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(−)-Patchouli alcohol protects against *Helicobacter pylori* urease-induced apoptosis, oxidative stress and inflammatory response in human gastric epithelial cells

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ABSTRACT

(−)-Patchouli alcohol (PA), the major active principle of Pogostemonis Herba, has been reported to have anti-*Helicobacter pylori* and gastroprotective effects. In the present work, we aimed to investigate the possible protective effect of PA on *H. pylori* urease (HPU)-injured human gastric epithelial cells (GES-1) and to elucidate the underlying mechanisms of action. Results showed that pre-treatment with PA (5.0, 10.0, 20.0 μM) was able to remarkably ameliorate the cytotoxicity induced by 17.0 U/mg HPU in GES-1 cells. Flow cytometric analysis on cellular apoptosis showed that pre-treatment with PA effectively attenuated GES-1 cells from the HPU-induced apoptosis. Moreover, the cytoprotective effect of PA was found to be associated with amelioration of the HPU-induced disruption of MMP, attenuating oxidative stress by decreasing contents of intracellular ROS and MDA, and increasing superoxide dismutase (SOD) and catalase (CAT) enzymatic activities. In addition, pre-treatment with PA markedly attenuated the secretion of nitric oxide (NO) and pro-inflammatory cytokines such as interleukin-2 (IL-2), interleukin-4 (IL-4) and tumor necrosis factor-α (TNF-α), whereas elevated the anti-inflammatory cytokine interleukin-13 (IL-13) in the HPU-stimulated GES-1 cells. Molecular docking assay suggested that PA engaged in the active site of urease bearing nickel ions and interacted with important residues via covalent binding, thereby restricting the active urease catalysis conformation. Our experimental findings suggest that PA could inhibit the cellular processes critically involved in the pathogenesis of *H. pylori* infection, and its protective effects against the HPU-induced cytotoxicity in GES-1 cells are believed to be associated with its anti-apoptotic, antioxidative, anti-inflammatory and HPU inhibitory actions.

1. Introduction

Helicobacter pylori, infecting over half of the world's population, is a vital etiological factor of many gastrointestinal diseases [1]. *H. pylori* infection produces various virulence factors in the stomach and induces mucosal apoptosis, reactive oxygen species (ROS) and inflammatory response with release of various cytokines, and subsequently cause many diseases of the stomach such as gastritis, peptic ulcer and gastric cancer [2,3].

To date, the most effective treatment against *H. pylori* infection is an antibiotics-based combination therapy. Despite the eradication of the pathogenic organism has been shown to reduce gastric inflammation and disorders, emerging antibiotic resistance and undesirable side effects have increasingly compromised the clinical efficacy of antibiotics-based regimen. Furthermore, the antibiotics currently used for *H. pylori* eradication normally possess a broad antibacterial

Abbreviations: Ala, alanine; Arg, arginine; Asn, asparagine; Asp, aspartate; CAT, catalase; Cys, cysteine; ELISA, enzyme-linked immunosorbent assay; FITC, fluorescein isothiocyanate; GES-1, human gastric epithelial cells; Gly, glycine; His, histidine; HPU, *Helicobacter pylori* urease; IL-2, interleukin-2; IL-4, interleukin-4; IL-13, interleukin-13; JC-1, 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimi- dazolylcarbocyanine iodide; Lys, lysine; MDA, malonyldialdehyde; Met, methionine; MMP, mitochondrial membrane potential; MTT, methyl thiazolyl tetrazolium; NO, nitrite oxide; PA, (−)-Patchouli alcohol; PI, propidium iodide; ROS, reactive oxygen species; RMSD, root-mean-square deviation; SOD, superoxide dismutase; TNF-α, tumor necrosis factor-α.

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spectrum, and their use would inevitably affect the normal gut flora, leading to a series of gastrointestinal side effects [4]. Hence, there is an urgent need to search for safer and more effective non-antibiotic agents with selective anti-*H. pylori* action for gastric diseases.

H. pylori urease (EC 3.5.1.5; urea amidohydrolase, HPU) plays an essential role in the pathogenesis of *H. pylori* infection by protecting the bacteria from the acid environment of the stomach, and promoting bacterial colonization via inducing the production of ammonia. Previous reports have shown that HPU promoted apoptosis, oxidative stress and the production of inflammatory mediators in gastric epithelial cells (GES-1), and lead to altered epithelial proliferation and oxidative DNA damage [5,6]. Therefore, HPU is deemed as an important target for selective anti-*H. pylori* agents in the management of the gastric disease [7].

Identification of effective and safe HPU inhibitors derived from medicinal plants is becoming an important area of research for the development of new therapeutic agents against *H. pylori* infections [8]. Pogostemonis Herba, the dried aerial parts of *Pogostemon cablin* (Blanco) Benth (Labiatae), is traditionally used in Chinese medicine for the treatment of a wide range of ailments, including gastrointestinal disorders, such as dyspepsia, gastritis and peptic ulcer [9]. Previous researches have revealed that the aqueous extract of Pogostemonis Herba could effectively protect the intestinal barrier function [10] and improve the digestive function [11]. (−)-patchouli alcohol (CAS number 5986-55-0, PA), a sesquiterpene officially used as a chemical marker for the quality assessment of Pogostemonis Herba in the Chinese Pharmacopoeia [12], has proved to exhibit various biological effects, including free radical-scavenging, anti-inflammatory, and immunomodulatory activities [13–15]. Recent work from our laboratory has also demonstrated the effectiveness of PA as a gastroprotective, anti-*H. pylori* and anti-urease agent. PA pre-treatment cleared *H. pylori* from the stomach of infected mice and relieved gastric inflammation caused by the bacterium. In addition, PA was demonstrated to selectively inhibit *H. pylori* functioning by inhibiting its HPU, while exerted no effect on other major normal gastrointestinal bacteria [16–18]. However, the possible modulatory effect of PA on the HPU infected GES-1 cells has not been investigated.

Based on the critical roles of gastroprotective and anti-*H. pylori* activities in the management of gastric diseases, the present study was, therefore, aimed to evaluate the possible cytoprotective effect of PA on the HPU-induced injury in GES-1 cells and to elucidate the underlying mechanisms of action.

2. Materials and methods

2.1. Plant materials

PA (Fig. 1) was isolated in our laboratory from patchouli oil with purity above 99.0% by gas chromatographic analysis and the optical rotation ($[\alpha]_D^{25}$) was measured to be-120° (*c* 2.0, $CHCl_3$) as reported previously [15]. Its structure was confirmed by melting point, infrared spectroscopy (IR), as well as by ^1H and ^{13}C nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry (MS) as described in our previous work [19]. PA was dissolved in dimethyl sulfoxide (DMSO) and the solvent concentration was ensured to be <0.1% in all experiments [20]. The GES-1 cells grown in the medium containing an equivalent amount of DMSO without PA were used as control.

2.2. Preparation and activity determination of HPU

H. pylori (ATCC 43504; American Type Culture Collection, Manassas, VA, USA) were grown in *Brucella* broth supplemented with 10% heat-inactivated horse serum for 24 h at 37 °C under microaerobic conditions (5% O_2, 10% CO_2, and 85% N_2). HPU was prepared and its activity was determined following the reported regime [16]. The resultant urease solution was added to an equal volume of glycerol and stored at 4 °C until use in the experiment.

2.3. Cell culture and treatments

Human GES-1 cells were kindly provided by the First Affiliated Hospital of Guangzhou University of Chinese Medicine. They were maintained and grown in high glucose DMEM supplemented with 10% (v/v) heat-inactivated fetal bovine serum at 37 °C in humidified atmosphere of 95% air and 5% CO_2.

To study the possible protective effect of PA, GES-1 cells were divided into control group (without PA and HPU treatment), HPU group (only 17.0 U/mg HPU for 24 h); and HPU plus PA (5.0, 10.0, 20.0 μM) groups. For HPU plus PA groups, GES-1 cells were pre-treated with PA for 4 h prior to co-cultivation with HPU for 24 h. The dose of PA was selected based on the results of our previous investigation and preliminary experiment.

2.4. Cell viability assay

Cell viability was assessed by the MTT assay, which is a test of normal metabolic status of cells based on the reduction of MTT to a purple formazon product by mitochondrial dehydrogenases of viable cells.

2.5. Annexin V-FITC-propidium iodide (PI) assay

The number of apoptotic cell death induced by the HPU was measured by flow cytometry using Annexin V-FITC-PI kit (Genzyme). Samples were analyzed within 1 h using a flow cytometer (BD Biosciences, CA, USA). The Annexin V and PI emissions were detected in the FL1-H and FL2-H channels of a FACS Vantage low cytometer, using emission filters of 525 and 575 nm, respectively. Fluorescence minus one controls were used to set the positive/negative cell gates and validate the flow cytometric results.

2.6. Measurement of the mitochondrial membrane potential (MMP, ΔψM)

The changes in the mitochondrial transmembrane potential (MMP, ΔψM) were determined using JC-1 dye (5,5′,6,6′-tetrachloro-1,1′,3,3′-

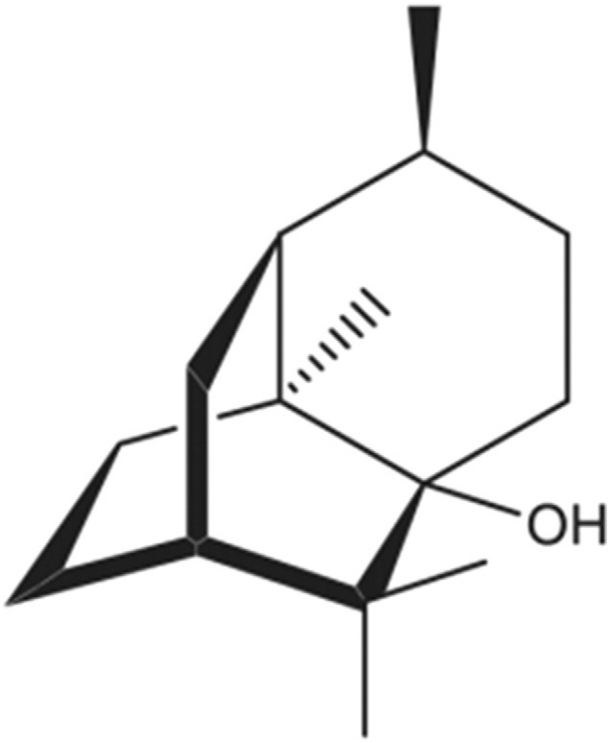

Fig. 1. Chemical structure of (−)-patchouli alcohol.