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Research Paper

In vitro antioxidant and anti-inflammatory activities of Radix Isatidis extract and bioaccessibility of six bioactive compounds after simulated gastro-intestinal digestion

Ping Xiao, Hongzhi Huang, Jianwei Chen, Xiang Li *

College of Pharmacy, Nanjing University of Chinese Medicine, Nanjing 210023, PR China

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Epigoitrin (Pubchem CID: 3032313)
Adenosine (Pubchem CID: 60961)
Isolariciresinol (Pubchem CID: 160521)
DPPH (Pubchem CID: 2735032)
PGE_2 (Pubchem CID: 5280360)
Lipopolysaccharide (Pubchem CID: 11970143)
Vitamin C (Pubchem CID: 54678501)
Hydrochloric acid (Pubchem CID: 313)
Nitro blue tetrazolium (Pubchem CID: 9281)
Nicotinamide-adenine dinucleotid (Pubchem CID: 5893)

ABSTRACT

Ethnopharmacological relevance: Radix Isatidis called "Ban-Lan-Gen" is one of the most commonly-used traditional Chinese medicines for antiviral, anti-inflammatory, antioxidant and antipyretic purposes. Investigate the bioaccessibility of uridine, epigoitrin, adenosine, clemastanin B, indigoticoside A and isolariciresinol as well as the antioxidant and anti-inflammatory activities during an *in vitro* gastro-intestinal digestion of the Radix Isatidis extract (RIE).

Materials and methods: High performance liquid chromatography (HPLC) technique was adopted to determine the bioaccessibility of six bioactive compounds in RIE. Antioxidant activities of RIE in different digestive stages were determined by 1,1-Diphenyl-2-picrylhydrazyl (DPPH), superoxide anion and hydroxyl radical scavenging abilities. Anti-inflammatory activity was assayed by the inhibitions of inflammatory cytokines such as nitrous oxide (NO), prostaglandin E_2 (PGE_2) and tumor necrosis factor α (TNF-α) producted by lipopolysaccharide (LPS) stimulated RAW264.7 cells.

Results: The bioaccessibility of uridine, epigoitrin, adenosine, clemastanin B, indigoticoside A and isolariciresinol were 15.38%, 18.28%, 24.01%, 6.50%, 8.65% and 17.78%, respectively. Also, the digestion products still possessed certain antioxidant activities. The antioxidant activity was highly correlated with lignans (clemastanin B, indigoticoside A and isolariciresino). The anti-inflammation activity of the three samples decreased in the order: IN sample (the solution that had diffused into the dialysis tubing) > Nondigested sample (RIE solution) > Gastric sample (post-gastric digestion) > OUT sample (material that remained in the gastro-intestinal tract).

Conclusions: Results obtained in this research reveal the amount of bioactive compounds from RIE that could be available for absorption *in vivo*. The antioxidant activity decreased significantly but the anti-inflammatory activity was enhanced in serum-available fraction after gastro-intestinal digestion *in vitro*. This study could provide a scientific basis for a deeper pharmacological activity study of Radix Isatidis and a simple method for pharmacodynamic material basis research.

1. Introduction

Radix Isatidis, the dried root of the plant *Isatis indigotica* Fort. belonging to the Cruciferae family, is widely distributed in the northern and central of China (Pan et al., 2013). Many studies have proven that Radix Isatidis has anti-inflammatory, antioxidant, anti-virus, anti-bacterial, bantitumor and immune regulatory effects (Hamburger, 2002; Recio et al., 2006; Shin et al., 2010; Du et al., 2013). As a well used traditional Chinese medicine, Radix Isatidis is an official herbal medicine for the treatment of infection and inflammation in China. Chemical studies showed that Radix Isatidis contains various constituents including alkaloids, nucleosides and lignans and organic acid. Epigoitrin has been used as the marker of antiviral efficacy in Radix Isatidis in the 2010 edition of the Chinese Pharmacopoeia (Xu et al., 2005; Xie et al., 2011). Uridine and adenosine are the main nucleosides in Radix Isatidis. The latest studies showed that nucleosides were important bioactive compounds related to anti-inflammatory, immunoregulation, antitumor and antivirus activities (El-Sayed et al., 2009; Ndhlovu et al., 2010; Shi et al., 2011; Da et al., 2012). The RIE strongly inhibited the production of NO, TNF-α and the expression of iNOS in LPS-stimulated RAW 264.7 cells (Du et al., 2012). Previous studies showed that the content of adenosine in Radix Isatidis had an incomplete relationship with anti-inflammatory activities and the adenosine was

* Corresponding author. Tel.: +86 25 85811512; fax: +86 25 85811524.
E-mail address: lixiang_8182@163.com (X. Li).

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likely to create synergies with other components (Ling, 2012). 7S,8R,8′R-(-)-lariciresinol-4,4′-bis-O-β-D-glucopyranoside (clemastanin B), (+)-lariciresinol-4-O-β-D-glucopyranoside (indigoticoside A) and isolariciresinol are the major lignans isolated from Radix Isatidis (Peng et al., 2005; He et al., 2006a, 2006b). Our previous study showed that the fraction containing these lignans had a strong antioxidant activity (Chen et al., 2012).

In vitro digestion and dialysis methods for simulating the gastro-intestinal conditions are extensively used since they are simple, rapid, inexpensive and reproducible (You et al., 2011). A valid method has been developed for bioaccessibility assessment such as the changes in dietary components during the gastro-intestinal stage, and has already been widely used in many food researches (Bouayed et al., 2011; de Lacey et al., 2012; Marquez et al., 2013; Miranda et al., 2013; Horner and Beauchemin, 2013). However, few studies have been conducted to evaluate the bioaccessibility and pharmacological activities of Chinese herb extract after digestion (Weathers et al., 2014). Radix Isatidis has been proven to have good anti-inflammation and antioxidant activities (Shin et al., 2010; Du et al., 2013). Whereas, the bioaccessibility, anti-inflammation and antioxidant activities after the digestion process have not been studied.

The aim of this research was to investigate the bioaccessibility of uridine, epigoitrin, adenosine, clemastanin B, indigoticoside A and isolariciresinol as well as the antioxidant and anti-inflammatory activities during an *in vitro* gastro-intestinal digestion of the RIE.

2. Materials and methods

2.1. Chemicals

Pepsin from porcine gastric mucosa, pancreatin from porcine pancreas and bile salts (mixture of sodium cholate and sodium deoxycholate) were purchased from Shanghai Solarbio Biosience&Techology Co., Ltd. (Shanghai, China). DPPH was obtained from Aladdin (Shanghai, China). Vitamin C and other chemicals were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The cellulose dialysis tubing (molecular mass cut off, 3500 Da) was obtained from Yuan Ye Biotechnology Co., Ltd. (Shanghai, China). Deionized water was prepared using a Millipore MilliQ-Plus system (Millipore, Bedford, MA). The solvents and chemicals used were of analytical grade or HPLC grade. Uridine, epigoitrin and adenosine were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Clemastanin B, indigoticoside A and isolariciresinol were separated and purified in the Lab of the Department of Traditional Chinese Medicine Chemistry, School of Pharmacy, Nanjing University of Chinese Medicine. The purity of all compounds was above 98%, which was determined by HPLC–DAD–ELSD. The structures (Fig. 1) were elucidated by their UV, IR, MS, ^{1}H NMR, ^{13}C NMR and 2D NMR data (Peng et al., 2005; He et al., 2006a, 2006b).

2.2. Materials and extraction procedure

Radix Isatidis was purchased from a medical market in Tongling city of Anhui province (China). Its botanical origin was identified by Professor Jianwei Chen, Nanjing University of Chinese Medicine. The voucher specimens were deposited at the Herbarium in Nanjing University of Chinese Medicine (Nanjing, China).

About 100 g of Radix Isatidis powder was added to a round bottom flask and extracted by reflux with 1000 ml volume of water/ethanol (30:70, v-v) for 2 h. The mixture was filtered and the filtrate was collected. Then, the extract was concentrated by rotary vaporization at 60 °C under reduced pressure to remove the ethanol, and the concentrate was obtained.

2.3. In vitro simulated gastro-intestinal digestion

The digestion procedure mimicking the physiological situation in the upper tract (stomach and small intestine) was adapted from a published method (Gil-Izquierdo et al., 2002) with some modifications. Briefly, the extract was resolved in suitable volume of distilled water to a working concentrate. The simulated stomach solution was prepared as follows: about 3.2 g pepsin and 2 g NaCl were dissolved in distilled water, and then adjusting to pH 1.7 with 5 M HCl. The extract solution (5 ml) was added to 20 ml simulated stomach solution and the mixture was incubated at 37 °C in a shaking water bath for 2 h. At the end of the post-gastric digestion, the mixture was immediately cooled down with ice bath and then an aliquot of 2 ml was removed, frozen and taken for analysis of the six compounds. The remainder was placed in a 250 ml glass beaker containing 4.5 ml of 4 mg/ml pancreatin and 25 mg/ml bile salts mixture. A segment of cellulose dialysis tubing (molecular weight cut-off 3500 Da) containing sufficient $NaHCO_3$ to neutralize the samples titratable acidity was added and the beaker was sealed with parafilm. After 2 h incubation at 37 °C, the solution outside the dialysis tubing was taken as the OUT sample representing material that remained in the gastro-intestinal tract (= colon available) and the solution that had diffused into the dialysis tubing was taken as the IN sample (= serum-available material). All samples were stored at −80 °C until analysis.

2.4. Antioxidant activity

2.4.1. DPPH radical scavenging activity

Experiments were carried out as published by Liu et al. (2009) with some modifications. Briefly, 2.0 ml of test sample mixed with 2.0 ml of 0.15 mM DPPH that was dissolved in 95% ethanol.

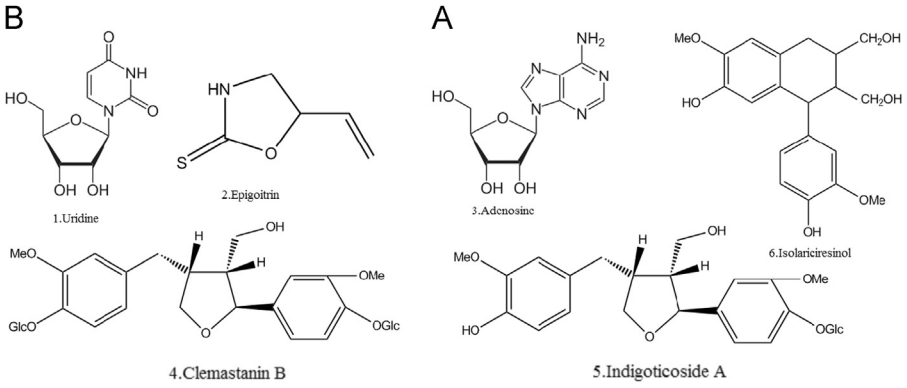

Fig. 1. Chemical structures of the investigated compounds.