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Effect of isoorientin on intracellular antioxidant defence mechanisms in hepatoma and liver cell lines

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ABSTRACT

Isoorientin (ISO) is considered one of the most important flavonoid-like compounds responsible for health benefits, including the prevention of liver damage as well as antioxidant, anti-inflammatory, and anti-nociceptive activities. Our previous study showed that ISO inhibited the proliferation of hepatoma cells through increasing intracellular ROS levels. Interestingly, ISO protects rat liver cells against hydrogen peroxide-induced oxidation stress by decreasing intracellular ROS levels. Why are there different effects of ISO on ROS in different physiological and pathophysiological circumstances? The present study investigated the effect of ISO on mitochondrial respiratory chain complexes and phase II detoxifying enzyme activities in human hepatoblastoma cancer cells (HepG2), buffalo rat liver cells (BRL-3A) and human liver cancer cells (HL-7702). The results showed that intracellular ROS levels and the protein expression of the respiratory chain complexes was significantly ($p < 0.01$) higher in the HepG2 cells than in the BRL-3A and HL-7702 cells. Additionally, ISO notably ($p < 0.01$) increased ROS levels in the HepG2 cells, while no significance was found in the BRL-3A and HL-7702 cells. Furthermore, in the HepG2 cells, the protein expression of the respiratory chain complexes and the phase II detoxifying enzyme activities and GSH content were decreased by ISO ($p < 0.01$), while ISO, in a certain range, enhanced the expression of the protein complexes and the phase II detoxifying enzyme activities and GSH content in BRL-3A and HL-7702 cells. All of these results demonstrated, for the first time, that ISO possesses a notable hepatoprotective effect, which might be mediated through the respiratory chain complexes and phase II detoxifying enzyme activities.

1. Introduction

Reactive oxygen species (ROS), as a "redox messenger", are involved in and regulates a number of intracellular pathological processes, including ageing, apoptosis, autophagy, cellular injury and so on [1,2]. Accumulating evidence shows that higher ROS levels induce oxidative modifications of cellular macromolecules, inhibit protein function and promote apoptotic cell death. In previous study, we found that some nature plant active components, such as momordin Ic [3], chicoric acid [4], and herbacetin [5], inhibited the proliferation of hepatoma cells by increasing intracellular ROS levels. Interestingly, in a human hepatoblastoma cancer cell line (HepG2), isoorientin increased intracellular ROS levels, while in buffalo rat liver cells (BRL-3A), isoorientin protected the cells against hydrogen peroxide-induced oxidation stress by decreasing ROS levels [6,7]. The reason for the different effects of isoorientin on intracellular ROS levels in different cell line remain unknown.

Isoorientin (3′,4′,5,7-tetrahydroxy-6-C-glucopyranosyl flavone; ISO) is a common C-glycosyl flavone (Fig. 1) and can be isolated from several plant species, including *Phyllostachys pubescens* [8], *Patrinia* [9], *Drosophyllum lusitanicum* [10], buckwheat [11], corn silks [12] and so on. ISO exhibits a significant hepatoprotective effect against carbon tetrachloride (CCl_4)-induced oxidative damage in rats [13] and tertiary-butylhydroperoxide (t-BOOH)-induced oxidative damage in HepG2 cells [14]. Furthermore, ISO possesses 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity, iron(III) reductive activity and an inhibitory effect on linoleic acid peroxidation and MDA formation [15].

Abbreviations: ISO, isoorientin; HepG2 cells, human hepatoblastoma cancer cell line; BRL-3A, buffalo rat liver cells; HL-7702, human liver cell line; ROS, reactive oxygen species; complex I, NADH-coenzyme Q oxidoreductase; complex III, coenzyme Q-cytochrome c oxidoreductase; complex IV, cytochrome c oxidase; SOD, superoxide dismutase; CAT, catalase; GSH, glutathione; GSH-Px, glutathione peroxidase.

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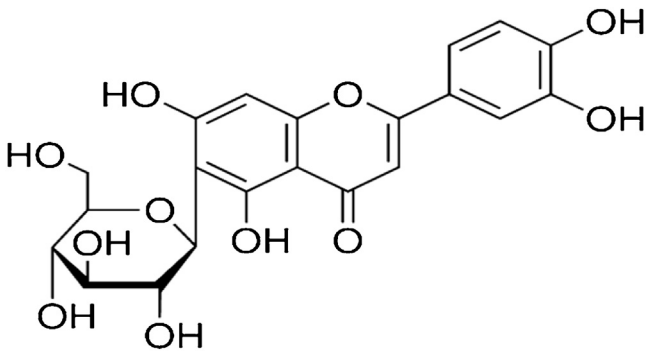

Fig. 1. Chemical structure of isoorientin (ISO).

Numerous studies show that the mitochondrial respiratory chain is a major source of ROS, and phase II detoxifying enzyme systems are typical mediators of ROS balance [16,17]. To investigate the underlying mechanisms of why isoorientin shows different effects on intracellular ROS levels in various physiological conditions, human hepatoblastoma cancer cells (HepG2), buffalo rat liver cells (BRL-3A) and human liver cells (HL-7702) were used to study the effects of ISO on the stimulation of the mitochondrial complexes and the induction of phase II detoxifying enzymes, which are two of the most important factors for cells in fighting against oxidative stress.

2. Materials and methods

2.1. Reagents and antibodies

Isoorientin (purity ≥98%) was purchased from Forever Biotechnology, Ltd. (Shanghai, China). RPMI-1640 cell cultures, DMEM high glucose cell cultures, foetal bovine serum (FBS) and the BCA protein kit were purchased from Thermofisher (Shanghai, China). The SOD, CAT, GSH and GSH-Px assay kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China). Antibodies against mitochondrial respiratory chain complex I (NADPH-diaphorase) (SC-20493), III (cytochrome reductase) (SC-69064) and GAPDH (SC-25778) were purchased from Santa Cruz Biotechnology (Santa Cruz, USA). The monoclonal antibody specific to IV (cytochrome oxidase) (AC610) was provided by the Beyotime Institute of Biotechnology (Jiangsu, China). All of the other chemicals were made in China and were of analytical grade.

2.2. Cell culture

The human hepatoblastoma cell line, HepG2, was obtained from the Fourth Military Medical University (Xian, China) and cultured in RPMI-1640 medium with 10% FBS and 1% penicillin/streptomycin at 37 °C in a humidified incubator (5% CO_2, 95% air). The buffalo rat liver cells (BRL-3A) and the human liver cell line (HL-7702) were obtained from Kunming Institute of Zoology, Chinese Academy of Science (Kunming, China), and were cultured in RPMI-1640 medium and DMEM high glucose medium with 10% FBS and 1% penicillin-streptomycin at 37 °C in a humidified incubator (5% CO_2, 95% air), respectively.

2.3. Detection of intracellular ROS production

Cellular ROS was measured with 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA; Sigma). This dye is a stable nonpolar compound that diffuses readily into cells and yields DCFH. Intracellular ROS, in the presence of peroxidase, changes DCFH to the highly fluorescent compound DCF. Thus, the fluorescence intensity is proportional to the amount of ROS produced by the cells. After treatment, 10 μM H_2DCFDA was added to the wells for 30 min at 37 °C. Then, the cells were washed twice with PBS and lysed. The cell lysates were centrifuged at 15,000*g* for 10 min at 4 °C. The DCF fluorescence intensity of the supernatant was measured via a fluorescence microplate reader at a 485 nm excitation and a 535 nm emission. Cellular ROS levels are expressed as the relative DCF fluorescence per microgram of protein [18].

2.4. Determination of phase II detoxifying enzyme activities and GSH level

After treatment, cells were rinsed with PBS (pH7.4), and lysed in cell lysates (P0013, Beyotime Institute of Biotechnology, Jiangsu, China). The samples were centrifuged at 15,000*g* for 10 min at 4 °C. An aliquot of the supernatant was used for the assay of phase II detoxifying Enzyme Activities. The protein concentrations in supernatants were measured using the BCA protein assay kit (Thermo Fisher, Shanghai, China) and BSA was used as the standard protein. The total SOD, CAT, and GSH-Px activity, and GSH level were determined by using an assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's protocol. The absorbance of T-SOD was read at 550 nm, CAT was read at 405 nm, GSH was read at 405 nm and GSH-Px was read at 412 nm.

2.5. SDS-PAGE and western blot analysis

For the preparation of the samples from the in vitro studies, the HepG2 cells were washed twice with PBS (pH 7.4), lysed with 1% of PMSF and 20 mM NaF and then incubated for 10 min on ice. The extracted proteins were removed from the culture dish by gentle scraping with a rubber policeman and transferring the solution to a microcentrifuge tube. The samples were centrifuged at 15,000*g* for 10 min at 4 °C, and then the supernatant was transferred to a new tube. The total protein concentration was determined using the BCA Protein Kit. The homogenates prepared from the cells were combined with SDS sample buffer and then immediately heated at 95 °C for 10 min. The proteins were separated by SDS-PAGE and electro-transferred onto a polyvinylidene fluoride membrane (0.45 μm, Millipore) using a semidry transfer apparatus. Blocking was carried out for 2 h in 5% nonfat dry milk in TBST (20 mM Tris, 166 mM NaCl, 0.05% Tween 20, pH 7.5). Then, the membrane was washed three times for 15 min at room temperature using a Table concentrator (70 r/min). The primary antibodies were added as per the manufacturer's recommended dilutions in TBST buffer overnight at 4 °C. The next day, after three washes in TBST, the secondary antibodies were added and incubated at 25 °C for 2 h, which was followed by three washes with TBST. The blots were detected with a chemiluminescent substrate (Thermofisher, China) and developed using the Molecular Imager Chemidoc XRS System (Bio-Rad, Shanghai, China).

2.6. Statistical analysis

All of the experiments were performed three times, and all the data are presented as the mean ± standard error (SE). Significant differences between the measurements for the control and treated samples were analysed using a one-way factorial analysis of variance (ANOVA) followed by Duncan's post hoc test (SPSS 16.0).