PARP inhibitor reduces proliferation and increases apoptosis in breast cancer cells

Yan Shi¹, Fang Zhou¹, Feng Jiang², Hong Lu¹, Jianjun Wang¹, Chuanyao Cheng¹

¹Department of Oncology, ²Department of Orthopedics, Henan University Huaihe Hospital, Kaifeng 475000, China

Correspondence to: Fang Zhou, Professor. Department of Oncology, Henan University Huaihe Hospital, 8# Baogonghu North Road, Kaifeng 475000, China. Email: 314144163@qq.com.

Objective: Apoptosis is a reliable marker of chemotherapeutic efficacy. Olaparib and paclitaxel inhibit proliferation and induce apoptosis in a variety of cancers. We investigated the effects of paclitaxel combined with olaparib on apoptosis in breast cancer Bcap37 cells.

Methods: Proliferation and apoptosis were detected by MTT assay and PI staining. Degradation of pro-caspase-3 and poly(ADP-ribose) polymerase (PARP) was analyzed by Western blotting.

Results: Compared with paclitaxel alone, paclitaxel combined with 100 mg olaparib significantly reduced survival in Bcap37 cells at all tested treatment durations (P<0.05); inhibition increased with increasing olaparib dose and treatment time (P<0.01). Combined treatment yielded significantly higher rates of apoptosis (P<0.05), which also increased with time (P<0.01). Fluorescence micrographs showed that early and late apoptotic cells increased with treatment time. Pro-caspase-3 and PARP degradation was induced by paclitaxel and enhanced by olaparib in a dose-dependent manner. Thus, combined treatment was substantially more effective than treatment with paclitaxel alone.

Conclusions: Our findings suggest that paclitaxel and olaparib inhibit breast cancer Bcap37 cell proliferation and induce apoptosis. Combined treatment further reduced cell growth and enhanced apoptosis, suggesting that this combination therapy may be a promising treatment for breast cancer.

Keywords: Breast cancer; paclitaxel; poly(ADP-ribose) polymerase inhibitor (PARP inhibitor); apoptosis

Submitted Jan 05, 2014. Accepted for publication Feb 25, 2014.
doi: 10.3978/j.issn.1000-9604.2014.02.13
View this article at: http://www.thecjcr.org/article/view/3691/4568

Introduction

Breast cancer is a major threat to women’s health and its incidence and fatality rate are gradually increasing (1-6). The pathogenesis of breast cancer is associated with genetic and environmental factors, as well as essential cellular pathways such as those controlling metastasisms, cell cycle progression, proliferation, and apoptosis (7,8).

Poly(ADP-ribose) polymerase (PARP) inhibitors target DNA repair defects in hereditary breast cancer. PARP inhibitors as monotherapy or in combination therapy have yielded promising results against different cancers in recent clinical trials (9). Olaparib is a novel PARP inhibitor that induces synthetic lethality in BRCA-deficient cells. PARP plays an important role in base excision repair of single-strand DNA breaks: inhibition of PARP results in the accumulation of single-strand DNA breaks, leading to the formation of double-strand breaks after stalling and collapse of the DNA replication fork (10). These double-strand breaks are usually repaired by the error-free homologous recombination repair pathway, of which the tumor-suppressor genes BRCA1 and BRCA2 are key components (11-15).

Paclitaxel, one of the most effective chemotherapeutic drugs, was isolated from the bark of the Pacific yew (Taxus brevifolia). The drug promotes and stabilizes microtubules and inhibits the late G2 and M phases of the cell cycle, thereby causing cell death. It is mainly used to treat lung, ovarian, and breast cancers (16,17).

Several studies have shown that paclitaxel or PARP inhibitors such as rucaparib, PJ34 and veliparib inhibit
tumor cell proliferation and promote apoptosis (18-20). Only a few studies have addressed the efficacy of olaparib in breast cancer. Therefore, we designed a study to determine the efficacy of paclitaxel combined with olaparib and explore the mechanism of olaparib-induced apoptosis in breast cancer cells.

Materials and methods

Cell culture and reagents

Human breast cancer Bcap37 cells were purchased from the Cell Bank of the Shanghai Institute of Biochemistry and Cell Biology of the Chinese Academy of Sciences. The cells were cultured in RPMI-1640 medium containing 10% fetal bovine serum (FBS) (Invitrogen, Carlsbad, CA, USA). Paclitaxel and olaparib were purchased from Sigma (St. Louis, USA) and LC Laboratories (Woburn, MA, USA) and dissolved in 100% dimethyl sulfoxide (DMSO, Sigma, USA). Cells were treated with paclitaxel alone (50 μg/mL) or in combination with the PARP inhibitor olaparib at 100, 200, or 400 mg.

MTT assay

We used 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assays to measure survival in breast cancer Bcap37 cells treated with the PARP inhibitor. Bcap37 cells were digested and diluted to 2×10^5/mL; 100 μL of this cell suspension was seeded in 96-well culture plates (Falcon, Oxnard, CA, USA). After 24 h, the cells were divided into untreated, paclitaxel, and paclitaxel+100/200/400 mg olaparib groups. Cells were harvested by scraping and suspending in medium after 24-h treatment. MTT solution (100 μL, Promega, USA) was added and the plates were incubated at 37 °C and 5% CO₂ for another 4 h. DMSO (200 μL) was added to dissolve the MTT crystals; the supernatant was removed by centrifugation and absorbance was measured at 560 nm. Treatments were performed in triplicate wells.

Annexin FITC-V/PI double staining and flow cytometry

Annexin V-fluorescein isothiocyanate/propidium iodide (Annexin FITC-V/PI) double staining and fluorescence microscopy were used to quantitate apoptosis. Adherent cells were trypsin-digested and collected. The cells were washed twice and resuspended in 500 μL binding buffer and 5 μL annexin FITC-V and then in 5 μL PI. Flow cytometry was performed to detect the DNA concentration and percentage of apoptotic cells.

Western blotting

Cultured Bcap37 cells were collected by trypsin digestion and washed twice with phosphate-buffered saline (PBS). Total cell protein was extracted, quantified by the Bio-Rad Dc protein quantitative assay, and separated by 12.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The proteins were transferred to polyvinylidene fluoride (PVDF) membranes and incubated with primary antibody overnight at 4 °C with shaking. The membranes were washed several times with Tris-buffered saline/Tween-20 and incubated with horseradish peroxidase (HRP)-labeled secondary antibody for 1 h at room temperature with shaking. Band intensity was detected by ECL detection reagent (Amersham, UK) with β-actin as an internal control.

Statistical analysis

Data analysis was performed with SPSS 16.0 software (SPSS Inc., Chicago, IL, USA); results are presented as x±s. Analysis of variance (ANOVA) was used for multiple comparisons and Student’s t-test was used to compare the mean when there was significant variation between the treatment and control groups. P<0.05 was considered statistically significant.

Results

PARP inhibitor suppressed Bcap37 cell growth

MTT cytotoxicity analysis was used to measure Bcap37 cell proliferation. Growth inhibition by paclitaxel alone or in combination with olaparib is shown in Figure 1. Survival decreased with increasing incubation time. Bcap37 cells treated with paclitaxel combined with various concentrations of olaparib (100, 200 or 400 mg) showed significantly greater inhibition than cells treated with paclitaxel alone (P<0.05); inhibition increased with increasing olaparib dose and treatment time (Table 1, P<0.01).

PARP inhibitors induced Bcap37 cell apoptosis

Apoptosis of Bcap37 was assessed by Annexin V/PI staining
and fluorescence microscopy (Table 2, Figures 2, 3). Apoptosis was induced by paclitaxel alone and in combination with olaparib. Combined treatment yielded significantly higher rates of apoptosis than paclitaxel alone (P<0.05) and the rates increased with time (P<0.01). Fluorescence micrographs showed that early and late apoptotic cells increased with treatment time. Olaparib inhibited Bcap37 cell proliferation and promoted apoptosis, and this effect enhanced in combination with paclitaxel.

**PARP inhibitors induced degradation of pro-caspase-3 and PARP in Bcap37 cells**

Pro-caspase-3 and PARP were detected by Western blotting of Bcap37 cells treated with paclitaxel alone or in combination with olaparib (Figure 4). Pro-caspase-3 was degraded and PARP was cleaved into two fragments. Pro-caspase-3 and PARP degradation was induced by paclitaxel and enhanced by olaparib in a dose-dependent manner.

**Discussion**

Breast cancer is one of the most common malignancies in women; it is a complex and intrinsically heterogeneous disease, and its incidence and mortality continue to rise (21).

Olaparib is a potent oral PARP inhibitor with acceptable levels of toxicity and monotherapeutic activity in patients who have breast or ovarian cancer with a germline BRCA1 or BRCA2 mutation. The maximum tolerated dose and initial signs of efficacy in BRCA-deficient ovarian cancers have been reported. Tutt and colleagues evaluated the efficacy, safety and tolerability of olaparib alone in BRCA1-
or BRCA2-mutated females with advanced breast cancer. The findings of their study provided robust evidence that supports the concept of PARP inhibition in BRCA1-deficient

breast cancers and showed a favorable therapeutic index for a novel targeted treatment strategy in patients with tumors genetically depleted of BRCA1-associated or BRCA2-associated DNA repair functions (22,23).

Many studies have addressed the anticancer efficacy of the plant-derived compound, paclitaxel. Paclitaxel is a broad-spectrum anticancer drug, providing therapeutic benefits for advanced cancers that are resistant to conventional therapeutic agents, and may exhibit significant curative effects for many clinical malignancies (24). There have been intensively used in clinical practices for paclitaxel since 1987 (25). Paclitaxel regulates microtubule stabilization, induces cell apoptosis and modifies immune functions. In particular, like taxinol which targets the microtubule system, paclitaxel promotes polymerization and inhibits degradation of the microtubules, thereby arresting cell cycle in G2/M stage and inducing tumor cell apoptosis. Paclitaxel is used to treat advanced ovarian cancer, breast cancer, non-small cell lung cancer, and esophageal cancer (14). Paclitaxel is one

Figure 3 Flow cytometry analysis of Bcap37 apoptosis in cells treated with paclitaxel alone or in combination with olaparib. (A) Control; (B) paclitaxel; (C) paclitaxel +100 mg olaparib; (D) paclitaxel +200 mg olaparib; (E) paclitaxel +400 mg olaparib. Q1, mechanically damaged cells; Q2, late apoptosis; Q3, normal cells; Q4, early apoptosis.

Figure 4 Apoptosis-related protein expression in Bcap37 cells treated with PARP inhibitor. Lane 1, paclitaxel; Lane 2, paclitaxel +100 mg olaparib; Lane 3, paclitaxel +200 mg olaparib; Lane 4, paclitaxel +400 mg olaparib.
of the most effective drugs for the treatment of esophageal cancer, with a monotherapy efficiency of 32%. A number of phase I/II clinical trials have demonstrated the efficacy of paclitaxel in the treatment of locally advanced and metastatic esophageal cancer (26). In addition, gemcitabine and paclitaxel are reported in a study by Sandler as new cytotoxic agents which have both been used as single agents or in combination, especially with cisplatin, in the therapy of non-small cell lung cancer with encouraging outcomes (27). Paclitaxel improves therapeutic efficacy when used in combination with other anticancer drugs and has thus come into broad use. Chemotherapy aims to regulate cell growth and apoptosis to eliminate cancer cells. Paclitaxel, gefitinib, gemcitabine, and vinorelbine inhibit proliferation and induce apoptosis in breast cancer (28-34).

We assessed the proliferation and apoptosis of Bcap37 cells treated with paclitaxel alone or in combination with olaparib. The results suggested that PARP inhibitors and paclitaxel function by different mechanisms, but both inhibit tumor proliferation and promote apoptosis; in combination, they yield a synergistic effect. Pro-caspase-3 is a key mediator of apoptosis and PARP is the main substrate for caspase-3; both are important in DNA damage repair and apoptosis. Our study showed that increasing the dose of olaparib led to increased degradation of pro-caspase-3 and PARP, thus confirming that olaparib induces Bcap37 apoptosis. Combined paclitaxel and olaparib inhibited DNA damage repair and may provide an ideal drug option for chemotherapy, although additional research is needed to determine the mechanism of the actions.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References