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Original article

Propofol enhances the cisplatin-induced apoptosis on cervical cancer cells via EGFR/JAK2/STAT3 pathway

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ABSTRACT

Objective: The main purpose of this study was to evaluate propofol and its combined effect with cisplatin on apoptosis of cervical cancer cells and molecular mechanisms of this phenomenon.
Methods: The effects of propofol and cisplatin on cell viability and apoptosis were detected by cell counting kit-8 (CCK-8) assay, colony formation assay and flow cytometry assay. Besides, protein expression of EGFR/JAK2/STAT3 pathway was determined by western blot. STAT3 was over-expressed in cervical cancer cells by STAT3 cDNA. Expression of EGFR and STAT3 protein of human tissues was evaluated by immunohistochemistry (IHC) assay.
Results: In this study, we found that not only propofol alone could inhibit cervical cancer cells viability but also could increase the inhibitory effect of cisplatin on cervical cancer cells growth. Meanwhile, propofol sensitized cervical cancer cells to cisplatin-induced apoptosis but not affected normal cervical cells. In genetic level, propofol could enhance the anti-tumor effect of cisplatin through EGFR/JAK2/STAT3 pathway. Further studies indicated that overexpression of EGFR and STAT3 is related to poor prognoses in cervical cancer patients, which contributed to confirm the clinical role of combined application of propofol and cisplatin.
Conclusion: Propofol enhances the cisplatin-induced cell apoptosis cervical cancer cells via EGFR/JAK2/STAT3 pathway and may be developed as a potential therapeutic agent to treat cervical cancer.

1. Introduction

Cervical cancer is the second most commonly diagnosed cancer and third leading cause of cancer death among females in less developed countries, accounting for an estimated 3.7% (527,600) new cancer cases and 3.2% (265,700) deaths worldwide in 2012 [1]. Concurrent administration of cisplatin and pelvic radiotherapy is the standard treatment for surgically treated early-stage cervical cancer patients with high risk factors [2,3]. However, chemotherapy showed limited effect on improving overall survival or quality of life of patients because some patients' responses to chemotherapy are of short duration. Drug resistance is a serious clinical problem that contributes to the limited effect of chemotherapy [4]. New approaches are needed to overcome patients' resistance to drugs.

Propofol (2, 6-diisopropylphenol, PPF), a general sedative and hypnotic reagent, is widely used for induction and maintenance of general anesthesia [5]. A variety of studies have demonstrated its protective effects against oxidative stress in neuron cells [6] and in endothelial cells [7,8]. Apart from its multiple anesthetic advantages, propofol exerts a number of non-anesthetic effects [9]. Animals studies demonstrated that propofol acting as gamma-aminobutyric acid type A agonist, resulted in neonatal brain cell death and functional deficits in adulthood [10]. Importantly, GABA receptor is also highly expressed in breast cancer metastases to brain [11]. So increasing studies have pointed out the anti-cancer properties of propofol. For breast cancer cells, propofol significantly restrained migration and invasion by inhibition of NF-κB

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pathways and NET1 expression, [12,13]. For lung cancer, Wu et al. found that propofol inhibits MMP-2 and −9 expressions to suppress cancer cell invasion and migration [14]. In human colon carcinoma, propofol stimulation inhibits cancer cell invasion due to ERK1/2-dependent down-regulation of MMPs [15]. In human esophagus cancer, propofol suppresses proliferation, invasion and angiogenesis via ERK-VEGF/MMP-9 signal pathway [16]. In pancreatic cancer, propofol can suppress proliferation and invasion by up-regulating microRNA-133a expression [17]. In cervical cancer, propofol can promote cell apoptosis via inhibiting HOTAIR mediated mTOR pathway [18]. There are also some studies concerning the synergism of propofol and conventional anti-tumor drugs. In pancreatic cells, it has been reported that propofol can increase the sensitivity of gemcitabine-induce apoptosis by inhibition of nuclear factor-κB activity [19]. In ovarian cancer cells, propofol can inhibit invasion and enhance paclitaxel-induced apoptosis through the suppression of the transcription factor slug [20]. Our study focuses on the synergy mechanism of cisplatin and propofol, which is of great importance to clinical practice.

Epidermal growth factor receptor (EGFR) is a 170 kDa transmembrane tyrosine kinase, which activates signaling pathway that promotes cell proliferation, angiogenesis, migration and adhesion and inhibits apoptosis [21]. Epidermal Growth Factor Receptor (EGFR) can be a target in cervical cancer as its overexpression ranges from 6% to 90%, and it has been associated with poor prognosis in some studies [22]. However, EGFR levels were increased in cisplatin-resistant cancers compared with those in cisplatin-sensitive cancers [23]. Cisplatin was known to induce EGFR tyrosine phosphorylation and it has been shown that nuclear localization of EGFR contributes significantly to DNA damage repair after cisplatin treatment [24]. This activation results in resistance to apoptosis, which leads to poor clinical outcomes for cancer patients. Based on these results, we hypothesized that propofol might block multiple intracellular signaling pathways that confer chemoresistance by cervical cancer cells, abrogating either primary or acquired chemoresistance.

Our study found that propofol could enhance the chemotherapeutic efficacy of cisplatin on cell proliferation in cervical cancer cells by inhibiting EGFR/JAK2/STAT3 pathway. This preclinical study documented that sensitization of cancer cells to cisplatin-induced inhibitory effect was achieved by propofol, as shown by stronger cell cytotoxicity compared with single agent treatment.

2. Materials and methods

2.1. Cell lines

The established human cervical cancer cell lines MS751, Caski, Siha, Hela and C33A were obtained from American type culture collection (ATCC). All cells were grown in Dulbecco's modified eagle's medium (DMEM, HyClone, Thermo Scientific, USA) supplemented with 10% fetal bovine serum (Gibco, Life technologies, USA), 100 U/ml penicillin (Biowest, Nuaillé, France) and 1% penicillin/streptomycin. All the cells were incubated at 37 °C in a humidified atmosphere with 5% CO2.

Human primary normal cervical epithelial cells were taken from the cervical tissue of the hysteromyoma patients who underwent complete hysterectomy in Fudan University Shanghai Cancer Center (FUSCC). The primary cells were maintained in Gibco® Medium 199 (Gibco, Life technologies, USA), supplemented with 1 μg/mL epidermal growth factor (EGF), 15% fetal bovine serum (Gibco, Life technologies, USA), 100 U/ml penicillin (Biowest, Nuaillé, France), and 100 U/ml streptomycin (Biowest, Nuaillé, France) and incubated at 37 °C in a humidified atmosphere with 5% CO2.

2.2. Drug treatment

Cisplatin and propofol was purchased from Sigma-Aldrich (St. Louis, MO, USA). Different concentrations of propofol and cisplatin were used to treat cervical cancer lines for cell viability assay for different concentrations. For single agent treatment, all cervical cells were treated with 10–100 μmol/L propofol for 72 h, or 2.5, 5, 10, μmol/L cisplatin for 72 h. For combined treatment, MS751 and Caski cells were treated with 10–100 μmol/L propofol for 24 h, and then were exposed to 2.5–20 μmol/L cisplatin for an additional 48 h. In apoptosis analysis, propofol and cisplatin were used with the most suitable concentration calculated from the cell viability assay.

2.3. Cell viability assay

To evaluate cell viability rate, we plated 8×10^3 cervical cancer cells per well in 96-well plates with 100 μl maintenance medium. The next day, the cells were treated with various concentrations of anticancer drugs. Cell Counting Kit-8 (CCK-8) (Dojindo Laboratories, Kumamoto, Japan) was used to monitor cell viability after 24 h and the number of viable cells was assessed by measurement of absorbance at 450 nm after two-hour incubation by a Microplate Reader (BioTek Instruments, Winooski, VT, USA). The viability rate was calculated as experimental OD value/control OD value.

2.4. Colony formation assay

Cells were pretreated with cisplatin(10 μmol/L)for 72 h, propofol (50 μmol/L) for 72 h, cisplatin(10 μmol/L)and propofol (50 μmol/L) for 72 h 500Cells were plated in a six-well plate in triplicate and separately treated with propofol (50 μmol/L), cisplatin (10 μmol/L), propofol (50 μmol/L, 24 h) plus cisplatin (10 μmol/L, 48 h) for 72 h. The cells were filled with fresh medium and allowed to grow for 2 weeks before being fixed with ice-cold methanol and stained with Crystal violet. The experiments were done at least three times for final analyses.

2.5. Apoptosis analysis by flow cytometry

Cells were treated with cisplatin(10 μmol/L)for 72 h, propofol (50 μmol/L) for 72 h, cisplatin(10 μmol/L)and propofol (50 μmol/L) for 72 h. Cells were labeled with FITC-conjugated Annexin V and PI using an Annexin V-FITC (fluorescein isothiocyanate) apoptosis detection kit (BD Biosciences, San Jose, CA, USA) according to the manufacturer's instructions, and subsequently analyzed by flow cytometry. The early apoptosis was determined based on the percentage of cells with Annexin V+/PI-, while the late apoptosis was that of cells with Annexin V+/PI+. The experiments were done at least three times for final analyses.

2.6. Western blot analysis

Western blot analysis was performed to determine the expression levels of various proteins in cells. Cells were treated with cisplatin(10 μmol/L)for 72 h, propofol (50 μmol/L) for 72 h, cisplatin(10 μmol/L) and propofol (50 μmol/L) for 72 h. Cells were rinsed in ice-cold 1 × PBS twice, and lysed with RIPA lysis buffer containing protease inhibitors (150 mM NaCl, 50 mM Tris–HCl, pH 8.0, 0.05 MEDTA, 1% Triton X-100, 0.1%SDS and 0.005 × protease inhibitor cocktail) for 30 min on ice, then centrifuged at 12,000*g* for 15 min at 4 °C. The total protein concentration was determined by BCA protein assay kit (Beyotime). Equal amounts (30 μg per load) of protein samples were resolved on 10% SDS-PAGE electrophoresis and transferred on to polyvinylidene fluoride (PVDF) membranes (Merck Millipore, Darmstadt, Germany). The blots were blocked in