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Full Length Research Paper

Cytotoxic activities of *Coriolus versicolor* (Yunzhi) extracts on human liver cancer and breast cancer cell line

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The CVP (*Coriolus versicolor* polysaccharide) is well known as anti-tumor drug in clinical applications. Although recent studies have demonstrated that CVP can inhibit the proliferation of cancer cells *in vitro* and *in vivo*, the different purity level of CVP has a different affect on various cancer cells. In this study, the crude CVP was extracted from *C. versicolor* dry fruit bodies by hot-water extraction and ethanol precipitation. The content of CVP was 7.74% in the fruit bodies, while 54.69% in the crude extracts by phenol-vitriolic colorimetry. After that, the *in vitro* cytotoxic activities of the CVP were examined on the four human liver cancer (7703, HepG2, 7721, PLC) and four human breast cancer (Bcap37, ZR75-30, MCF-7, T-47D) cell lines using a MTT cytotoxicity assay. The results showed that the CVP inhibited the proliferation of 7703, Bcap37, T-47D in low concentration; the IC_{50} values on 7703, Bcap37 and T-47D were 18.37, 14.42 and 9.29 mg/l, respectively. The CVP also inhibited the proliferation of MCF-7 and ZR75-30, but at high concentration, the IC_{50} values on MCF-7 and ZR75-30 were 39.26 and 34.59 mg/l, respectively. The CVP does not inhibit the proliferation of HepG2, 7721, PLC and human normal liver cell line (WRL). The CVP was found to selectively inhibit the proliferation of human liver cancer and human breast cancer.

Key words: *Coriolus versicolor*, fruit body, polysaccharide, anti-tumor.

INTRODUCTION

Coriolus versicolor, also known as Yun Zhi (YZ), belonging to the family Basidiomycotina, is a mushroom widely used in traditional Chinese herbal remedies. Its medical value correlate to *C. versicolor* extracts. Of the *C. versicolor*-derived therapeutics extracts, polysaccharopeptides are commercially the best established. The polysaccharopeptides were obtained from *C. versicolor* known as *C. versicolor* polysaccharides (CVP), is a complicated protein-bound polysaccharide extracted from its mycelium or fruiting body. The composition of the polysaccharopeptide appears to depend on the source of the material and the method of recovery used, such as polysaccharopeptide Krestin (PSK) obtained from the extraction of *C. versicolor* (CM-101) strains in China and polysaccharopeptide (PSP) obtained from the extraction of *C. versicolor* (Cov-1) strains in Japan. Both products have similar physiological activities but are structurally different (Chu et al., 2002). The major bioactive CVP is a β-(1→3)-glucan branching at 4’ and 6’ positions. The CVP mainly consists of neutral polysaccharides of glu-

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Abbreviations: **YZ**, Yun Zhi; **CVP**, *Coriolus versicolor* polysaccharides; **PSK**, polysaccharopeptide Krestin; **PSP**, polysaccharopeptide; and **MTT**, 3-(4, 5-dimethylthiazolyl)-2, 5-diphenyl- tetrazolium bromide.

cose units; the main chain of β-1-3 consisted of β-D-1, 4-Glc and β-D-1, 3-Glc, and branch chains were situated, β-D-1, 3, 6-Glc and β-D-1, 4, 6-Glc (Zhang et al., 2001). The substance contained a branched glucan core with (1→3)-β-, (1→4)-β- and/or (1→6)-β-linkages, has a molecular weight of about 100 KDa and is highly water-soluble (Ng, 1998; Wang et al., 1996).

The CVP have many pharmacological activities, including immunopotentiation, immunosuppressive, improvement of appetite and liver function, calming of the central nervous system and enhancement of pain threshold. Historically, the CVP have been considered as important remedies for maintaining health, enhancing overall immune status, and prevention and treatment of chronic diseases (Ng, 1998). Presently, CVP is considered as a potential candidate for drug development in treatment and prevention of human cancers because of its immunological properties as well as its ability to distinguish cancerous cells from normal cells. Based on a statistics and analysis of anti-tumor plant drugs in a hospital of Guangdong province, the frequency of using CVP is the highest in various fungal polysaccharides during the years of 2000-2002 (Liu et al., 2005). *In vitro* studies reveal that PSP acts selectively on B-cell lymphoma cell line (Raji), human promyelocytic leukemia cell lines (HL-60, NB-4) (Lau et al., 2004; Hsieh et al., 2002), human breast cancer cell lines (T-47D, MCF-7, MDA-MB-231) (Aoyagi et al., 1997; Chow et al., 2003), prostate cancer cell lines (PC-3, DU-145) (Hsieh and Wu, 2001). Although the CVP suppress proliferation of many human cancer cell lines *in vitro* and *in vivo*, not all cancers seem to respond to *C. versicolor* polysaccharopeptides. Normal lymphocytes, human normal liver cell line (WRL) and human breast cancer cell line (BT20) are not affected by PSP (Hsieh et al., 2002; Lau et al., 2004; Ho et al., 2005). The anti-tumor activity of the extract from *C. versicolor* appears to depend on the strains-derived (Yang et al., 2000), the habitat in which it grows (Monro, 2003), the source material (Matsunaga et al., 1996) and the method of recovery used (Chen et al., 2003). The CVP can be produced from *C. versicolor* mushrooms harvested in the wild or cultivated commercially or from mycelial growth of *C. versicolor* in submerged fermentation. The polysaccharopeptides isolated from different sources (mushroom, mycelium, and biomass-free broth) differ somewhat in structure, composition, and physiological activity. The present study aimed to examine the *in vitro* cytotoxic activities of a culture-grown of *C. versicolor* hot-water extract in eight cell lines and to verify if the crude CVP can be extracted from the fruit body. This study provides a method of extract prepared CVP from cultivated fruit bodies, and farther revealed that the CVP significantly suppressed the proliferation of four human breast cancer cells in a dose-dependent manner, and four human liver cancer cells in a selectively manner *in vitro*.

MATERIALS AND METHODS

Reagents

The cells used were human liver cancer cell lines (7703, HepG2, 7721, PLC), human breast cancer cell lines (Bcap37, ZR75-30, MCF-7, T-47D) and human normal liver cell line (WRL) provided by the Cancer Institute of Shanghai Jiao Tong University. The RPMI-1640 medium and fetal calf serums were purchased from the Gibco Laboratories (Grand Island, NY, USA). The MTT (3-(4, 5-dimethylthiazolyl)-2, 5-diphenyl-tetrazolium bromide) and the dimethyl sulfoxide (Me_2SO) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Other chemicals used were reagent grade which were purchased from local chemical agent store.

Preparation of *C. versicolor* extract

C. versicolor fruiting bodies were cultivated by Shaanxi Key Laboratory of Bio-Resources, Shaanxi University of Technology (Zhou and Lin, 1999). The polysaccharides were obtained from the cultivated fruit bodies. The crude polysaccharide was extracted according to the method described as follows: the fruiting bodies were selected, cleaned, quantified and crushed. Based on a Soxhlet extraction method with petroleum ether as the extractive solvent, the materials was degreased, the residual was air-dried, and then extracted with hot-water at proper proportion and temperature. After being filtrated and discolored, the solution was concentrated to 10% of its original volume using reduced pressure method, followed by precipitation with 95% ethanol. The free proteins and pigment of the extracts were removed by Sevage method and active carbon, and then the extract was refined. The major components of the extract are polysaccharides, the amount of which were measured and extracting rate was calculated by following formula:

$$\text{Polysaccharides extracting rate} = \frac{\text{Dry-weight of ethanol sediments}}{\text{Dry-weight of fruit bodies}}$$

The experiment was replicated three times (Tian et al., 2003).

Determination of polysaccharide content

The contents of polysaccharides were measured by the phenol-sulphuric acid method using glucose as standard (Cuesta et al., 2003). The basic protocol was followed, with the modifications indicated below. The sugar solution (2 ml) and the phenol solution (2 ml) were added to each screw cap tube (18 × 180 mm), which was capped and vortex-stirred. Then 10 ml of concentrated sulfuric acid was added slowly down along the side of the tube. The tubes were then closed, vortex-stirred for 5 s and incubated for 2 min at 100°C water bath. All tubes were allowed to cool down to room temperature before measuring the absorbance at 490 nm using distilled water as blank in the Multiskan Spectrum Microplate Spectrophotometer (Thermo Labsystem).

Cytotoxicity assays

Cells were maintained as monolayer cultures in RPMI-1640 medium supplemented with 10% fetal calf serum and incubated at 37°C in a humidified incubator at 5% CO_2. Toxicity tests were described as before (Campling et al., 1991), nine sorts of cells were seeded independently in 96-well plate with the final volume 100 μl containing 1×10^4 cells per well. The plates were incubated at 37°C for 48 h. CVP in PBS was proportionally diluted with RPMI-1640, and 50 μl of each solution was added to triplicate wells. After 72 h, 0.15 mg (30 μl of 5 g/l) MTT was added to each well and incubated at 37 °C for a further 4 h. The medium was removed and 100 μl Me_2SO was added into each well after the plate was shaken thoroughly for 10 min. The absorbance of the samples was measured at 570 nm with a Multiskan Spectrum Microplate Spectrophotometer (Thermo Labsystem).

RESULT AND DISCUSSION

Extracting and determining of CVP

With hot-water extraction and ethanol precipitation, the content of the resulting extract (crude polysaccharide) from *C. versicolo* fruit body is about 7.74%. After free proteins and pigment being removed, the extract was refined. The content of purified polysaccharide from extract sample was determined as 54.69%. These results were consistent with previous studies (Tian et al., 2003). The glucose was used as standard to determine content of polysaccharide in the refined extracts. The results showed that linear concentrations, regression equation and regression coefficient were 0~180 μg/ml, y = 0.115+1.337X, R^2 =0.99867 respectively. CVP is commonly recovered by precipitation from the concentrated extract. Ethanol precipitation (Tian et al., 2003; Kim et al., 2001) and ammonium sulfate fractionation are used frequently. Conventional ion exchange chromatography on DEAE Sephadex and DEAE cellulose, and advanced HPLC methods have also been used effectively in the final recovery stages to purify CVP, but those are generally impractical in large-scale processing (Cui and Chisti, 2003). However, considering any commercial production process, the number of individual steps in the product recovery train should be kept to a minimum. Then, we select a simple and economic process for recovering CVP and determining the content of polysaccharide in the extracts, which is more practical.

Effects of CVP on the cytotoxicity of human cancer cells

As other fungal-derived bioactive polysaccharides, the CVP also inhibited proliferation of intact cells (Chang, 2002; Ebina, 2003). The CVP inhibited the proliferation of cancer cells of 7703, Bcap37, T-47D in low concentration; the IC_{50} of CVP on 7703, Bcap37, and T-47D were 18.37, 14.42 and 9.29 mg/l, respectively. The CVP also inhibited the proliferation of cancer cells of MCF-7 and ZR75-30, but at high concentration, the IC_{50} of CVP on MCF-7 and ZR75-30 were 39.26 and 34.59 mg/l (Figure 1). The CVP does not inhibit the proliferation of cancer cells of HepG2, 7721, PLC and human normal liver cell line (WRL). The study results show that the CVP can selectively and dose-dependently inhibit the proliferation of four breast cancer cell lines, with ascending order of IC_{50} values: T-47D, Bcap37, ZR75-30, MCF-7, while it can only inhibit the proliferation of one liver cancer cell line (7703). Three

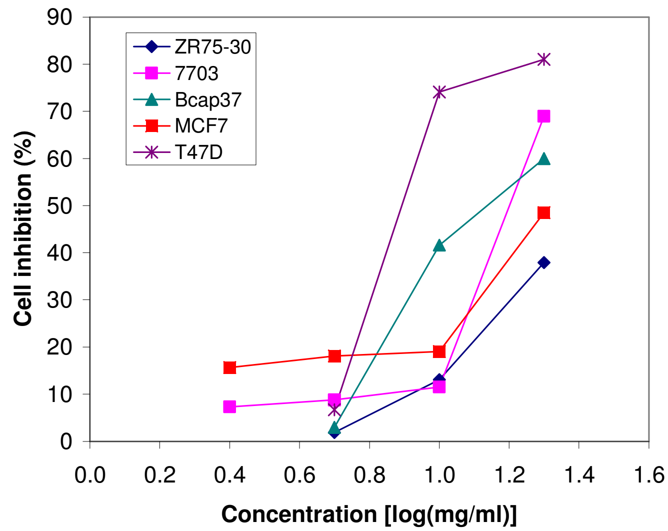

Figure 1. Determination of antitumor activity and cytotoxicity of CVP on one human liver cancer cell lines (7703) and four human breast cancer cell lines (ZR75-30, Bcap37, MCF-7, T-47D).

human liver cancer cell lines (HepG2, 7721, PLC) and human normal liver cell line (WRL) are not affected by CVP. This result suggests that CVP is more actively in the inhibition of proliferation of human breast cancer cells than human liver cancer cell cells. The CV extract can selectively suppress the proliferation of various cell and cell lines are consistent with previous reports (Lau et al., 2004; Ho et al., 2005). The recent studies demonstrated the *in vitro* anticancer mechanism(s) of CVP which include retardation of cancer proliferation by delaying cell cycle and induction of apoptosis in breast and cervical tumor cell lines, as well as leukemia and lymphoma cell lines (Ho et al., 2005). Previous research reports that CVP at 50 to 800 μg/ml dose-dependently suppressed the proliferation of B-cell lymphoma (Raji) and human promyelocytic leukemia (HL-60, NB-4) cell lines by more than 90% ($p<0.01$), with ascending order of IC_{50} values: HL-60 (147.3 g/l), Raji (253.8 g/l) and NB-4 (269.3 g/l). The CVP was found to selectively and dose-dependently inhibit the proliferation of lymphoma and leukemic cells possibly via an apoptosis-dependent pathway (Lau et al., 2004). Another research found out that the CVP dose-dependently suppressed the proliferation of three breast tumor cell lines, T-47D, MCF-7, MDA-MB-231, while BT-20 cells were not significantly affected (Ho et al., 2005). The anti-proliferative effects of the CVP on MDA-MB-231, MCF-7 and T-47D cells are mediated through apoptosis induction, which in turn is differentially regulated depending on p53 and Bcl-2 expression. The CVP significantly suppressed the proliferation of T-47D cell via up-regulation of the p53 protein expression and down-regulation of Bcl-2 protein expression, but in MCF-7 cell it is via down-regulation Bcl-2 protein expression only. But the expression of p53 and Bcl-2 protein are unaffected in

MDA-MB-231 cell treated with CVP. The study results further support the contention that CVP selectively inhibits the tumor cell growth. The mechanism of action of the selective effect of CVP causing tumor cell death may involve different parameters of apoptosis.

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