Kaohsiung Journal of Medical Sciences (2015) 31, 229–234

Available online at www.sciencedirect.com

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ORIGINAL ARTICLE

Clinically acceptable colchicine concentrations have potential for the palliative treatment of human cholangiocarcinoma

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Received 11 November 2014; accepted 19 January 2015
Available online 10 March 2015

KEYWORDS
Animal study;
Cholangiocarcinoma;
Colchicine;
Proliferation

Abstract Microtubules are an ideal target for anticancer drugs because of their essential role in mitosis. Colchicine is a microtubule destabilizer. Whether the clinically acceptable colchicine concentrations had anticancer effects on human cholangiocarcinoma cells was investigated. Two human cholangiocarcinoma cell lines (C14/KMUH, C51/KMUH) were investigated using clinically acceptable plasma colchicine concentrations (2 ng/mL and 6 ng/mL for the *in vitro* experiment, 0.07 mg colchicine/kg/d × 14 days for the nude mouse experiment). Our results showed that colchicine caused significantly dose-dependent antiproliferative effects on both cell lines (all $p < 0.0001$). Nude mouse (BALB/c-nu) experiments showed that the increased tumor volume ratios in colchicine-treated mice were significantly lower than control mice started from the 11th day of treatment ($p = 0.0167$). The tumor growth rates in colchicine-treated mice after 14 days of treatment were significantly lower than in control mice (0.147 ± 0.004/d vs. 0.274 ± 0.003/d, $p = 0.0015$). In addition to the well-known direct colchicine–tubulin interaction as a common anticancer mechanism of colchicine, microarray and quantitative reverse transcriptase-polymerase chain reaction showed that the antiproliferative effects of both 2 ng/mL and 6 ng/mL colchicine on C14/KMUH cells could be partially explained by downregulations of both *HSD11B2* and *MT-COI*. There was no effect of colchicine

Conflicts of interest: All authors declare no conflicts of interest.

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http://dx.doi.org/10.1016/j.kjms.2015.01.008

on *MT-COI* expression in C51/KMUH cells, however, 6 ng/mL colchicine also downregulated *HSD11B2* in this cell line. In conclusion, clinically acceptable colchicine concentrations can inhibit the proliferation of human cholangiocarcinoma cells. This drug has good potential for the palliative treatment of cholangiocarcinoma due to its low cost and our long-standing prescription experience.

Introduction

Cholangiocarcinoma (CC) is one of the most difficult-to-treat intra-abdominal malignancies. Although surgical resection of the tumor and liver transplantation may provide the opportunity for long-term survival, these modalities can only be applied in a small number of patients [1]. For patients unable to receive a surgical approach, several palliative treatments including radiotherapy, chemotherapy, and photodynamic therapy have been applied but the effects were limited [2].

The common characteristic of cancer cells is increased rate of mitosis, which means that cancer cells are more vulnerable to mitotic poison than are normal cells. Microtubules have long been considered as an ideal target for anticancer drugs because of their essential roles in mitosis and forming the dynamic spindle apparatus. Colchicine is a cheap alkaloid agent that has been used in medicine for a very long time [3—5]. It is a microtubule destabilizer that has a very strong binding capacity to tubulin to perturb the assembly dynamics of microtubules [6—9]. It 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 [10]. The cost of colchicine is much more affordable than other microtubule-interfering agents such as paclitaxel and docetaxel. Moreover, oral intake of colchicine is a very convenient and safe treatment when it is appropriately used and contraindications have been excluded [3—5]. The peak plasma concentrations after oral administration of 0.6—1 mg colchicine range from approximately 2 ng/mL to 6 ng/mL [11—13]. The lowest reported lethal doses of oral colchicine are 7—26 mg and acute ingestions of colchicine exceeding 0.5 mg/kg have a high fatality rate [5]. Our recent *in vitro* and *in vivo* experiments showed that the clinically acceptable colchicine concentrations had significantly dose-dependent anticancer effects on hepatocellular carcinoma (HCC) cells [14]. The anticancer effects of colchicine on HCC cells originated not only from the well-known direct colchicine—tubulin interaction [6—10] but also from colchicine-induced differential expressions of several antiproliferative genes [14]. Whether the clinically acceptable colchicine concentrations also had significant anticancer effects on CC cells is still unknown. This study was done to clarify this issue for the investigation of the potential role of colchicine in the palliative treatment of CC. All gene names are according to the official symbols from the HUGO Gene Nomenclature Committee provided by the US National Center for Biotechnology Information.

Materials and methods

Cell lines

Two human CC cell lines (C14/KMUH, C51/KMUH) established by our institution were investigated. The detailed methodology for the establishment of these cell lines was described in our previous study [15]. These cell lines were verified using positive for periodic acid-Schiff stain and stain for cytokeratin 19 but negative for monoclonal mouse antihuman hepatocyte antigen. All procedures to establish these cell lines were approved by the Institutional Review Board of our hospital, and patients provided informed consent. All cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. The serum-containing culture medium consisted of 10% fetal bovine serum, 90% Dulbecco Modified Eagle high glucose medium, supplemented with 20mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin (HyClone, Logan, Utah, USA). Colchicine was purchased from Sigma-Aldrich Corporate (St. Louis, MO, USA).

Proliferative experiment

Each cell line seeded in a 96-well culture plate was incubated with serum-containing medium for 24 hours. Next, the medium was replaced with serum-free medium with various concentrations of colchicine (0 ng/mL, 2 ng/mL, and 6 ng/mL). The cells were incubated for an additional 72 hours for 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 a soluble formazan by viable cells. The amount of formazan dye formed detected by the spectrophotometer expressed as optical density directly correlates to the number of metabolically active cells in the culture. The experimental procedures were carried out following 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. Then, the results were analyzed using an automated microplate reader (MRX, Dynex Technologies, Inc., Chantilly, VA, USA). Absorbance was measured at a wavelength of 450 nm (reference wavelength 630 nm). In all experiments, 16 replicate wells were used for statistical calculation.