Chemico-Biological Interactions 288 (2018) 12–23

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

Procyanidin from peanut skin induces antiproliferative effect in human prostate carcinoma cells DU145

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ARTICLE INFO

Keywords:
Procyanidins
Peanut skin
Prostate cancer
Apoptosis
DU145

ABSTRACT

In this study, the antiproliferative activity of peanut skin procyanidins (PSP) and six fractions (PSP-1 ~ 6) isolated from PSP by several chromatographic steps on the human prostate cancer DU145 cells were evaluated. The results showed that PSP and PSP-1 ~ 6 significantly inhibited the proliferation of DU145 cells. PSP-2 was the most effective fraction, which was identified as procyanidin B_3 mainly and procyanidin dimer [(E)C-luteolin or keampferol] secondarily. Moreover, the mechanism of antiproliferative activity of PSP-2 was investigated. It was observed that PSP-2 induced apoptotic cell death and cell cycle arrest at S phase in DU145 cells. PSP-2 caused the increase of intracellular ROS level and the decrease of Bcl-2/Bax ratio, and triggered the activation of p53 and caspases-3 in DU145 cells. Our findings demonstrated that procyanidins from peanut skin have the potential to be developed as an anti-prostate cancer agent.

1. Introduction

Prostate cancer (prostatic carcinoma, PCa) is one of the most frequently diagnosed cancer among males worldwide [2]. There are the data indicated that African Americans have the highest rates of prostate cancer incidence. Meanwhile, PCa is the second leading cause of cancer death among men in USA [32]. In addition, the trends in mortality rates and incidence rates for prostate cancer have been increasing in China [7]. Therefore, more research into the treatment of prostate cancer is necessary. To date, the current methods for the treatment of prostate cancer are commonly involving surgery, radiotherapy, chemotherapy, hormone therapy, and focal ablative therapies [19]. However, some obvious adverse reactions have emerged in most existing chemotherapies, for instance urinary incontinence and erectile dysfunction [44]. Hence, balancing the benefits of effectiveness and the harm of therapy is the key to the treatment of prostate cancer.

Nowadays, more and more studies have confirmed that natural products have profound effects on the prevention and treatment of prostate cancer both in vitro and in vivo [8,17,41,47]. And the phytochemicals such as flavonoids and procyanidins are rich in natural food, which are attracting growing interest. Some epidemiological studies have also showed that a high intake of flavonoids and procyanidins is correlated with a decreased risk of prostate cancer [11,42].

Peanut (*Arachis hypogaea* L.) is one of the most valuable cash crops in many countries of the world. The total production of peanut is approximately 29 million metric tons per year all over the world, with China, India, and USA as the top three leading countries. However, peanut skin (PS), as the other edible part of peanut, is being discarded as waste or used as an animal feed ingredient [13]. Several reports have suggested that the procyanidins from peanut skin have antioxidant, anti-inflammatory and anti-melanogenic activities [9,36,45]. Thus, there has been growing attention on the peanut skin procyanidins (PSP) because of their wide range of potential health benefits [3,4,29,46].

Procyanidins have been widely studied for their health benefits, which have been known to have anti-angiogenic activity and cytotoxic properties, inhibit high glucose-induced mitochondrial dysfunction and apoptosis, and induce apoptosis and necrosis in human prostate cancer cells [6,16,30,40]. However, the effects of procyanidins from peanut skin have not been investigated in prostate cancer. Few data are available on the procyanidins from peanut skin with antiproliferative activity on human prostate cancer cells.

On the basis of the above considerations, the aims of this present study are to purify the procyanidins from peanut skin, assess the

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https://doi.org/10.1016/j.cbi.2018.04.008
Received 12 December 2017; Received in revised form 13 February 2018; Accepted 10 April 2018
Available online 11 April 2018
0009-2797/

inhibitory effects of the procyanidins on human prostate cancer DU145 cell proliferation, identify the procyanidins with the strongest inhibitory effect, and investigate the underlying mechanism of anti-proliferative activity of procyanidins on the DU145 human prostate cancer cells.

2. Materials and methods

2.1. Materials and chemicals

ToyoPearl HW-40S resin was provided by Tosoh Co., Ltd. (Tokyo, Japan). MEM medium and fetal bovine serum (FBS) were purchased from Gibco (USA). Penicillin/streptomycin and trypsin were obtained from Genivew (USA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and dimethyl sulfoxide (DMSO) were purchased from Sigma (USA). Annexin-FITC/PI cell apoptosis detection kit, reactive oxygen species assay kit with DCFH-DA and PI cell cycle detection kit were supplied by Nanjing Jiancheng Biotechnology Institute (Nanjing, China). RIPA lysis buffer and Hoechst 33258 staining kit were purchased from Beyotime Institute of Biotechnology (Jiangsu, China). Trizol was obtained from Aidlab Biotechnologies (Beijing, China). Primary antibody of cleaved caspase-3 was purchased from Cell Signaling Technology, Inc. (USA), and primary antibodies of p53, Bax and Bcl-2 were obtained from Proteintech Group, Inc. (USA). Hiscript Reverse Transcriptase and SYBR Green/Flourescein qPCR Master Mix were purchased from Vazyme Biotechnologies (Nanjing, China). Chemiluminescence ECL assay kit was purchased from Thermo (USA). All of the other reagents were commercial products of the highest available purity.

2.2. Preparation of procyanidins from peanut skin

Peanut skin was extracted by 70% ethanol and purified by AB-8 macroporous resin with 40% ethanol as eluent to obtain peanut skin procyanidins (PSP). PSP separations were carried out by injecting methanolic solutions containing PSP onto a 25 × 400 mm glass column prepared with ToyoPearl HW-40S resin. The compounds were eluted with methanol at a flow rate of 1 mL/min and monitored at 280 nm. Six fractions (PSP-1 ~ 6) were collected over the time intervals indicated in Fig. 1, evaporated under reduced pressure at 40 °C, and finally lyophilized. The dried powder was stored at −20 °C until further analysis.

2.3. Cell culture

Human prostate cancer cell line (DU145) was provided by the Cell Bank of Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). DU145 cells were cultured in MEM medium, supplemented with 10% FBS and 1% penicillin/streptomycin. The cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2.

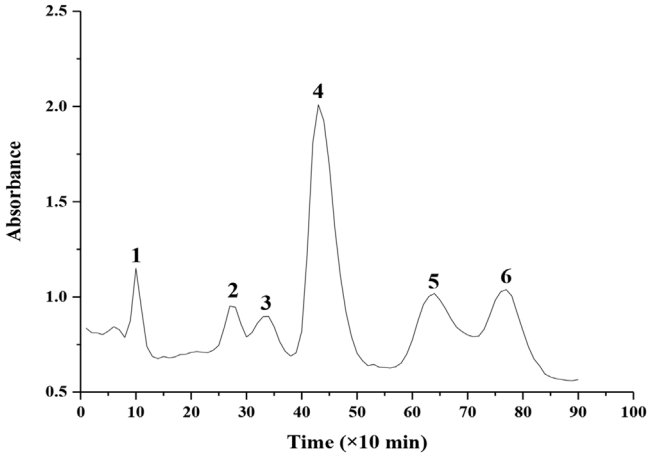

Fig. 1. Six fractions separated from PSP.

Table 1
Primer Sequences for qRT-PCR.

Name	Primer	Sequence	Size
Homo GAPDH	Forward	5′- TCAAGAAGGTGGTGAAGCAGG -3′	115 bp
	Reverse	5′- TCAAAGGTGGAGGAGTGGGT -3′	
Homo Caspase-3	Forward	5′- AGAACTGGACTGTGGCATTG -3′	190 bp
	Reverse	5′-CTTGTCGGCATACTGTTTCA-3′	
Homo p53	Forward	5′-AGGTTGGCTCTGACTGTACC-3′	195 bp
	Reverse	5′-GATTCTCTTCCTCTGTGCGC-3′	
Homo Bax	Forward	5′-TTTTGCTTCAGGGTTTCATC-3′	114 bp
	Reverse	5′-GACACTCGCTCAGCTTCTTG-3′	
Homo Bcl-2	Forward	5′-ACTTCGCCGAGATGTCCA-3′	258 bp
	Reverse	5′-CATCCCAGCCTCCGTTAT-3′	

2.4. Cell proliferation assay

The effects of the PSPs (PSP and PSP-1 ~ 6) on the proliferation of DU145 cells were measured using a MTT assay. The cells were grown in 96-well plates at a density of 6×10^3 cells/well. After 24 h, the cells were treated with PSPs. After incubation for 24 h, the cells were re-washed and incubated with 200 μL of MTT (0.5 mg/mL) for 4 h. Finally, 150 μL of DMSO was added to each well after removal of the supernatant and the absorbance at 490 nm was measured with a microplate reader (Multiskan GO, Thermo Fisher, USA). The viability of cells was quantified as a percentage compared to the control.

2.5. UPLC-Q-TOF-MS/MS

UPLC-Q-TOF-MS/MS analysis was performed on an Acquity UPLC H-Class Xevo G2-XS TOF mass spectrometry (Waters, USA). The column used was a ACQUITY UPLC® BEH C18 (2.1 mm × 100 mm, 1.7 μm) (Waters, USA). Separation was achieved with a gradient of 0.1% aqueous formic acid (A) and acetonitrile (B) at 0.4 mL/min in a 15-min program as follows: 0–0.5 min, 5% B, 0.5–8 min, increase to 50% B, 8–10 min, increase to 95% B, 10–12 min, 95% B, 12–15 min, decrease to 5% B. The samples were dissolved in methanol to 0.05 mg/mL and filtered through membrane (0.22 μm). The injection volume was 1 μL, and the eluate was monitored at 280 nm using a diode array detector, meanwhile, the eluent was also detected by mass spectrometer. A source temperature of 120 °C, negative ion mode was used with a sprayer needle voltage of 2.5 kV. The gas flow rate was 50 L/h and the capillary temperature was 450 °C. The full scan mass spectra from *m/z* 50 to 1500 were measured using 1000 ms for collection time.

2.6. Cell morphology

Briefly, the DU145 cells were seeded in 24-well plates at a density of 5×10^4 cells/well. After incubation for 24 h, the cells were treated with PSP-2 for another 24 h, then washed with PBS twice and stained with 1 μg/mL of Hoechst 33258 at room temperature for 5 min. The nuclear morphology of cells was observed with a positive fluorescence microscopy (Leica, Germany). The morphology of cells without staining was also observed with an optical inverted microscopy (Nikon, Japan).

2.7. Transmission electron microscopy (TEM)

The DU145 cells were seeded at a density of 2×10^6 cells per flask. Cells treated with PSP-2 (50 μg/mL) were harvested and washed with PBS twice, and then added with 2.5% glutaraldehyde fixative for ultramicrotome sectioning. TEM was performed with a transmission electron microscope (Hitachi, Japan).