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ORIGINAL ARTICLE—ALIMENTARY TRACT

The synthesized 2-(2-fluorophenyl)-6,7-methylenedioxyquinolin-4-one (CHM-1) promoted G2/M arrest through inhibition of CDK1 and induced apoptosis through the mitochondrial-dependent pathway in CT-26 murine colorectal adenocarcinoma cells

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Abstract

Background In this study, we investigated the effects of 2-(2-fluorophenyl)-6,7-methylenedioxyquinolin-4-one (CHM-1) on cell viability, cell cycle arrest and apoptosis in CT-26 murine colorectal adenocarcinoma cells.

Methods For determining cell viability, the MTT assay was used. CHM-1 promoted G2/M arrest by PI staining and flow cytometric analysis. Apoptotic cells were evaluated by DAPI staining. We used CDK1 kinase assay, Western blot analysis and caspase activity assays for examining the CDK1 activity and proteins correlated with apoptosis and cell cycle arrest. The in vivo anti-tumor effects of CHM-1-P were evaluated in BALB/c mice inoculated with CT-26 cells orthotopic model.

Results CHM-1 induced CT-26 cell viability inhibition and morphologic changes in a dose-dependent and time-dependent manner and the approximate IC_{50} was 742.36 nM. CHM-1 induced significant G2/M arrest and apoptosis in CT-26 cells. CHM-1 inhibited the CDK1 activity and decreased CDK1, Cyclin A, Cyclin B protein levels. CHM-1 induced apoptosis in CT-26 cells and promoted increasing of cytosolic cytochrome *c*, AIF, Bax, BAD, cleavage of pro-caspase-9, and -3. The significant reduction of caspase-9 and -3 activity and increasing the viable CT-26 cells after pretreated with caspase-9 and -3 inhibitor indicated that CHM-1-induced apoptosis was mainly mediated a mitochondria-dependent pathway. CHM-1-P improved mice survival rate, and enlargement of the spleen and liver metastasis were significantly reduced in groups treated with either 10 mg/kg and 30 mg/kg of CHM-1-P and 5-FU in comparison to these of CT-26/BALB/c mice.

Conclusions Taken together, CHM-1 acted against colorectal adenocarcinoma cells in vitro via G2/M arrest and apoptosis, and CHM-1-P inhibited tumor growth in vivo.

Keywords CHM-1 and CHM-1-P · CT-26 colorectal adenocarcinoma cells · Apoptosis · G2/M arrest · Orthotopic model

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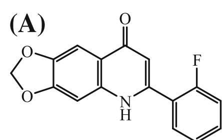
Introduction

Colorectal cancer is one of the major causes of deaths in the world, and it is the third leading cause of cancer death in Taiwan. According to the People Health Bureau of Taiwan, there are about 37.4 new cases per 100,000 people per year [1]. Colorectal cancer is a multi-step process

involving progressive disruption of intestinal epithelial cells proliferation [2]. The 5-fluorouracil (5-FU) is one of the used chemotherapeutic agents in first-line therapy for colorectal cancer, but serious side effects such as nausea, fatigue, and a decline in the number of blood cells, and limits its clinical usefulness [3, 4]. New compounds that are not detrimental to normal cells and exert cytotoxic effects only on colorectal cancer cells are considered to be the most chemotherapeutic effective. The progression of the cell cycle has been intensively investigated, particularly CDK1/Cyclin B complex's important role in the regulation of G2/M phase [5, 6]. Anti-mitotic agents affecting microtubule assembly or disassembly have been shown to interfere with the progression of the cell cycle, and some of them have shown significantly clinical anti-cancer effects [7, 8]. Numerous evidences have shown that induction of cell death in tumor cells is through cell cycle arrest and apoptosis [9, 10].

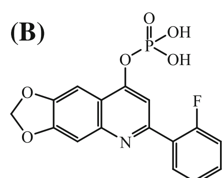
Selectively inducing apoptosis in colorectal cancer cells has been increasingly recognized as a promising therapeutic approach for colorectal cancer. Recently, we have designed and synthesized a series 2-phenyl-4-quinolone series compounds as novel anti-mitotic agents, CHM-1 (2-(2-fluorophenyl)-6,7-methylenedioxyquinolin-4-one, as shown in Fig. 1a) is the potential compound for anti-cancer activities. CHM-1 significantly inhibited tubulin polymerization and showed cytotoxic effect on many human tumor cell lines (SK-Hep-1, HA22T, Hep3B, HepG2 and U2-OS cells) [11, 12]. We also have designed and synthesized CHM-1-P (2-(2-fluorophenyl)-6,7-methylenedioxyquinolin-4-yl dihydrogen phosphate, as shown in Fig. 1a), and it was quickly converted into its active parent compound CHM-1 in vivo by pharmacokinetic analysis. However, neither the cytotoxic effects of CHM-1 and CHM-1-P on colorectal cancer cells nor the molecular mechanisms underlying its anti-cancer activity have been revealed. Therefore, in this study, we investigated the molecular mechanisms of its anti-cancer effects on CT-26 cells in both in vitro and in vivo assays.

(A)



2-(2-fluorophenyl)-6,7-methylenedioxyquinolin-4-one (CHM-1)

(B)



2-(2-fluorophenyl)-6,7-methylenedioxyquinolin-4-yl dihydrogen phosphate (CHM-1-P)

Fig. 1 The structure of CHM-1 (**a**) and CHM-1-P (**b**)

Materials and methods

Chemicals and reagents

CHM-1 (2-(2-fluorophenyl)-6,7-methylene-dioxyquinolin-4-one) and CHM-1-P (2-(2-fluorophenyl)-6,7-methylene-dioxyquinolin-4-yl dihydrogen phosphate; Fig. 1) were synthesized and obtained from Dr. Sheng-Chu Kuo in Graduate Institute of Pharmaceutical Chemistry, College of Pharmacy, China Medical University. Potassium phosphates, dimethyl sulfoxide (DMSO), Propidium iodide (PI), Triton X-100, Tris–HCl, Ribonuclease-A were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Caspase-9 inhibitor (Z-LEHD-FMK), caspase-3 inhibitor (Z-DEVD-FMK) (R&D, USA) were dissolved in DMSO and diluted in cell culture medium before use.

Cell culture

CT-26 colorectal adenocarcinoma cells, *N*-nitroso-*N*-methyl urethane-induced mouse colon carcinoma cells of BALB/c origin, were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultured in RPMI-1640 medium (GIBCO, Grand Island, NY, USA) supplemented with 10% heat-inactivated fetal calf serum (Hyclone, Logan, UT, USA) at 37°C in a humidified 5% CO_2 and 95% air at 1 atm incubator. Full grown monolayer cultures were trypsinized (0.25% trypsin-EDTA), harvested and passaged several times for expansion [13].

Determinations of cell viability by MTT assay

The CT-26 cells were seeded onto 96-well cell culture plates at 2.5×10^4 cells/well then incubated with 0, 250, 500, 750 and 1000 nM of CHM-1 for 24, 48 and 72 h. Then the cells were treated by the addition of MTT dye to each well. After an additional 4 h incubation, the growth medium was removed and the formazan crystals, formed by oxidation of the MTT dye, were dissolved with 0.04 N HCl in isopropanol. The absorbance was measured at 570 nm and the cell survival ratio was expressed as a percentage of the control [14].

Determinations of cell cycle and apoptosis by PI staining

The CT-26 cells were seeded onto 24-well cell culture plates at 2.5×10^5 cells/well then incubated with 750 nM of CHM-1 for 0, 12, 24 and 48 h. The cells were harvested and washed by centrifugation. For cell cycle and apoptosis determination, cells were fixed gently by putting 70% ethanol in −20°C overnight and then re-suspended in PBS

containing 40 μg/ml PI and 0.1 mg/ml RNase and 0.1% Triton X-100 in dark room for 30 min. The cell cycle and apoptotic nuclei were determined by flow cytometry (FACS Calibur™, Becton Dickinson, Franklin Lakes, NJ, USA) as described previously [15].

DAPI staining

The CT-26 cells were seeded onto 6-well cell culture plates at 2.5×10^5 cells/well then incubated with 750 nM of CHM-1 for 0 and 24 h. After incubation for the indicated periods of time, the cells were fixed gently by 70% ethanol, then the cells were stained with DAPI (4,6-diamidino-2-phenylindole dihydrochloride), then photographed using a fluorescence microscope as described previously [16].

CDK1 kinase assay

CDK1 kinase activity was analyzed according to the protocol of Medical & Biological Laboratories CDK1 kinase assay kit (MBL, Nagoya, Japan). About 1×10^6 cells were suspended in a buffer containing, in a final volume of 0.2 ml, 50 mM Tris–HC1, pH 7.5, 1 mM phenylmethylsulfonyl fluoride, 50 pg/ml leupeptin, 10 mM 2-mercaptoethanol, 1 mM MgCl, 2 mM EGTA, 0.5 mM dithiothreitol, 0.01% Brij35, 25 mM β-glycerophosphate, and 0.5 M NaCl. Cell suspensions were sonicated and centrifuged at $100{,}000\times g$ for 30 min. To determine the CDK1 kinase assay condition using MV Peptide, and determined by measuring OD 492 as described previously [17].

Caspase activity assay

The CT-26 cells were seeded onto six-well cell culture plates at 2.5×10^6 cells/well then incubated with 750 nM of CHM-1 or caspase-3 inhibitor (Z-DEVD-FMK), caspase-9 inhibitor (Z-LEHD-FMK) for 1 h prior to treatment with CHM-1 for 6, 12, 18 and 24 h. Cytosolic fraction proteins were prepared and determined as described previous [17, 18]. About 50 μg of cytosol proteins were incubated with caspase-3 and -9 specific substrates [Ac-DEVD-pNA and Ac-IETD-pNA (R&D System)] for 1 h at 37°C. The caspase activity was determined by measuring OD 405 as described previously [17, 18].

Western blotting

The CT-26 cells were seeded onto 10-cm dish at an initial concentration of 1.0×10^7 cells and incubated with 750 nM of CHM-1 for 6, 12 and 24 h. Cytosolic fraction and total protein were prepared and determined as described previous [17, 18]. Equal amount of proteins (30 μg) were separated by SDS-PAGE and transferred onto PVDF membrane. Blots were blocked in PBST buffer (0.05% Triton X-100 in PBS) buffer containing 5% non-fat milk for 1 h, the membrane was incubated overnight at 4°C with specific primary antibodies (Cyclin A, Cyclin B, CDK1, Cytochrome *c*, Pro-caspase-9, Pro-caspase-3 AIF, Bax, BAD, Bcl-2, Bcl-xL and Actin). The membrane was washed with PBST buffer and incubated with horseradish peroxidase (HRP) conjugated secondary antibodies. The specific protein was detected by using enhanced chemiluminescence kits (Amersham ECL Kits) [15–17].

In vivo anti-colorectal cancer activity in CT-26 orthotopic model

About 80 male BALB/c mice of 22–28 g in weight at the age of 8 weeks were obtained from Laboratory Animal Center, College of Medicine, National Taiwan University (Taipei, Taiwan). The BALB/c mice were maintained at the Animal Center of China Medical University for 2 weeks under animal guidelines before the grouping and experiments. The BLAB/c mice were divided into four groups and each group contained twenty animals. Group I was injected i.p. with 1.0×10^6/100 μl of CT-26 cells as a control. Group II was injected with CT-26 cells and treated with (10 mg/kg, oral) CHM-1-P in PBS. Group III was injected with CT-26 cells and treated with (30 mg/kg, oral) CHM-1-P in PBS. Group IV was injected with CT-26 cells and treated with (30 mg/kg, oral) 5-fluorouracil (5-FU, i.p.). Anti-colorectal cancer activity was assessed as the survival time (each group contained ten animals) in the treatment and un-treated group. Furthermore, the CHM-1 and 5-FU groups (each group also contained ten animals) were treated on 35 days then the animals were weighed and killed. Anti-tumor activity was assessed as the ratio of median survival time (MST) in the treatment group (T) to MST in the control group (C), and the results are shown as T/C: life span T/C (%) = (MST of drug-treated group/MST of control group) × 100. Long-term survivors were recorded until each animal died. The colon, liver and spleen samples were isolated and weighed individually for histopathology. The total tumor volume in each animal was estimated according to the formula: total tumor volume (mm^3) = $L \times W^2/2$, where L is the length and W is the width [19].

Statistical analysis

In vitro study, Student's t test was used to analyze differences between CHM-1-treated and control groups. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. In vivo study, mice body and spleen weight results were expressed as mean ± SD and the difference between the groups were

tested by one way ANOVA. Significant differences between values for CT-26/BALB/c mice and various treatment are shown $***p < 0.001$.

Results

Next, we examine the effects of CHM-1 on cell viability and morphology of CT-26 cells. CT-26 cells were treated with CHM-1 (Fig. 1a) at 250, 500, 750 and 1000 nM. The cell viability was determined by MTT method at 24, 48 and 72 h later. As shown in Fig. 2a, CHM-1 reduces CT-26 cell viability in a dose- and time-dependent manner. The approximate IC_{50} was 742.36 nM. Apoptotic morphological examinations in Fig. 2b by DAPI staining and the result show that CHM-1 induced DNA condensation and apoptosis in the CT-26 cells.

CHM-1 induced G2/M arrest and apoptosis in CT-26 cells. We investigated whether CHM-1 could induce cell cycle arrest and apoptosis in CT-26 cells. Cells were treated with 750 nM of CHM-1 for 12, 24 and 48 h then were analyzed cell cycle distribution by PI staining and flow cytometry. As shown in Fig. 3a, CHM-1 induced a time-dependent accumulation of G2/M population in CT-26 cells (13.82% G2/M at 0 h; 44.63% G2/M at 12 h; 53.26% G2/M at 24 h; 66.84% G2/M at 48 h), and then the cells underwent apoptosis (1.02% Sub-G1 at 0 h; 18.68% Sub-G1 at 12 h; 45.68% Sub-G1 at 24 h; 61.05% Sub-G1 at 48 h). To expose the mechanisms of CHM-1 induced G2/M arrest, we investigated the protein expressions of CDK1, Cyclin A, Cyclin B by Western blotting, and the CDK1 activity determination. After exposure to 750 nM of CHM-1, CT-26 showed a decrease in the protein levels of CDK1, Cyclin A and Cyclin B that were examined by Western blotting (Fig. 3b). CDK1 kinase assays on CHM-1 treated CT-26 cells showed a significant decrease in the activity of CDK1 at 12 and 24 h after CHM-1 treatment (Fig. 3c).

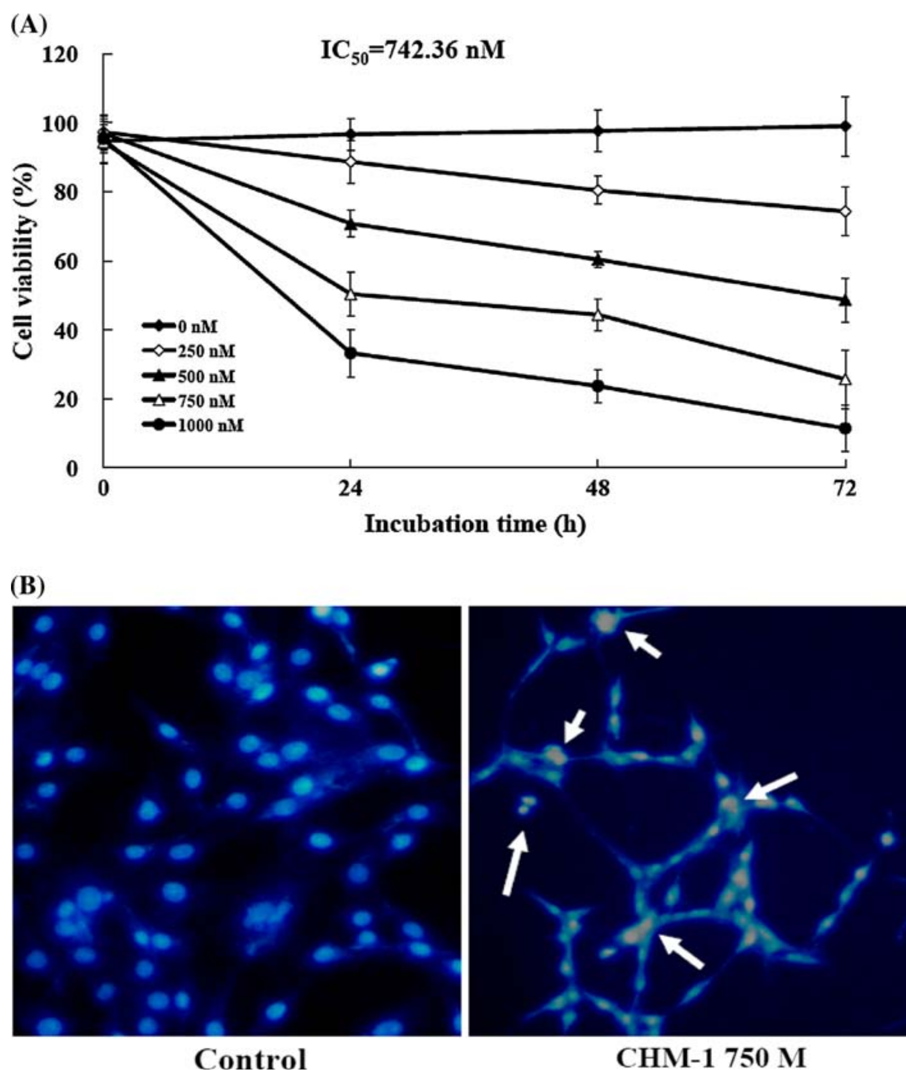


Fig. 2 Effects of CHM-1 on the percentage of CT-26 cell viability (**a**) and DNA condensation by DAPI staining (**b**). Cells were cultured with 0, 250, 500 and 1000 nM of CHM-1 for 24, 48 and 72 h. For percentages of viable cells were detected by MTT assay described in "Materials and methods". Each point is a mean ± SD as of three experiments. For DAPI staining, the cells were examined and photographed under fluorescence microscopy (×400)

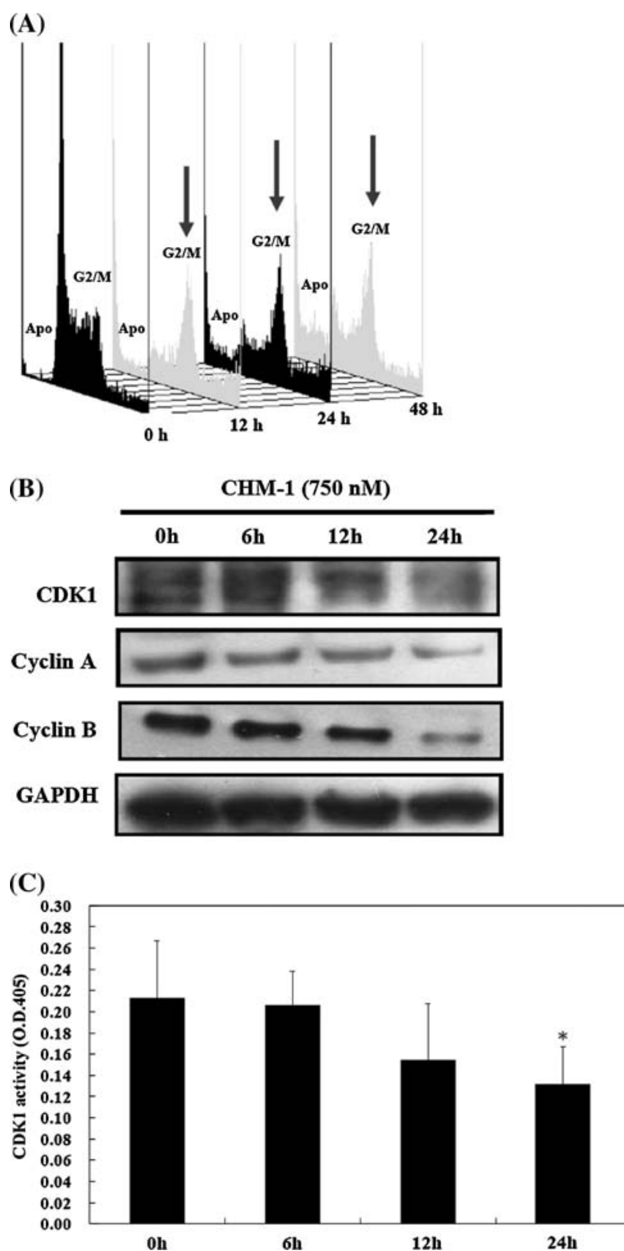


Fig. 3 Effects of CHM-1 on cell cycle distribution, associated proteins levels, and CDK1 kinase activity in CT-26 cells. Cells were treated with 750 nM CHM-1 for 0, 12, 24 and 48 h. Cell cycle distribution (**a**) were determined by flow cytometric assay as described in "Materials and methods". Cells were treated with 750 nM CHM-1 for 0, 6, 12 and 24 h, then total proteins were prepared then detected for CDK1, cyclin A, cyclin B by Western blotting (**b**) and CDK1 kinase activity (**c**). CDK1 kinase activity was measured as described in "Materials and methods". Data represents mean ± SD of three experiments. $*p < 0.05$

Effects of CHM-1 on the levels of apoptosis associated proteins in CT-26 cells. To examine whether the mitochondrial-mediated apoptotic pathway was involved in CHM-1-induced apoptosis, we examined the expression levels of these Bcl-2 family proteins (Bax, BAD, Bcl-2 and Bcl-xL) and cytosolic cytochrome *c*, pro-caspase-9, pro-caspase-3 and AIF. As shown in Fig. 4a, CHM-1 promoted the levels of cytosolic cytochrome *c* and AIF but decreased the expression of pro-caspase-9 and pro-caspase-3. In Fig. 4b, 750 nM of CHM-1 decreased the protein expression of Bcl-2, Bcl-xL and increased the protein expression of Bax and BAD. These results suggest that CHM-1 induced apoptotic response is mediated by mitochondrial pathway. Therefore, we investigated the CHM-1-treated CT-26 cells for the caspase-9 and caspase-3 activities by caspase activity assay. Both caspase-9 and caspase-3 activities increased at 12 h after CHM-1 treatment (Fig. 4c, d), whereas the activity of caspase-8 was not affected (data not shown). Moreover, pre-incubation with caspase-9 inhibitor (Z-LEHD-FMK) or caspase-3 inhibitor (Z-DEVD-FMK), strongly reduced the CHM-1-induced caspase-9 and caspase-3 activities and an increase in the percentage of viable cells in CT-26 cells (Fig. 4e). This confirms that CHM-1-induced apoptosis is mediated by induction of caspase-9 and -3 activities.

Anti-colorectal cancer activity of CHM-1-P on CT-26 orthotopic model. CHM-1-P was quickly converted into its active parent compound CHM-1 by in vivo pharmacokinetic analysis. On the basis of the significant growth-inhibitory effect of CHM1 in vitro, we tested the effects of CHM-1-P on the survival time of CT-26 orthotopic model (CT-26/BALB/c mice). We orally administered 10 and 30 mg/kg of CHM-1-P and i.p. administered 30 mg/kg of 5-FU in BALB/c mice after they had been inoculated with CT-26 cells. As shown in Fig. 5a, 10 and 30 mg/kg of CHM-1-P significantly prolonged the survival time of CT-26/BALB/c colorectal cancer mice. The average life span on CHM-1-P (10 mg/kg) was prolonged by 144.44% and on CHM-1-P and 5-FU (30 mg/kg) was prolonged by 155.56%. CHM-1 showed a broad spectrum and high degree of in vivo antitumor activity. In addition, we observed no significant difference in body weight between control and CHM-1-P-treated animals (Fig. 5b). The total tumor volume on colon, spleen and liver tissues were excised from individual animals after 35 days treatment. The photograph and spleen weight were examined and representative results are presented. Our data showed that spleen enlargement (Fig. 5c), total tumor volume (Fig. 5d) and liver metastasis (Fig. 5e) were significantly reduced in the CHM-1-P and 5-FU treated mice in comparison to those in CT-26/BALB/c mice. The results supported that CHM-1-P and 5-FU in CT-26/BALB/c mice were due to anti-colorectal cancer activity.

Discussion

Anti-mitotic agents interact in the tubulin/microtubule system constitute an important concept in anti-cancer drug

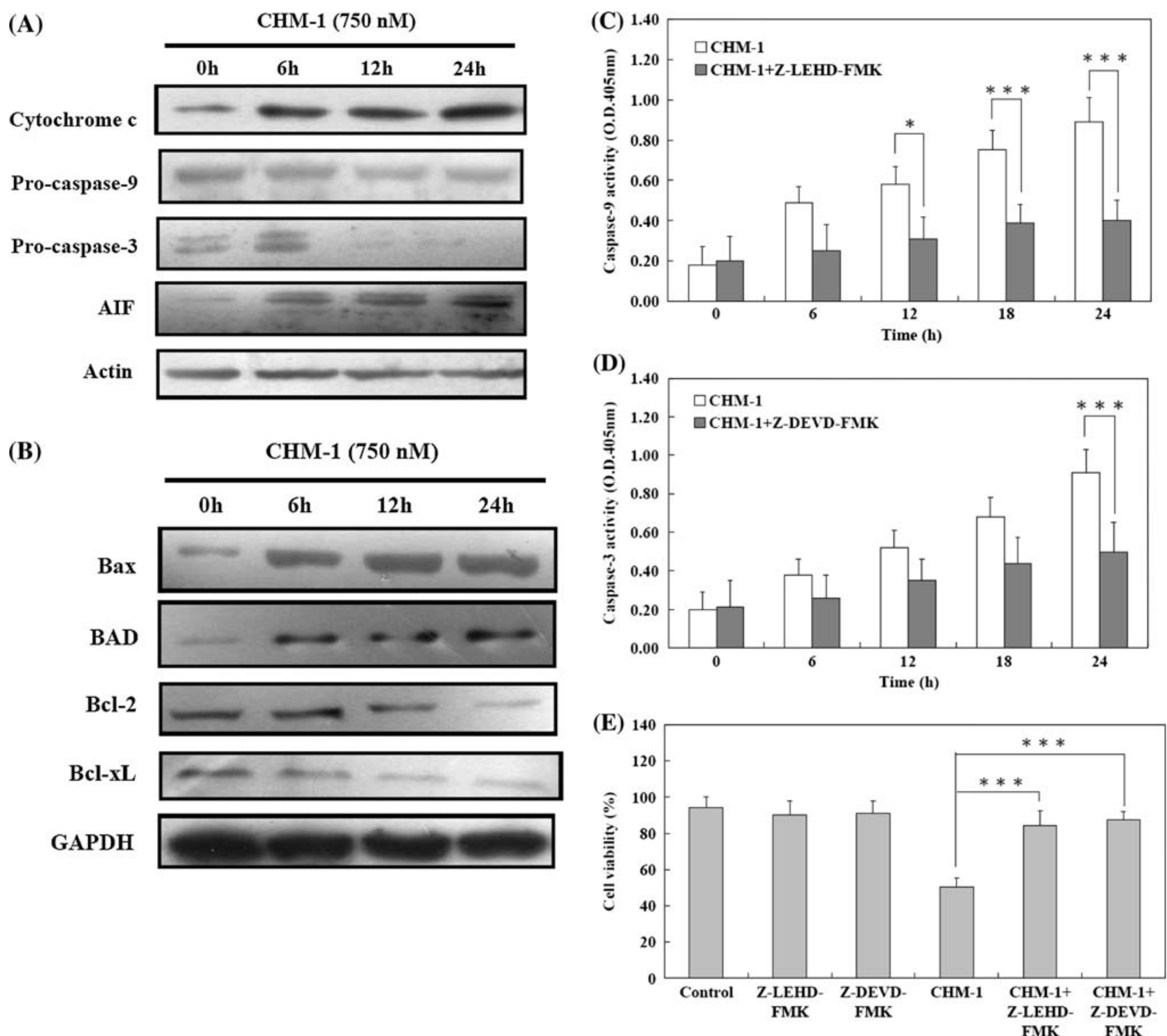


Fig. 4 Effects of CHM-1 on the levels change of apoptosis associated proteins levels in CT-26 cells. Cells were treated with 750 nM CHM-1 for 0, 6, 12 and 24 h, total or cytosolic proteins were detected (**a**) the levels of cytochrome *c*, pro-caspase-9, pro-caspase-3 and AIF; (**b**) the levels of Bax, BAD, Bcl-2 and Bcl-xL expressions by Western blotting as described in "Materials and methods". Cells were treated with 750 nM CHM-1 in the presence or absence 20 μM of caspase-9 and -3 inhibitors for various time periods then cells were collected for caspase-3 (**c**) and caspase-9 (**d**) activities determination and cell viability (**e**) according to the manufacturer's instructions as described in "Materials and methods". Data represents mean ± SD of three experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

discovery [8, 20]. Several microtubule-binding agents have been developed as clinical anti-cancer agents such as Taxol and Vinblastine [8, 20]. CHM-1 was developed in hopes of reducing the side effects associated with other microtubule inhibitors. Our data showed that 750 nM of CHM-1 significantly inhibited the proliferation of CT-26 colorectal adenocarcinoma cells. However, Teng et al. demonstrated that CHM-1 was less toxic to normal cells, including human fibroblasts (MRC5) and mouse hepatocytes [11, 12], but no report has addressed the effects of CHM-1 on colorectal cancer. We firstly investigated CHM-1 and CHM-1-P in CT-26 cells for anti-cancer activities with associated molecular mechanisms in vitro and in vivo. Our results indicated that CHM-1 induced cytotoxicity in CT-26 cells in a dose- and time-dependent manner, acting through G2/M arrest and apoptosis. We also demonstrated that 10 mg/kg and 30 mg/kg of CHM-1-P in BALB/c mice inoculated with CT-26 cells models were due to anti-colorectal cancer activity. Teng et al. demonstrated that CHM-1 induced a significant concentration-dependent growth inhibition and induced apoptosis, then produced dose-dependent tumor regression and prolonged the

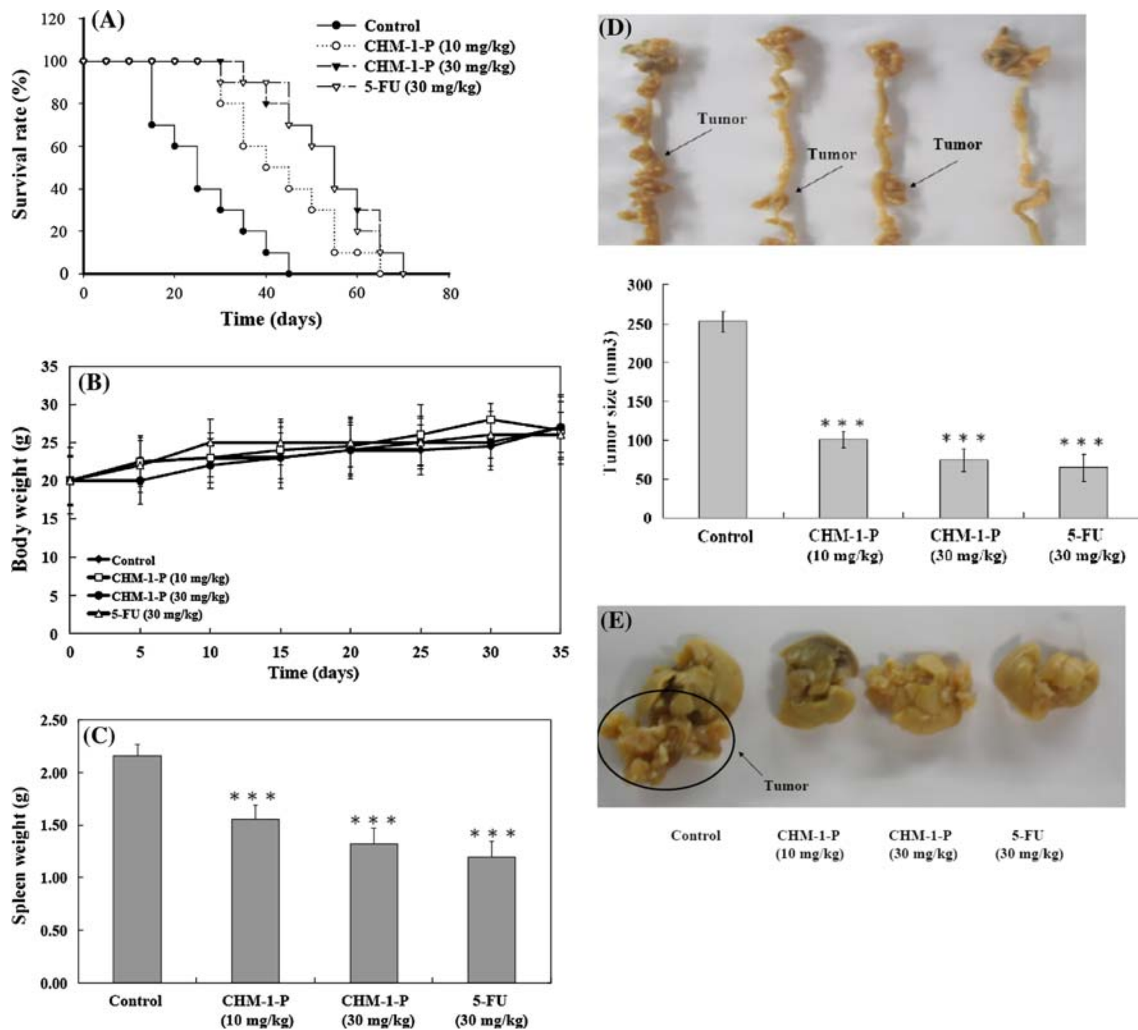


Fig. 5 In vivo anti-tumor activity of CHM-1-P. CT-26/BALB/c mice orally administered 10 and 30 mg/kg of CHM-1-P or i.p. administered 30 mg/kg of 5-FU. *Each value* represents the ratio of survival animal (**a**), body weight (**b**), spleen weight (**c**), total tumor volume (**d**) and liver (**e**) of CT-26/BALB/c mice with CHM-1 administrations ($n = 5$). Body and spleen weight results were expressed as mean ± SD and the difference between the groups were tested by one way ANOVA. Significant differences between values for CT-26/BALB/c mice and various treatment are shown ***$p < 0.001$

lifespan of mice carrying liver cancer xenografts, indicating its in vivo efficacy [11]. We suggest that CHM-1 represents a promising candidate as an anti-cancer agent with low toxicity to normal cells.

Microtubule interacting agents may interfere with the dissociation of chromosomes, thus inducing G2/M arrest and/or apoptosis. The G2/M checkpoint plays an important role in providing time for DNA repair, whereas apoptosis may function to remove damaged cells. Our data showed that CHM-1 started to induce G2/M phase arrest at 12–48 h of treatment. The CDK1/Cyclin B complex is one of the major regulators leading the G2 to M progression or apoptosis [16]. Our results showed a decrease in the protein level of CDK1, Cyclin A and Cyclin B, and a decrease in the activity of CDK1 at 12 and 24 h after CHM-1 treatment. These results are similar to previous reports regarding the regulation of CDK1/cyclin activity [16]. It was reported that anti-mitotic agents induced apoptosis through decreased CDK1 kinase activity. We are now in the progress of elucidating the possibility by directly blocking CDK1 activity or blocking its down stream targets (such as Bad, Bcl-2 and survivin).

Activation of the caspase is the major mechanism that promotes apoptosis in response to death-inducing signals from cell surface receptors and mitochondria stress [21]. In this study, we observed the induction of caspase-9 and caspase-3 specific activities by CHM-1. We also found that caspase-9 and caspase-3 inhibitors individually prevented CHM-1-induced apoptosis in CT-26 cells. Many reports have shown that during apoptosis, some pro-apoptotic factors, including pro-caspase and caspase-independent factors such as AIF, can be released from mitochondria into

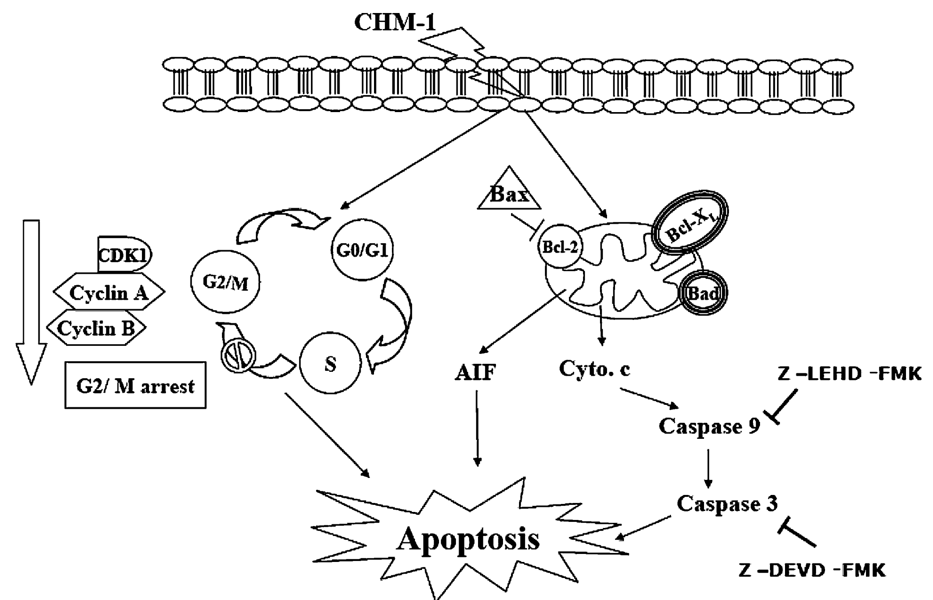


Fig. 6 The proposed model of CHM-1-mediated G2/M arrest and apoptosis in CT-26 colorectal adenocarcinoma cells

cytosol [22–24]. In Western blot analysis, our results showed that AIF also involved in the CHM-1 induced apoptosis. Teng et al. demonstrated that CHM-1 induced AIF translocation and nuclear condensation in HA22T cells [11]. We suggested that CHM-1-induced apoptosis in CT-26 cells is mediated through the activation of caspase cascades, AIF translocation and mitochondria-dependent pathway. These differences in results between the two studies may be study in different cells types and treatment conditions.

Bcl-2 family members have been reported in the regulation of mitochondria-mediated apoptotic pathways [25]. It is also reported that Bcl-2 and Bax locate in the mitochondrial outer-membrane and the Bcl-2/Bax ratio regulates the release of mitochondrial cytochrome *c* to cytosol [25, 26]. Our results indicated that CHM-1 promoted pro-apoptotic Bax, BAD levels and inhibited the levels of anti-apoptotic Bcl-2, Bcl-xL, which lead to the changes of the ratio of Bax/Bcl-2, which lead to the release of cytochrome *c*, pro-caspase-9 and AIF from the mitochondrial to the cytosol. After cytochrome *c* is released from the mitochondria, it binds to Apaf-1 and ATP and then binds to pro-caspase to form apoptosome, which activates caspase-3, leading to apoptosis.

Concentrated and selected accumulation of anti-cancer drugs at the tumor site is essential for the success of drug treatment in vivo. Teng et al. have reported that CHM-1 injected i.p. inhibited tumor growth and prolonged the lifespan in mice inoculated with HA22T cells [11]. Furthermore, CHM-1-P can significantly improve the water insolubility of CHM-1, prolong the circulation times in the blood, and enhance anti-tumor efficacy. Our studies showed that both 10 mg/kg and 30 mg/kg of CHM-1-P significantly prolonged the survival time of CT-26/BALB/c colorectal cancer mice. In addition, spleen enlargement, colon tumors, and liver metastasis were significantly reduced in CHM-1-P and 5-FU treated mice compared to those of CT-26/BALB/c mice. The proposed signaling pathways of CHM-1-induced G2/M arrest and apoptosis in CT-26 colorectal adenocarcinoma cells are shown in Fig. 6.

Conclusion

We demonstrated that CHM-1 and CHM-1-P exhibit novel anti-colorectal adenocarcinoma agents, and they have the potential to develop into a clinical trial candidate in the future.

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