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Original Article

Polyphyllin I (PPI) increased the sensitivity of hepatocellular carcinoma HepG2 cells to chemotherapy

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Abstract: In this study the antitumor effects of polyphyllin I (PPI) were investigated in hepatocellular carcinoma HepG2 cells. Our data showed that PPI treatment exerted dose-dependent cytotoxicity on HepG2 cells as previously reported. Furthermore, PPI could sensitize HepG2 cells to cisplastin treatment in concentration-dependent manner. The molecular mechanisms of PPI actions involved nuclear factor-κB (NF-κB) and its downstream gene products. PPI treatment dose-dependently could decrease the constitutive phosphorylation of NF-κB subunit p65 protein and its downstream target genes expression, such as Bcl-2, c-Myc and VEGF. PPI could also inhibit cisplatin-evoked increase of p65 protein phosphorylation and its downstream genes expression, which could be further decreased by combination with NF-κB specific inhibitor, PDTC. The cytotoxicity and chemosensitization effects of PPI on HepG2 cells were greatly potentiated by concomitant treatment with PDTC. Taken together, our data confirmed the cytotoxicity of PPI on hepatocellular carcinoma HepG2 cells and provided new findings about PPI sensitizing HepG2 cells to chemotherapy. Moreover, our data also indicated the involvement of NF-κB signaling pathway in PPIactions for the first time.

Keywords: Hepatocellular carcinoma, polyphyllin I (PPI), cisplatin, nuclear factor--κB (NF-κB), cytotoxicity, chemotherapy, chemosensitizing effects

Introduction

Hepatocellular carcinoma (HCC) is the main form of liver cancer, which represents the third and fifth leading cause of death from cancer worldwide in men and women, respectively. The main attributable factors of HCC include persistent infection of hepatitis virus and contamination of foodstuff with aflatoxins [1, 2]. For many HCC patients the cancers are considered as non-resectable when diagnosed. And for these patients chemotherapy is the only choice of treatment. However, drug resistance developed in cancer cells after treatment is always a major obstacle to the successful management of liver cancer [3, 4]. So patients with HCC usually show poor prognosis and low 5-year survival rate (approximately 5-6%) because of the low effectiveness of available treatments [1]. And therefore, development of new adjuvant therapeutic agents that can chemosensitize cancer cells is still in great demand.

Traditional Chinese medical herb, Rhizoma of *Paris polyphyllin,* also known as Chong-Lou in China, has been widely prescribed by herbal practitioners to treat a number of tumors including pancreas, urinary bladder and liver tumor [5]. As one of the main active components of Rhizoma of *Paris polyphyllin* [6], polyphyllin I (PPI), a steroidal saponin, has been studied to show antitumor effects in many types of cancers, such asgastric cancer [7, 8], breast cancer [9], glioblastoma [10], ovarian cancer [11], lung cancer [6] and hepatocellular cancer [12, 13] through inhibiting tumor cell growth, metastasis [6, 9-12, 14], and inducing cell cycle arrest [8] and mitochondrial pathway-mediated apoptosis [13, 15]. However, whether PPI cansensitize tumor cells to chemotherapy has not been investigated so far. In this study we determined the chemosensitizing effects of PPI on hepatocellular carcinoma HepG2 cells and further explored the molecular mechanisms.

Materials and methods

Materials

HepG2 hepatocellular carcinoma cells were purchased from American Type Culture Collection (ATCC, Manassas, Virginia, USA). Polyphyllin

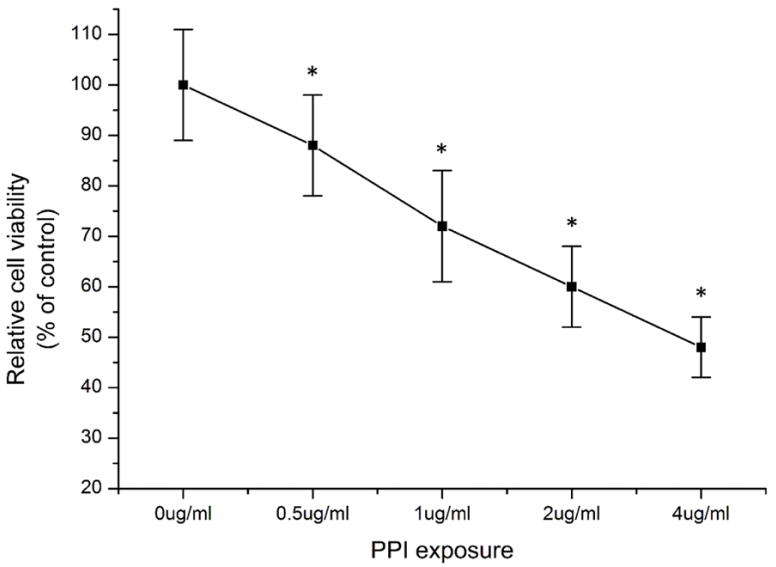

Figure 1. Polyphillin I (PPI) induced cytotoxicity in human hepatoma HepG2 cells. HepG2 cells were treated with different dose of PPI (0.5 ug/ml-4 ug/ml) for 24 hours. Cell viability was measured using commercially available CCK-8 kit as described in Materials and Methods and presented as the percentage of control. Data were presented as mean ± standard deviation (SD). *P<0.05 *vs.* control (without PPI treatment).

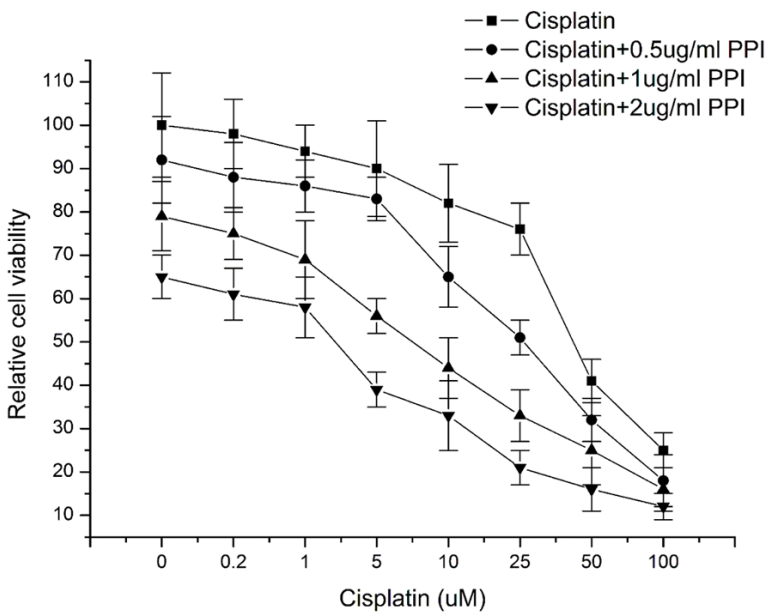

Figure 2. Polyphyllin I (PPI) sensitized HepG2 cells to cisplatin-induced cytotoxicity. HepG2 cells were treated with different concentration of cisplatin (0.2 uM-100 uM) for 24 hours in the presence or absence of different dose of PPI (0.5 ug/ml-2 ug/ml). Cell viability was measured using commercially available CCK-8 kit as described in Materials and Methods and presented as the percentage of control. Data were presented as mean ± standard deviation (SD).

I (PPI) was bought from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Ammonium pyrrolidinedithiocarbamate (PDTC), Aprotinin, leupeptin and cisplatinwere purchased from Sigma Chemical Co. (St. Louis, MO, USA). Primary antibodies against actin, phosphor-p65, Bcl-2, c-Myc, VEGF were purchase from Cell Signaling Technology (Denver, Colorado, USA). Secondary antibodies were obtained from Jackson ImmunoResearch (Baltimore, Maryland, USA).

Cell culture

HepG2 cells were cultured in RPMI 1640 medium (Gibco-BRL, Gasthersburg, MD, USA) supplemented with 10% (v/v) heat-inactivated fetal calf serum (FCS) (Gibco-BRL, Gasthersburg, MD, USA), 2% penicillin/streptomycin (10,000 U/ml penicillin, 10 mg/ml streptomycin), at 37°C, 5% CO_2.

Treatment and cell viability assay

The day before treatment HepG2 cells were plated in 96-wells culture plate at the density of 2×10^4 cells per well. Then cells were treated with drugs for 24 hours or 48 hours. After that, cell viability was determined by CCK-8 assay kits purchased from Dojindo (Kumamoto, Japan). The percentage of cell viability was calculated as $OD_{drug}/OD_{control}\times100\%$.

Western blot

HepG2 cells were lysed in lysis buffer (1% (w/v) SDS, 1 mM Na_3VO_4, 10 mM Tris. HCl pH 7.4, 5 mM $MgCl_2$) supplemented with 2 ug/ml aprotinin, 5 ug/ml leupeptin and 1 mM PMSF. Subsequently, 25 ug of protein were separated

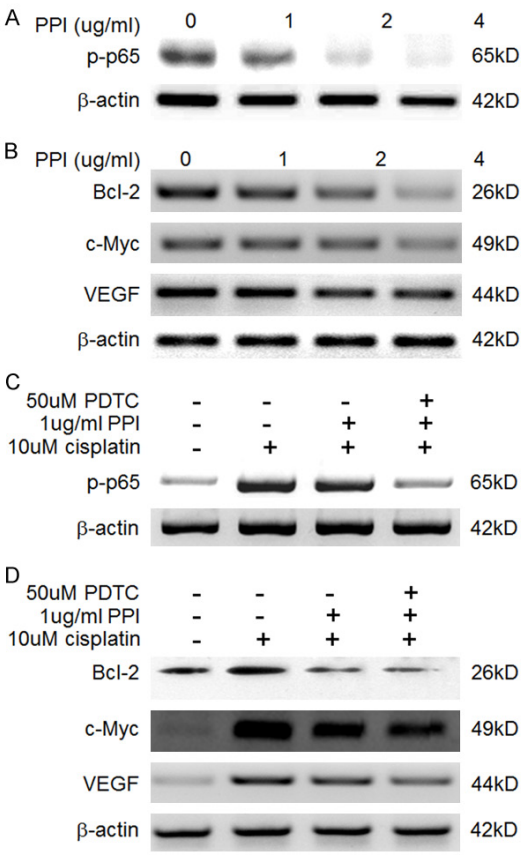

Figure 3. Inhibitory effects of polyphyllin I (PPI) on nuclear factor-κB (NF-κB) activation (A) and its downstream target genes expression (B) HepG2 cells were incubated with PPI (0.5 ug/ml-4 ug/ml) for 24 hours and then were harvested for further western blotting analysis. Inhibitory effects of PPI (1 ug/ml) and its combination with PDTC (50 uM) on cisplatin (10 uM)-induced NF-κB activation (C) and its downstream target genes expression (D) HepG2 cells were incubated with cisplatin (10 uM) for 24 hours in the presence or absence of PPI (1 ug/ml) or PPI (1 ug/ml) + PDTC (50 uM). Then cells were harvested for further western blotting analysis.

by 12% sodium dodecyl sulfate-polyacrylamide gelelectrophoresis (SDS-PAGE) and transferred to PVDF (polyvinylidene fluoride) membrane. Membranes were incubated with appropriate dilutions of specific primary antibodies overnight at 4°C. After incubation of horseradish peroxidase (HRP)-conjugated secondary antibody (dilution, 1:20000), the blots were developed with enhanced chemiluminescence detection kit (ECL, Millipore, Darmstadt, Germany).

Statistical analysis

IBM SPSS statistics 21.0 (SPSS Inc., Chicago, Illinois, USA) was utilized to analyze the data obtained in this study. Numeric variables were compared between groups through one-way analysis of variance (one-way ANOVA). $P<0.05$ was considered as statistically significant.

Results

Cytotoxic effects of polyphyllin I (PPI) on HepG2 cell line

Previous reports have indicated that polyphillin I harbored antitumor activities in many types of cancers, including gastric cancer [7, 8], breast cancer [9], glioblastoma [10], ovarian cancer [11], lung cancer [6] and hepatocellular cancer [12, 13] through inhibiting tumor cell growth, metastasis [6, 9-12, 14], and inducing cell cycle arrest [8] and mitochondrial pathway-mediated apoptosis [13, 15]. In this research we confirmed the cytotoxic effects of polyphyllin I in hepatocellular carcinoma cell line HepG2 cells. As illustrated in **Figure 1**, PPI could induce HepG2 cells death in dose-dependent manner.

Chemosensitizing effects of polyphyllin I (PPI) on HepG2 cell line

Although a lot of works have been done to investigate the antitumor activities of PPI, so far no research works were performed to explore the sensitizing effects of PPI on tumor cells. In this study our data showed that cell death induced by cisplatin treatment was further enhanced by combination with PPI treatment in concentration-dependent manner (**Figure 2**), which proved the chemosensitizing effects of polyphyllin I on HepG2 cells.

Involvement of nuclear factor-κB (NF-κB) signaling pathway in the anticancer activities of polyphyllin I (PPI)

In order to further elucidate the molecular mechanisms of PPI actions, we determined the influence of PPI on the NF-κB signaling pathway. As indicated in **Figure 3A**, PPI treatment could greatly inhibit the activation of NF-κB in concentration dependent manner, which was indicated by the phosphorylation level of p65 protein. The NF-κB regulated gene products such as Bcl-2, c-Myc, VEGF, were also dose-dependently decreased by PPI treatment (**Figure 3B**). PPI treatment not only inhibited the constitutive NF-κB activation in HepG2, but also inhibited cisplatin-evoked NF-κB activation (**Figure 3C**) and up-regulation of NF-κB regulat-

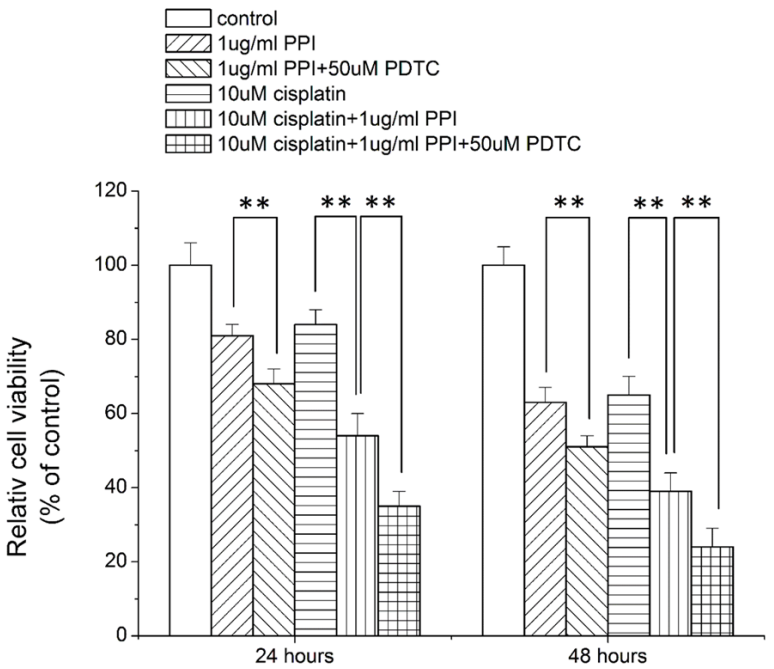

Figure 4. Nuclear factor-κB (NF-κB) inhibitor potentiated polyphyllin I (PPI) induced cytotoxicity and chemosensitization in HepG2 cells. In these assays NF-κB specific inhibitor PDTC was used. The cytotoxic enhancement of NF-κB inhibitor was investigated by incubation of HepG2 cells with 1 ug/ml PPI in the presence or absence of 50 uM PDTC. The chemosensitization enhancement of NF-κB inhibitor was studied by treating HepG2 cells with 10 uM cisplatin and 1 ug/ml PPI in the presence or absence of 50 uM PDTC. 24 hours or 48 hours later cell viability was measured and expressed as % of control. **P<0.05.

ed gene products including Bcl-2, c-Myc, VEGF (**Figure 3D**), all of which could alsobe further decreased by combination with 50 uM PDTC, an NF-κB specific inhibitor (**Figure 3D**).

How did NF-κB signaling pathway influence the cytotoxic and chemosensitizing effects of PPI on HepG2 cells? In order to answer these questions, HepG2 cells were treated with different combination of cisplatin, PPI and PDTC for 24 hours and 48 hours; then the cells viability was determined by CCK-8 assay kits. As shown in **Figure 4**, inhibition of NF-κB pathway by 50 uM PDTC could enhance the cytotoxic effects of PPI on HepG2 cells (**Figure 4**). Moreover, the chemosensitizing effects of PPI were also potentiated by 50 uM PDTC treatment (**Figure 4**).

Discussion

Because of multi-drug resistance and severe toxic side effects of current chemotherapy for cancers, developing new therapeutic agents against tumor is always needed. Nowadays, traditional Chinese medical herbs, as an important complementary and alternative therapy, attract more and more eyeballs, and more and more natural chemical compounds from Chinese medical herbs have been isolated and identified with antitumor activities [16-19]. Polyphyllin I (PPI), one of the main active steroidal saponins from Chinese medical herbs, Rhizoma of *Paris polyphyllin,* was previously proved to harbor antitumor effects in many cancers [6, 7, 13, 14, 20, 21]. However, the antitumor activities of PPI and its molecular mechanisms have not been thoroughly investigated so far. In present study, our results not only confirmed the previously reported cytotoxic effects of PPI on HepG2 cells [13, 22] (**Figure 1**), but also provided new fidnings that PPI could sensitize HepG2 cells to cisplatin treatment (**Figure 2**).

Previous investigations have demonstrated that PPI exertedits antitumor activities through inhibiting tumor cell growth, proliferation and metastasis [6, 9-12, 14], inducing cell cycle arrest [8] and mitochondrial pathway-mediated apoptosis [13, 15]. In present study NF-κB signaling pathway was found to be involved in PPI actions. Our results indicated that PPI treatment could inhibit not only the constitutive activation of NF-κB signaling pathway (**Figure 3A**) in HepG2 cells, but also the adaptive activation of NF-κB signaling pathway evoked by cisplatin treatment (**Figure 3C**), both of which were represented by phosphorylation of p65. Furthermore, PPI treatment decreased NF-κB regulated genes expression, such as Bcl-2, c-Myc, VEGF (**Figure 3B** and **3D**). Because NF-κB and its regulated gene products were involved in chemo-resistance and radio-resistance in many tumors [23, 24], we suggested that it was through inhibition of NF-κB activation PPI exerted its antitumor and chemosensitizing effects in HepG2 cells. Exactlyas we supposed, combination treatment of PPI with NF-κB specific

inhibitor, PDTC, the cytotoxic effects and chmosensitizing activities were both greatly enhanced (**Figure 4**).

In conclusion, our results confirmed that PPI harbored cytotoxic effects on hepatocellular carcinoma HepG2 cells as previously reported [13, 22]. And furthermore, our study indicated that PPI could sensitize hepatocellular carcinoma HepG2 cells to chemotherapy. The cytotoxic and chemosensitizing activities of PPI might be accomplished through down-regulation of NF-κB signaling pathway.

Disclosure of conflict of interest

None.

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