involving CYP enzymes, thus suggesting a sufficient safety for its putative use as a nutraceutical taken together with drugs.

Introduction

Olive oil is a regular dietary component of the different countries in the world, in particular bordering the Mediterranean Sea (Owen et al. 2004). It is recognized as a healthy food from a nutritional point of view because of its content in monounsaturated fatty acids as well as bioactive components, such as polyphenols and pentacyclic triterpenes (Ghanbari et al. 2012). Among the latter, maslinic acid (MA) is the most abundant pentacyclic triterpene acid in the fruit of *Olea europaea L.* (Lozano-Mena et al. 2012). Interestingly, MA has a similar chemical structure compared with oleanolic acid (OA) (Fig. 1), which is found in plants worldwide (Kim et al. 2004). Until now, MA has been reported to possess several biological activities, such as antitumor (Li et al. 2010; Reyes-Zurita et al. 2011; Reyes-Zurita et al. 2009), antiinflammation (Huang et al. 2011), antioxidation (Montilla et al. 2003), antiallodynic (Nieto et al. 2013) and antidiabetogenic activities (Guan et al. 2011). Moreover, MA also is a marked component of hawthorn fruit (Rigelsky and Sweet 2002), a well-known fruit in China, which is used to make many forms of food, including jams, jellies, fruit drinks and other soft drinks (Chen et al. 1995).

In recent years, much attention has been paid to MA because of its beneficial effects on human health. For example, an extract from the skin of olive fruits containing 73.3% MA and 25.8% oleanolic acid inhibits proliferation and induces apoptosis in HT-29 human colon cancer cells (Juan et al. 2006). In fact, the antitumor effect has also been reported in different human tumor cell lines, such as astrocytoma (Martin et al. 2007), pancreatic cancer cells (Li et al. 2010), prostate cancer cells (Park et al. 2013) and human breast cancer cells (Allouche et al. 2011). Previous studies in our lab have shown that MA together with TNFα could be new promising agents in the treatment of pancreatic cancer because MA can improve the antitumor activities of TNFα and inhibit pancreatic tumor growth and invasion (Li et al. 2010). In addition, we also reported that MA suppresses osteoclastogenesis and prevents

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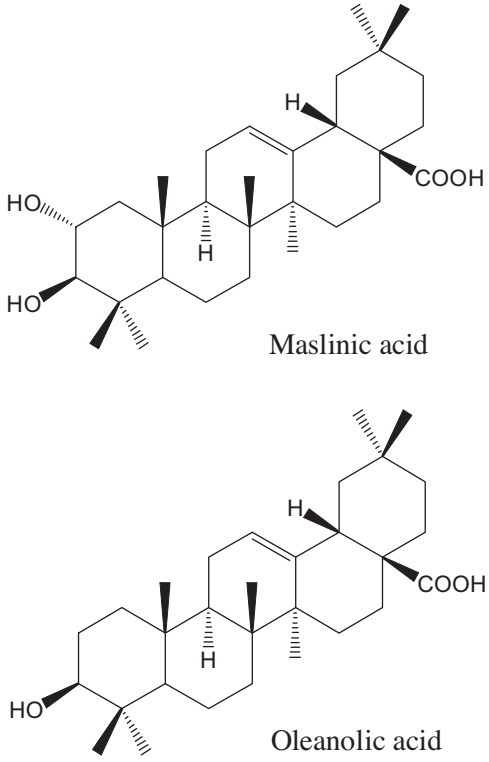

Fig. 1. Chemical structure of maslinic acid (MA) and oleanolic acid (OA).

ovariectomy-induced bone loss by regulating RANKL-mediated NF-κB and MAPK signaling pathways (Li et al. 2011). Hence, MA is considered as a promising anutraceutical or candidate for drug development. However, the studies about the metabolic-related properties of MA are limited. In particular, the interactions between MA and the cytochrome P450 (CYP) enzymes remain uncertain. When people use MA and/or olive oil, or some food containing MA with Western drugs together, these may cause unwanted food–drug interactions via drug metabolizing CYP enzymes.

The aim of this study was to determine the effects of MA on several major human and rat CYP enzymes responsible for the metabolism and disposition of a large number of drugs currently used, including CYP1A2, CYP2C9 (human)/2C11 (rat), CYP2D1 (rat)/2D6 (human), CYP2E1 and CYP3A2 (rat)/3A4 (human) isoforms. Enzyme kinetic studies using the model CYP probe substrates in the presence of various concentrations of MA were performed to investigate the mode of inhibition of the enzyme–substrate interactions. At the same time, it was also explored the structure–activity relationship of MA and OA with CYP activity in human liver microsomes.

Materials and methods

Chemicals and reagents

Pooled human liver microsomes and specific human CYP3A4 isoform were obtained from Corning Gentest Corporation (Woburn, MA, USA) and stored at −150 °C until use. All the experimental procedures involving humans have been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) guidelines. Phenacetin, paracetamol, metacetamol, oleanolic acid, tolbutamide, chlorpropamide, furafylline, dextromethorphan, 6β-hydroxytestosterone, glucose 6-phosphate (G6P), glucose 6-phosphate dehydrogenase (G6PDH), β-nicotinamide adenine dinucleotide phosphate (NADP), and tris (hydroxymethyl) aminomethane hydrochloride (Tris-HCl) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). 4-Hydroxytolbutamide, 6-hydroxychlorzoxazone and dextrorphan were obtained from Toronto Research Chemical (North York, Canada). Testosterone was purchased from Energy Chemical Co. (Shanghai, China). Corticosterone and ketoconazole were purchased from Tokyo Chemical Industry Co. (Shanghai, China). Chlorzoxazone was purchased from Alfa Aesar (Massachusetts, USA). Maslinic acid (purity >98%) was synthesized in our laboratory (Li et al. 2010). Acetonitrile and methanol (all HPLC grade) were purchased from Merck (Darmstadt, Germany). Acetic acid glacial (HPLC-grade) was purchased from TEDIA. Ethyl acetate (HPLC grade) was purchased from Fisher chemicals (Leicester, UK). Distilled water was purified in a Millipore system Milli Q.

Animals

Male Sprague–Dawley rats (200–250 g) were purchased from National Rodent Laboratory Animal Resources, Shanghai Branch of China. The animals were kept in animal holding room under standard conditions with 12 h light–dark cycles, with free access to rodent cubes and tap water. Animals were maintained according to the National Institutes of Health standards established in the 'Guidelines for the Care and Use of Experimental Animals', and all the experimental protocols were approved by the Animal Investigation Committee of the Institute of Biomedical Sciences and School of Life Sciences, East China Normal University.

Preparation of rat liver microsomes

In this study, male Sprague–Dawley rats (200–250 g) were fasted overnight and killed by cervical dislocation before removal of the liver. The liver was excised, rinsed with ice-cold saline (0.9% NaCl w/v), weighed and homogenized in a 0.05 mM Tris/KCl buffer (pH 7.4). The homogenate was centrifuged at 10,000 × *g* at 4 °C for 30 min. The supernatant was then centrifuged at 105,000 × *g* at 4 °C for 60 min. The pellet was reconstituted with 0.05 mM Tris/KCl buffer (pH 7.4) and stored at −150 °C until used. The protein concentration of the liver microsomes was determined by a protein quantitative assay using bicinchoninic acid (Brown et al. 1989).

Assays of CYP1A2 activity in human liver microsomes

CYP1A2 activity was assessed by formation of paracetamol from phenacetin by the method reported previously (Wang et al. 2009). The oxidative metabolism of phenacetin was measured in a system consisting of an NADPH-generating system and microsomes according to the method specified below. The incubation mixture (final volume of 250 μl in 0.1 M potassium phosphate buffer, pH 7.4) consisted of an NADPH-generating system (1.3 mM NADP, 3.3 mM G6P, 0.4 units/ml G6PDH, 3.3 mM magnesium chloride and 0.8 mg/ml pooled human liver microsomes). For inhibition study, 50 μM phenacetin was used. The concentrations of MA and OA used were from 1.25 to 100 μM. Furafylline, a selective CYP1A2 inhibitor, was used as positive control. The tubes were incubated in Eppendorf Themomixer at 800 rev/min, 37 °C. The reaction was initiated by adding protein to incubation mixture. After 30 min, incubations were terminated by adding 250 μl ice-cold acetonitrile. The tubes were then centrifuged in microcentrifuge at 13,000 × *g* for 12 min to precipitate protein. The supernatant was collected and metacetamol (15 μl of 500 μg/ml) was added as an internal standard. The whole mixture was then extracted with 500 μl ethyl acetate at 1400 rev/min in Thermomixer for 30 min at 25 °C. The tubes were then centrifuged at 8000 × *g* for 8 min. The organic layer was transferred to glass conical tube and evaporated at a heat block at 40 °C under a gentle stream of nitrogen gas. The residue was dissolved