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Original article

Lobaplatin induces apoptosis and arrests cell cycle progression in human cholangiocarcinoma cell line RBE

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ABSTRACT

The aim of this study was to determine the anticancer effects of lobaplatin in cholangiocarcinoma (CCA) RBE cells. We also explored the mechanism of action of lobaplatin by analyzing its influence on apoptosis and cell cycle. Our findings have shown that lobaplatin inhibits cell proliferations in human CCA cells with an IC50 value of approximately 5.26 ± 0.63 μg/mL. Flowcytometry assay confirmed that lobaplatin affected CCA cell survival by blocking cell cycle progression and inducing apoptosis. Lobaplatin treatment reduced Cyclin D1, CDK4, and CDK6 expression, which led to the blocking of G0/G1 transition. In addition, lobaplatin increased p53, Bax expression, caspase-3 and poly (ADP-ribose) polymerase (PARP) cleavage, and reduced Bcl-2 expression, which contributed to the apoptosis of CCA cells. Lobaplatin showed a good anti-tumour activity in in vitro models of human CCA cells. These results indicate that Lobaplatin, as the third-generation platinum antineoplastic agent, could be an effective chemotherapeutic agent in human CCA treatment through induction apoptosis and cell cycle arrest.

1. Introduction

Cholangiocarcinoma (CCA) is a malignant tumour originating from biliary tract epithelial cells [1,2]. CCA accounts for approximately 15% of the total liver cancer cases worldwide, and its incidence is rising [3]. Among primary liver tumors, CCA is the second most common primary hepatic tumour, which incidence is only less than that of liver cancer [4,5], and it is becoming the most common hepatic tumor-induced death [6].

For the management of carcinoma, systemic chemotherapy with anticancer agents offers a marginal survival benefit such as platinum compounds. Lobaplatin (D-19466; 1, 2-diammino-methyl-cyclobutane-platinum(II)-lactate), one of the third-generation platinum compound, has shown encouraging anticancer activity in a variety of tumor types such as human esophageal cancer, ovarian cancer, breast cancer and small cell lung cancer, colorectal carcinoma, and hepatocellular carcinoma, etc. [7–11], and has been approved in China for the treatment of chronic myelogenous leukemia (CML), metastatic breast cancer and small cell lung cancer [12].

But there was still no research of lobaplatin effects on CCA. To provide evidence for the clinical application of lobaplatin in CCA treatment, the effect of lobaplatin was assessed in human CCA cell line RBE and the underlying molecular mechanisms were explored in the present study.

2. Materials and methods

2.1. Materials

Lobaplatin was purchased from Hainan Chang'an International Pharmaceutical Co., Ltd (China). (USA). Cell Counting Kit-8 (CCK-8 kit) was obtained from Dojindo molecular technologies, Inc. (Japan).

2.2. Cell culture

The RBE cell line was obtained from the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). The cells were maintained in RPMI-1640 (Gibco BRL, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Gibco) and 100 unites/mL penicillin and 0.1 mg/mL streptomycin (Penicillin and Streptomycin Solution (100X), Gibco, USA) at 37 °C in a humidified atmosphere containing 5% CO_2.

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2.3. Proliferation assay

Cytotoxicity of lobaplatin to RBE cell was examined using cell proliferation assay. Cells were seeded in a 96-well microtiter plate at 5×10^3 cells/well, and cultured for 24 hours prior to exposure to lobaplatin of varying concentrations for 48 hours. Ten μL of CCK-8 solution was added to each well of the plate. Incubate the plate for 2 hours in the incubator. The absorbance was read at an optical density (OD) 450 nm using a SpectraMax 190 Microplate Reader (Molecular Devices, Sunnyvale, CA, USA). Six wells were used for each concentration. The inhibitory rate of cell proliferation was calculated by the following formula: inhibition rate (IR) = [1- (OD treated/OD control) × 100%]. The 50% inhibitory concentration (IC50) was calculated by nonlinear regression fit of the mean values of the data obtained in triplicate independent experiments. And cell morphologic changes were assessed using a phase-contrast microscope (CKX41, Olympus. Japan) after 24 hours treatment with lobaplatin.

2.4. Cell cycle distribution analysis

The effect of lobaplatin on RBE cell cycle distribution was determined by FACS analysis. Cells were exposed to different concentrations of lobaplatin respectively for 24 hours. After that, cells were collected, washed twice with PBS and fixed with 70% ethanol solution at 4 °C overnight. After centrifugation, cell pellets were stained with 5 μg/mL PI and 1 μg/mL RNase A at 4 C for 30 minutes. The samples were analyzed by FACSCan flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA.) with CellQuest software. The distributions of cell cycle were analyzed using ModFit LT software. All experiments were repeated and yielded similar results.

2.5. Cell apoptosis analysis

Cell apoptosis analysis was performed using Annexin V-FITC Apoptosis Detection Kit (BioVision, CA, USA.). RBE cells were exposed to different concentrations of lobaplatin (0–25 μg/mL) for 24 hours. After that, 5 × 105 cells were collected by centrifugation and resuspended in 500 μL of 1 × Binding Buffer. Five μL of Annexin V-FITC and 5 μL of propidium iodide (PI) were added to the cells. After incubation at room temperature for 5 minutes in the dark, cells were then analyzed by flowcytometry (Becton Dickinson, Franklin Lakes, NJ, USA.). All experiments were repeated and yielded similar results.

2.6. Western blot analysis

The proteins of apoptosis and cell cycle-related signaling pathways were examined by Western blot. RBE cells were treated with various concentrations of lobaplatin for 24 hours. After treatment culture, cells were lysed by ice-cold RIPA buffer (50 mM Tris-HCl, 10% glycerol, 1% Triton X-100, 150 mM NaCl, 100 mM NaF, 5 mM EDTA, 2 mM phenylmethylsulfonyl fluoride, 1 mM sodium orthovanadate, and 1 mg/mL leupeptin, pH 7.5).

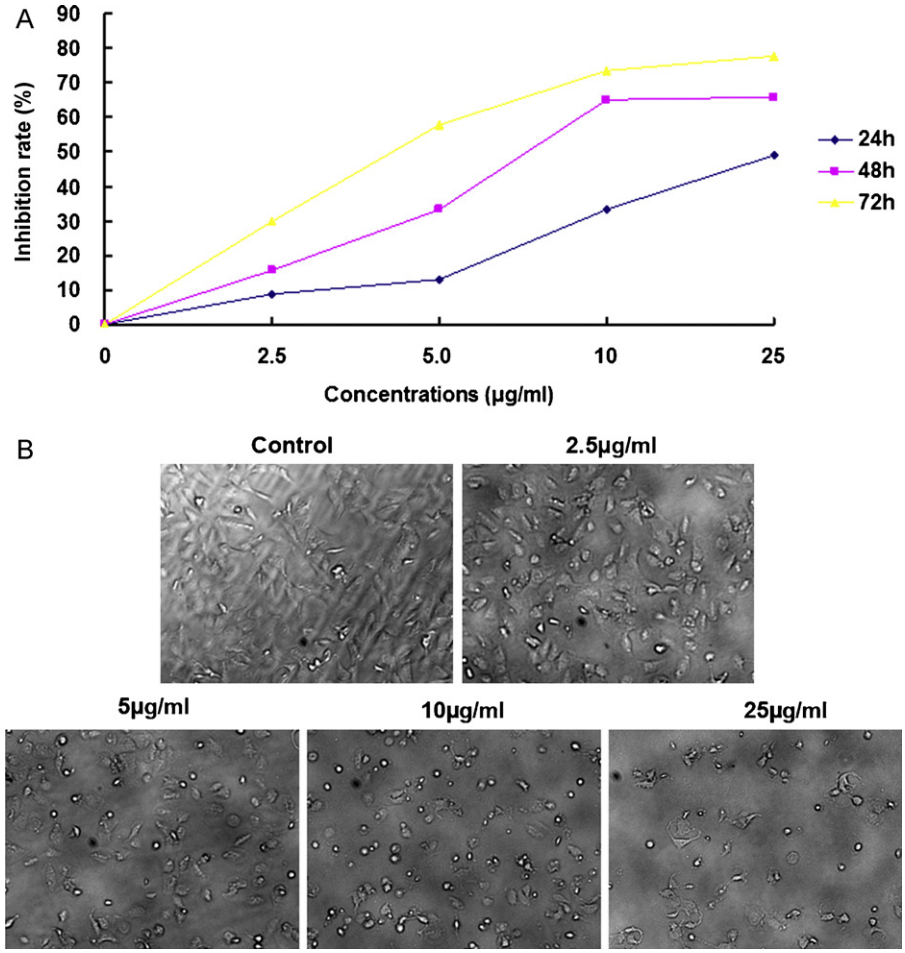

Fig. 1. Influence of lobaplatin on the cell proliferation (A) and morphology changes in human cholangiocarcinoma RBE cells (B). Cells were exposed to various concentrations of lobaplatin (0, 2.5, 5, 10, and 25 μg/mL) for 48 hours, followed by analysis using a CCK-8 assay. And cell morphologic changes were assessed using a phase-contrast microscope after 24 hours treatment.