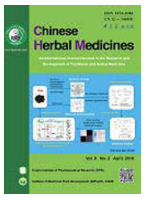

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Original article

Induction of Solasonine on Apoptosis of Human Breast Cancer Bcap-37 Cells through Mitochondria-Mediated Pathway

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ABSTRACT

Objective To study the *in vitro* antiproliferative effect and probable mechanism of solasonine on human breast cancer Bcap-37 cells, meanwhile, make comparison with solamargine. **Methods** The cytotoxicity was evaluated by MTT assay. The cell damage and type of cell death were examined through Hoechst33342/PI and Annexin V/PI staining, respectively. Mitochondrial membrane potential was detected by JC-1 staining. The expression of Bcl-2, Bcl-xL, Bax, and cytochrome c was determined by immunoblot method, and the activation of caspase-3 was analyzed by immunocytochemistry method. **Results** Solasonine showed the different extents of cytotoxicity on eight human tumor cell lines as well as four human normal cell lines, and the IC_{50} values of solasonine ranged from 12.73 to 37.15 μmol/L. Cell apoptosis and mitochondria depolarization were observed in Bcap-37 cells after treatment with solasonine for 24 h, respectively. In immunoblot and immunocytochemistry analysis, solasonine obviously induced the up-regulation of Bax and down-regulation of Bcl-2 and Bcl-xL, caused the release of cytochrome c from mitochondria into cytosol, and increased the expression of both pro- and cleaved caspase-3. Solamargine exhibited stronger antipoliferative activity than solasonine, but the similar mechanism in Bcap-37 cells in this study. **Conclusion** Solasonine possesses the antiproliferative effect on tumor cells. Regulation of the levels of Bcl-2, Bcl-xL, Bax, and activation of mitochondria cytochrome c-dependent apoptosis pathway might be one of its main antitumor mechanisms against breast cancer cells. In view of the cytotoxic effect of solasonine and solamargine also shown on normal cells, the safety needs concern when the antitumor activity is studied.

Key words

antitumor; breast cancer; mitochondria pathway; *Solanum nigrum*; solasonine

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1. Introduction

Solanum nigrum L. (Solanaceae), also known as "black nightshade", is an herbal plant that has been used as a traditional medicine in China for a long time due to its diuretic, antipyretic, and detumescent effects (Xu et al, 2014; Ding et al, 2012). Pharmacological studies have indicated that the extracts and/or components from *S. nigrum* possess a variety of biological activities including antifungal, anti-malarial, hepatoprotective, antitumor effects, and so on (Sun et al, 2012; Chen et al, 2010; Abdel-Rahim et al, 2014; Hu et al, 1999), among which the antitumor activity has attracted much attention from researchers recently because of its remarkable effects (Wang et al, 2010; Nawab et al, 2012). The multiple bio-activities of *S. nigrum* are based on its various medicinal components, such as glycoalkaloids (Ding et al, 2013), glycoproteins (Heo and Lim, 2005), polysaccharides (Li et al, 2009), steroidal saponins (Zhou et al, 2006), and polyphenolic compounds (Nawab et al, 2012), and alkaloids are considered to be one of its main antitumor active components (An et al, 2006; Li et al, 2008).

Solasonine (SS) and solamargine (SM) are two steroidal glycoalkaloids (SGAs) isolated from *S. nigrum*, and the structural difference between these two alkaloids is the carbohydrate moiety on C_3 side chain of steroidal aglycone, namely solasodine (Figure 1) (Ding et al, 2013). It has been proved that solasodine glycosides are very important antitumor agents (Cui et al, 2012), and both SS and SM possess antiproliferative activity against many human cancer cell lines (Munari et al, 2014). Studies on SM showed that it could produce antitumor activity through apoptosis-inducing effects (Kuo et al, 2000; Hsu et al, 1996). However, little is known about the antitumor mechanism of SS, since the reports are few. Although there is speculation that SS may have the similar antitumor mechanism to that of SM, exact experimental data are still lacking.

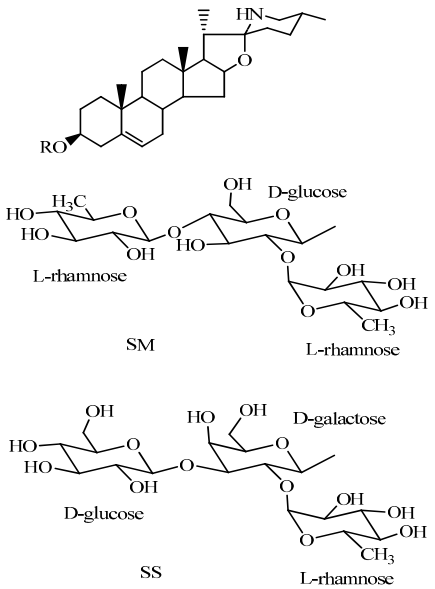

Figure 1 Chemical structures of SS and SM

In this paper, eight human tumor cell lines involving epidermoid carcinoma (A-431), gastric cancer (BGC-803), hepatocellular carcinoma (SMMC-7721) and especially breast cancer (Bcap-37, T47D, MCF-7, and MDA-MB-231, MDA-MB-453), as well as four human normal cell lines including liver cells (L-02), lung epithelial cells (BEAS-2B), kidney proximal tubule cells (HK-2) and breast epithelial cells (MCF-10A) were used to evaluate and compare the cytotoxic effect of SS and SM. Furthermore, the human breast cancer cells Bcap-37 was selected to investigate the antitumor mechanism of SS, with SM as comparison. Besides *Solanum nigrum*, SS and SM are existed in at least 100 species of *Solanum* (Ding et al, 2012; Munari et al, 2014). By this study, we hope to enrich the information about antiproliferative activity of SS and SM on both tumor and normal cells, especially antitumor mechanism of SS against breast cancer cells, and meanwhile remind the possible toxic effect of SS and SM on normal cells.

2. Materials and methods

2.1 Reagents and chemicals

RPMI 1640 medium, DMEM, and fetal bovine serum (FBS) (Gibco, USA). Annexin V-FITC apoptosis detection kit and BCA protein assay kit (Beyotime Institute of Biotechnology, China). Primary antibodies against Bcl-2, Bcl-xL, Bax, cytochrome c, GAPDH, and β-actin (Santa Cruz Biotech, CA). Mitochondrial membrane potential detection kit, SP immunocytochemistry detection kit, primary antibodies against caspase-3, activated caspase-3, and Cox4, enhanced chemiluminescence (ECL) kit (KeyGen technology, China). 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), trypsin, hoechst 33342, and prodium iodide (PI) (Sigma, USA). All other reagents were of analytical grade. Solasonine and solamargine (HPLC purity of 98%) were provided by Jiangsu Kanion Pharmaceutical Co., Ltd. (Tang et al, 2011). Compounds were dissolved in dimethyl sulfoxide (DMSO) respectively, to prepare stock solution, and diluted to the desired concentration with corresponding medium without serum before use. The DMSO in culture medium never exceeded 0.1%.

2.2 Cell lines and culture

The human epidermoid carcinoma cells (A-431), human hepatocellular carcinoma cells (SMMC-7721), human breast cancer cells (Bcap-37, MCF-7, MDA-MB-231, MDA-MB-453), and human normal liver cells (L-02) were obtained from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). The human gastric cancer cells (BGC-803) were presented by Prof. Ma HY (Nanjing University of Chinese Medicine, China). The human breast cancer cells (T47D), human lung epithelial cells (BEAS-2B), human kidney proximal tubule cells (HK-2), and human normal breast epithelial cells (MCF-10A) were obtained from KeyGen Technology (Nanjing, China). Cells