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ORIGINAL ARTICLE

Anticancer effects of clinically acceptable colchicine concentrations on human gastric cancer cell lines

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Abstract Colchicine is a very cheap microtubule destabilizer. Because microtubules are an ideal target for anticancer drugs, the purpose of this study was to investigate whether clinically acceptable colchicine concentrations have anticancer effects on gastric cancer cells, and its possible anticancer mechanisms. Two human gastric cancer cell lines (i.e., AGS and NCI-N87) were investigated by proliferative assay, microarray, quantitative reverse transcriptase-polymerase chain reaction, and a nude mice study using clinically acceptable colchicine concentrations (2 ng/mL and 6 ng/mL for *in vitro* tests and 0.07 mg colchicine/kg/d for *in vivo* tests). Our results showed that colchicine had the same inhibitory effects on the proliferation of both cell lines. The antiproliferative effects of colchicine on both cell lines were achieved only at the concentration of 6 ng/mL ($p < 0.0001$). In both cell lines, 18 genes were consistently upregulated and 10 genes were consistently downregulated by 6 ng/mL colchicine, compared with 2 ng/mL colchicine. Among these genes, only the upregulated *DUSP1* gene may contribute to the antiproliferative effects of colchicine on gastric cancer cells. The nude mice (BALB/c-nu) experiment showed that colchicine-treated mice after 14 days of treatment had lower increased tumor volume ratios ($p = 0.0199$) and tumor growth rates ($p = 0.024$) than the control mice. In conclusion, colchicine has potential for the

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palliative treatment of gastric cancer. However, the anticancer effects are achieved only at high clinically acceptable colchicine concentrations. Monitoring the colchicine plasma concentration is mandatory if this drug is applied for the palliative treatment of gastric cancer.

Introduction

Gastric cancer is a disease with one of the highest cancer mortality. Surgical resection of all lesions is essential for curative treatment. For patients with advanced gastric cancer, many regimens of palliative chemotherapy have been applied, but the results are modest [1—3]. Therefore, a search for a new anticancer drug suitable for the palliative treatment of patients with advanced gastric cancer is necessary.

Colchicine is a very cheap alkaloid agent that has been used in medicine for a long time [4—7]. Colchicine is a microtubule destabilizer that has very strong binding capacity to tubulin to perturb the assembly dynamics of microtubules [8—11]. Colchicine also can increase cellular free tubulin to limit mitochondrial metabolism in cancer cells through inhibition of the voltage-dependent anion channels of the mitochondrial membrane [12]. The clinical application of colchicine has been limited because of its toxicity, although oral colchicine is a safe treatment when appropriately used and contraindications have been excluded [4—7]. The peak plasma concentrations after oral administration of 0.6—1 mg colchicine range approximately 2—6 ng/mL [13—15]. The lowest reported lethal dose of oral colchicine is 7—26 mg, and an acute ingestion of colchicine exceeding 0.5 mg/kg has a high fatality rate [7]. Our recent study showed that clinically acceptable colchicine concentrations (i.e., 2—6 ng/mL) had potential for the palliative treatment of hepatocellular carcinoma [16] and cholangiocarcinoma [17]. The antiproliferative effects of 6-ng/mL colchicine on hepatocellular carcinoma cells were the same as 1 μg/mL epirubicin, which is near the maximum plasma concentration of epirubicin obtained by an intravenous bolus of 75 mg/m^2 body surface epirubicin in patients [18]. The purpose of this study was to investigate whether clinically acceptable colchicine concentrations also had anticancer effects on gastric cancer cells and its possible anticancer mechanisms. All gene names are in accordance with the official symbols from the HUGO Gene Nomenclature Committee provided by the United States National Center for Biotechnology Information (Bethesda, MD, USA).

Materials and methods

Cell lines

Two human gastric cancer cell lines—AGS and NCI-N87—purchased from the American Type Culture Collection (Rockville, MD, USA) were investigated. All cultures were maintained at 37°C in a humidified atmosphere of 5% carbon dioxide (CO_2) and 95% air. The serum-containing culture medium consisted of 10% fetal bovine serum, 90% Dulbecco's Modified Eagle's high glucose medium, supplemented with 20mM L-glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin (HyClone, Logan, Utah, USA). Colchicine was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Proliferative experiment

Each cell line was seeded in 96-well culture plate and incubated with serum-containing medium for 48 hours. The medium was then replaced with serum-free medium containing various concentrations of colchicine (0 ng/mL, 2 ng/mL, 6 ng/mL). The cells were further incubated for 72 hours for the proliferative assay. The premixed WST-1 cell proliferation reagent (Clontech Laboratories, Inc., A Takara Bio Company, Mountain View, CA, USA) was applied. The principle for this examination is that the stable tetrazolium salt, WST-1, is cleaved to soluble formazan dye by viable cells. The amount of formazan dye formed detected by spectrophotometer (expressed as optical density) is directly correlated with the number of metabolically active cells in the culture. The experimental procedures were performed by the manufacturer's protocols. The cells were incubated with reagent for 3 hours at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. The results were thereafter analyzed by an automated microplate reader (MRX; Dynex Technologies, Inc., Chantilly, VA, USA). The absorbance was measured at the 450-nm wavelength. (The reference wavelength was 630 nm.) In all experiments, 16 replicate wells were used for statistical calculation.

Microarray and quantitative reverse transcriptase-polymerase chain reaction experiments

Each cell line was seeded in three 25-cm^2 plastic culture flasks with a serum-containing medium for 24 hours. The medium was then replaced with serum-free medium containing various concentrations of colchicine (i.e., 0, 2 ng/mL, 6 ng/mL). The cells were further incubated for 24 hours. The total RNA in each flask was extracted by Trizol Reagent (Invitrogen/Life Technologies Corporation, NY, USA), and followed by RNAeasy Mini Kit (QIAGEN GmbH, Hilden, North Rhine-Westphalia, Germany). Purified RNA was quantified at optical density 260 nm by an ND-1000 spectrophotometer (Nanodrop Technology, Wilmington, DE, USA) and qualified by a bioanalyzer (Bioanalyser 2100; Agilent Technology, Santa Clara, CA, USA). Two microarrays (Agilent SurePrint G3 Human GE 8 × 60 k; Agilent Technologies, Santa Clara, CA, USA) were applied to investigate