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Biochemical Pharmacology 65 (2003) 1343–1350
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

Antitumor effect of the cinnamaldehyde derivative CB403 through the arrest of cell cycle progression in the G_2/M phase

Ha-Won Jeong[a], Dong Cho Han[a], Kwang-Hee Son[a], Mi Young Han[a], Jong-Seok Lim[a], Ji-Hong Ha[b], Chang Woo Lee[a], Hwan Mook Kim[a], Hyoung-Chin Kim[a], Byoung-Mog Kwon[a,*]

[a]*Korea Research Institute of Bioscience and Biotechnology, 52 Uendong Yoosunggu, Taejeon 305-600, South Korea*
[b]*Department of Genetic Engineering, Kyungpook National University, Daegu 702-701, South Korea*

Received 5 February 2002; accepted 9 September 2002

Abstract

Cinnamaldehydes have been shown to have inhibitory effects on farnesyl protein transferase (FPTase; EC 2.5.1.29) *in vitro*, angiogenesis, cell–cell adhesion, and tumor cell growth and to be immunomodulators. However, the mechanisms responsible for these effects remain unknown. To elucidate the molecular mechanism of the cinnamaldehyde derivative CB403 for growth inhibition, CB403 was synthesized from 2′-hydroxycinnamaldehyde. CB403-treated cells were weakly adherent to the culture dishes. In addition, CB403 inhibited tumor growth in these cells in a concentration-dependent manner. FACS analysis using human cancer cells treated with this compound showed cell cycle arrest in mitosis, which was correlated with a marked increase in the amount of cyclin B1. Furthermore, CB403 blocked *in vivo* growth of human colon and breast tumor xenografts without loss of body weight in nude mice. These results support the hypothesis that the cinnamaldehyde derivative CB403 exerts cytostatic properties by inducing mitotic arrest in cancer cells.
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Keywords: Cinnamaldehyde; Cell cycle; Cyclin; Antitumor

1. Introduction

Cell cycle control is the major regulatory mechanism of cell growth. In recent years, considerable advances have been made in understanding the role of cyclins, CDKs, CDKIs, retinoblastoma protein (pRb), and the E2F transcription factor family in cell cycle progression [1]. This process is regulated by the coordinated action of CDKs in association with their specific regulatory cyclin proteins. Primary regulators of G_1 progression are the D-type cyclins (D1, D2, D3), cyclin E, and CDKs. CDK4 and CDK6 are activated in early G_1 by interactions with D-type cyclins, whereas CDK2 is activated in late G_1 by interaction with cyclin E, and at the G_1/S boundary and throughout the S phase by interaction with cyclin A [2]. G_2 to M phase progression is regulated by a member of the CDK/cyclin family, CDK1/cyclin B. CDK1 protein, which is constitutively expressed throughout the cell cycle, is activated by binding to its partner cyclin B during S phase [2,3]. CDKIs including $p21^{Waf1/Cip1}$, $p27^{Kip1}$, and $p16^{Ink4}$ also contribute to the regulation of cell cycle progression by controlling CDK activity [4].

2′-Hydroxycinnamaldehyde, isolated from the stem bark of *Cinnamomum cassia* Blume (Lauraceae), was reported to have an inhibitory effect on farnesyl protein transferase activity *in vitro* [5]. The cinnamaldehydes have also been shown to have various activities such as anti-angiogenic activity, to be immunomodulators, and to be inhibitors of cell–cell adhesion, CDK4/cyclin D1 kinase [6–9], and the proliferation of several human cancer cell lines including those established from breast, ovarian, lung, and colon carcinomas and leukemias [8].

To examine the effects of these compounds on the cell cycle, we prepared a cinnamaldehyde derivative (CB403),

* Corresponding author. Tel.: +82-42-860-4557; fax: +82-42-861-2675.
E-mail address: kwonbm@kribb.re.kr (B.-M. Kwon).

Abbreviations: CDK, cyclin-dependent kinase; CDKI, cyclin-dependent kinase inhibitor; FACS, fluorescence activated cell sorter; Rb, retinoblastoma; FBS, fetal bovine serum.

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doi:10.1016/S0006-2952(03)00038-8

a potential CDK4/cyclin D1 inhibitor, which is relatively stable in comparison with the natural product 2′-hydroxycinnamaldehyde [9]. In the present study, we investigated the molecular effects of CB403 on the progression of MCF-7 breast and SW620 colon cancer cells through the cell cycle and its antitumor effects in nude mice.

2. Materials and methods

2.1. Materials

All the chemicals used for these experiments were purchased from the Sigma Chemical Co. CB403 (Fig. 1) was prepared by a reported method [9], and DMSO was used as the solvent for the drug stock and for dilution.

2.2. Cell culture and synchronization

The cell lines used were obtained originally from the ATCC. SW620 (a human colon cancer) and MCF-7 (a human breast cancer) cells were maintained in RPMI 1640 (Gibco/BRL) supplemented with 10% heat-inactivated FBS (Gibco/BRL) and 25 mM HEPES. NIH3T3 (a mouse fibroblast cell line) cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat-inactivated FBS. The cells were grown in an incubator at 37° under a humidified atmosphere of 5% CO_2. Exponentially growing cells were exposed to CB403 at the indicated concentrations for different lengths of time.

Cell synchronization at the G_1/S phase was performed as described by Jin *et al.* [10]. Cells were seeded at 0.5×10^6 per 100-mm plate. After 1 day, the cells were treated with thymidine (2 mM) for 24 hr and arrested in the S phase. Then cells were washed in PBS to release them from the S phase and were fed thymidine-free medium. After 9 hr, cells were subjected to a second thymidine treatment. After this 14-hr treatment, cells were washed with PBS to release them from the G_1/S block.

For cell synchronization at metaphase, cells were exposed to nocodazole (500 ng/mL) at 37° for 14–16 hr. After treatment, metaphase cells were collected by a gentle shake-off method, centrifuged at 500 *g* for 5 min at room temperature, and washed twice with fresh medium. To relieve cells from the M phase arrest, they were replated in a 60-mm cell culture dish (4×10^5/T25 flask) and incubated at 37° in fresh medium for various time periods.

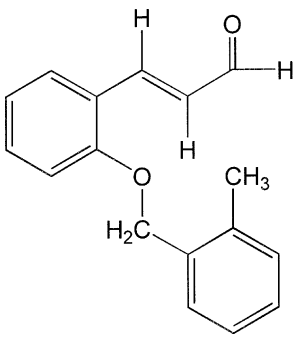

Fig. 1. Structure of CB403.

2.3. Cell proliferation assays

Cells (5000) were seeded into 96-well plates in RPMI 1640, or DMEM containing 10% FBS. After 20–24 hr, cells were replenished with fresh complete medium containing either a test compound or 0.1% DMSO. After incubation for 48 hr, the cell proliferation reagent WST-1 (Roche) was added to each well. The amount of WST-1 formazan produced was measured at 450 nm using an ELISA Reader (Bio-Rad).

2.4. Cell cycle analysis

To analyze the DNA content by flow cytometry, cells were trypsinized from the culture flask. After centrifugation at 300 *g* for 5 min at room temperature, the supernatant was removed. The cells were then washed twice with a PBS solution and fixed with 3 mL of ice-cold 70% EtOH overnight. Fixed cells were harvested by centrifugation at 300 *g* for 3 min at room temperature and washed twice with PBS containing 1% FBS. The collected cells were resuspended in PBS (100 μL/1×10^5 cells) and treated with 100 μg/mL of RNase A at 37° for 30 min. Then propidium iodide was added to a final concentration of 50 μg/mL for DNA staining, and 20,000 fixed cells were analyzed on a FACScalibur (Becton Dickinson). Cell cycle distribution was analyzed using the Modifit's program (Becton Dickinson).

2.5. Western blotting

Proteins (10 μg) were resolved by 7.5 or 12% SDS–PAGE (7.5% for Rb and 12% for the others), and then transferred to PVDF membranes (Roche). Membranes were blocked with 5% nonfat dry milk in TBS-T (50 mM Tris–HCl, pH 7.6, 150 mM NaCl, 0.1% Tween 20). The primary antibodies used were: polyclonal anti-cyclin D1 (sc-753), anti-CDK1 (sc-403), anti-CDK2 (sc-163), and anti-CDK4 (sc-601) (Santa Cruz Biotechnology); monoclonal anti-Cip1 (C24420) and anti-Kip1 (clone 57) (Transduction Laboratories); and anti-Rb (14001A) (Pharmingen). The secondary antibodies used were horseradish peroxidase-conjugated goat anti-rabbit IgG from Santa Cruz (sc-2004), and horseradish peroxidase-conjugated goat anti-mouse IgG from Transduction Laboratories (M15345). The antibodies were used at the dilution recommended by the manufacturers. Membranes were incubated with primary antibody for 2 hr at room temperature, washed five times with TBS-T, and visualized with Chemiluminescence POD reagents (Roche).

2.6. Nude-mouse xenograft assay

To evaluate the antitumor activity of CB403 *in vivo*, SW620 human colon adenocarcinoma cells (3×10^7/mL) and MDA-MB-231 human breast cells (5×10^7/mL) were implanted subcutaneously into the right flank of nude mice on day 0. The compound was dissolved in 0.5% Tween 80 and was administered i.p. at a concentration of 50 mg/kg per day for 17 days. Tumor volumes were estimated as length (mm) × width (mm) × height (mm)/2 [11]. To determine the toxicity of the compound, the body weight of the tumor-bearing animals was measured. On day 17, the mice were killed, and the tumors were weighed.

Table 1
Effect of CB403 on human tumor cell growth

Tumor cell line	GI_{50} (μg/mL)	Tumor cell line	GI_{50} (μg/mL)
PC-3	2.58	SW620	1.61
SNB19	7.72	MCF-7	2.83
SK-OV-3	4.29	MDA-MB-231	2.88
CAKI-1	6.59	HCC2998	10.77
UO-31	3.11	HT29	3.42
A498	8.02	NCI-H23	1.76
DLD-1	2.67	SK-MEL-2	4.69
KM12	2.91	UAC62	2.56
RPMI7951	3.04	M14	5.41
786-O	6.56	T27D	1.74

Results were obtained from two independent assays and are expressed as an average. Growth inhibition was measured as described in Section 2.

3. Results

3.1. Inhibition of tumor cell growth

CB403 activity on the proliferation of 20 tumor cell lines was examined using a cell proliferation assay kit. CB403 exhibited a concentration-dependent inhibition of cell growth in a broad range of concentrations. The GI_{50} values of CB403 for *in vitro* growth inhibition ranged from approximately 1.6 to 10.7 μg/mL; the SW620 cell line was relatively more sensitive than the other cell lines with a GI_{50} value of 1.61 μg/mL (Table 1).

3.2. Effects of CB403 on the cell cycle profile of asynchronous cells

To determine the effect of CB403 on the cell cycle, FACS analysis was performed using the human cancer cell lines SW620 and MCF-7. The concentration of CB403 was 5 μg/mL. Cells were harvested at 6, 12, 24, and 48 hr after treatment and analyzed with a FACScalibur. Because we previously reported that CB403 inhibits CDK4/cyclin D1 kinase activity *in vitro* [9], CB403 was expected to arrest the cell cycle in the G_1 phase. However, as shown in Figs. 2 and 3, CB403 treatment of MCF-7 and SW620 cells for 6, 12, and 24 hr induced an increase in DNA content to 4N. It should be noted that in the case of SW620, CB403 caused the development of polyploid cells (8N-DNA content) and detachment from the bottom of the culture flasks. CB403-treated SW620 cells floated in the culture medium as early as 6 hr after exposure. At 24 hr after treatment, half of the cells were floating in the medium. After 48 hr of treatment, there were no floating cells in the medium; all cells were attached at the bottom of the culture flasks. As for SW620 cells, CB403 treatment also induced MCF-7 cells to detach from the culture flasks. In mitosis, cells generally show

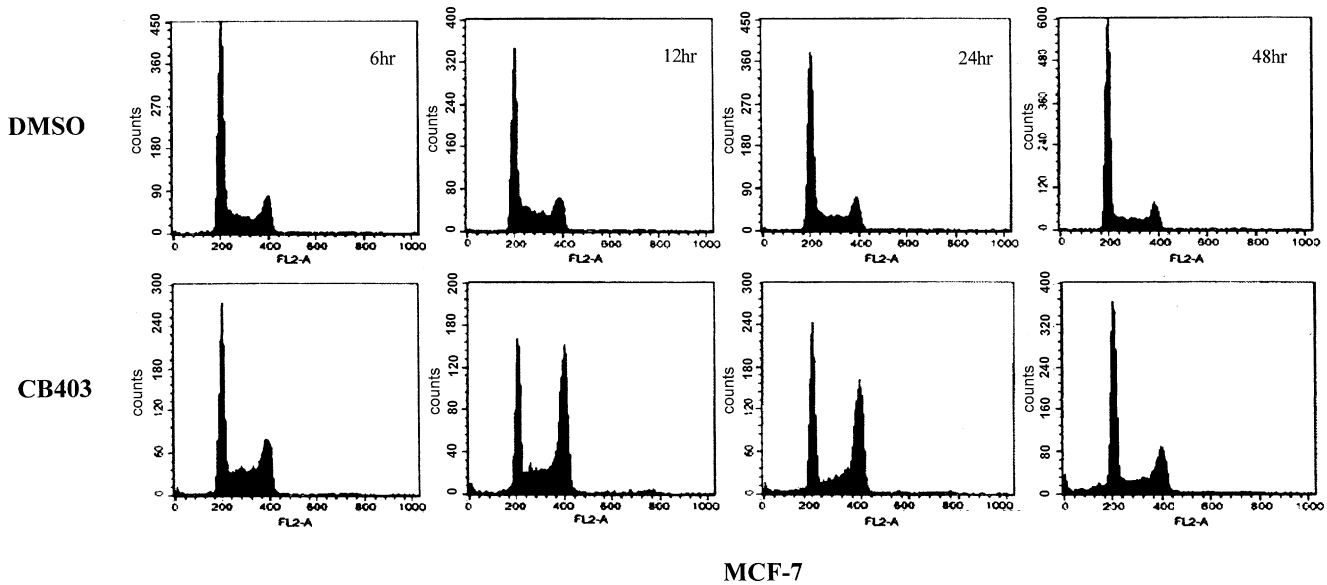

Fig. 2. G_2/M phase arrest induced by CB403 treatment in MCF-7 cells. Cells from the MCF-7 human breast cancer cell line was treated with CB403 (5 μg/mL) or with vehicle solvent (0.1% DMSO) for the indicated times, as described in Section 2. Cells were harvested, fixed, and stained with propidium iodide. Then 20,000 stained cells were subjected to FACScalibur analysis to determine the distribution of cells throughout the G_1, S, and G_2/M phases. Experiments were performed at least three times with consistent and repeatable results.

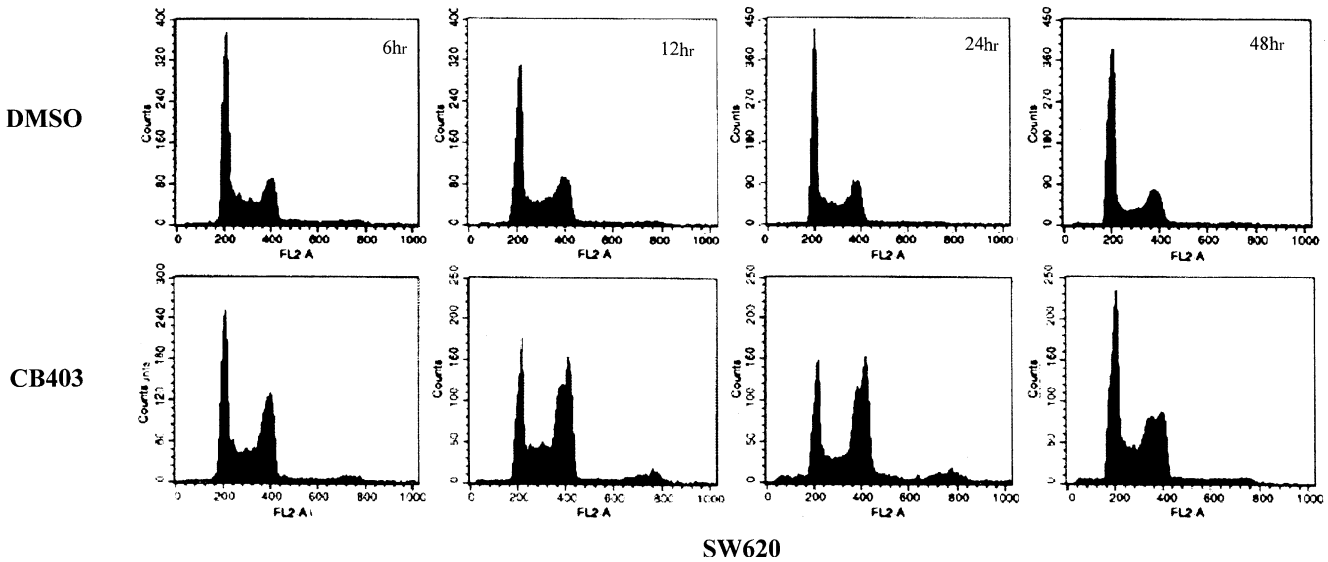

Fig. 3. Induction by CB403 treatment of G_2/M phase arrest and polyploid cells in SW620 cells. Human colon cancer SW620 cells were treated with CB403 (5 μg/mL) or with the vehicle solvent (0.1% DMSO) for the indicated times, as described in Section 2. Cells were harvested, fixed, and stained with propidium iodide. Then 20,000 stained cells were subjected to FACScalibur analysis to determine the distribution of cells throughout the G_1, S, and G_2/M phases. Experiments were performed at least three times with consistent and repeatable results.

weak binding affinity to the matrix and a round morphology; the floating cells in the CB403-treated cultures were estimated to be mitotic. Interestingly, 48 and 72 hr after treating MCF-7 and SW620 cells, respectively, it was hard to detect any floating cells in the culture medium, and the cell cycle profile was similar to that of the control cells. Similarly, mouse fibroblast NIH3T3 cells were treated with CB403, and the cell cycle profile was analyzed using a FACS. As shown in Fig. 4, CB403 showed no effect on the cell cycle profile of a non-tumorigenic cell line, NIH3T3, suggesting a cell-type specific activity for CB403.

3.3. Effect of CB403 on the cell cycle profile of synchronized cells

To determine whether CB403-treated cells were blocked in the G_2 or M phase, these cells were analyzed for progression from mitotic arrest. By destabilizing the microtubule structure, nocodazole specifically blocks the cell cycle at the metaphase-anaphase transition and allows for the collection of a cell population that is highly enriched with cells in the M phase of the cell cycle. To synchronize cells in mitosis, MCF-7 and SW620 cells were treated with nocodazole. After incubation, M-phase syn-

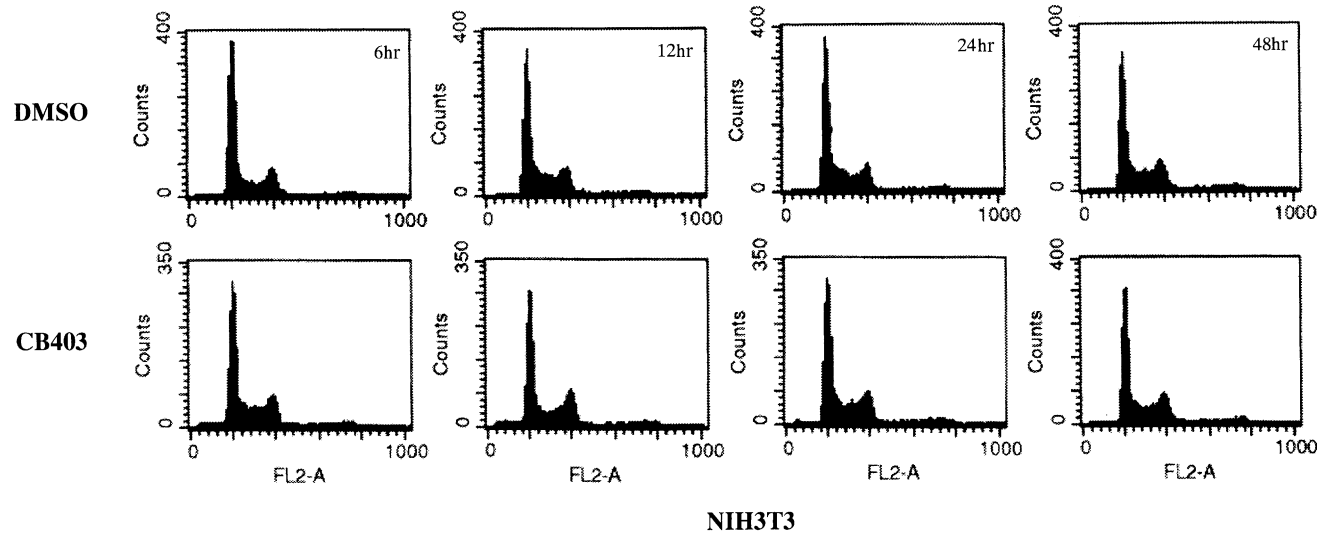

Fig. 4. Representative cell cycle distribution of NIH3T3 cells in the presence and absence of CB403. Cells from the NIH3T3 mouse fibroblast cell line were treated with CB403 (5 μg/mL) or with the vehicle solvent (0.1% DMSO) for the indicated times. The cells were then analyzed for DNA content by staining with propidium iodide followed by FACS analysis as described in Section 2. CB403 did not show any effect on the cell cycle profile of cells from the non-tumorigenic NIH3T3 cell line. Experiments were performed two times with consistent results.

chronized cells were replated in medium with or without CB403 (5 μg/mL). Cells were collected at the indicated time, and the cell cycle distribution was analyzed by a FACS. As shown in Fig. 5, MCF-7 cells were released from a nocodazole-induced arrest after replating in the absence of CB403 (left panel). However, replating cells with CB403 maintained the M-phase arrest, confirming the M-phase arrest and anti-mitotic activity of CB403. This mitotic arrest by CB403 lasted until 20 hr after nocodazole release. A similar result was also observed when SW620 cells were treated with this compound (data not shown). These results indicate that CB403 inhibits the proliferation of MCF-7 and SW620 cells via M-phase arrest.

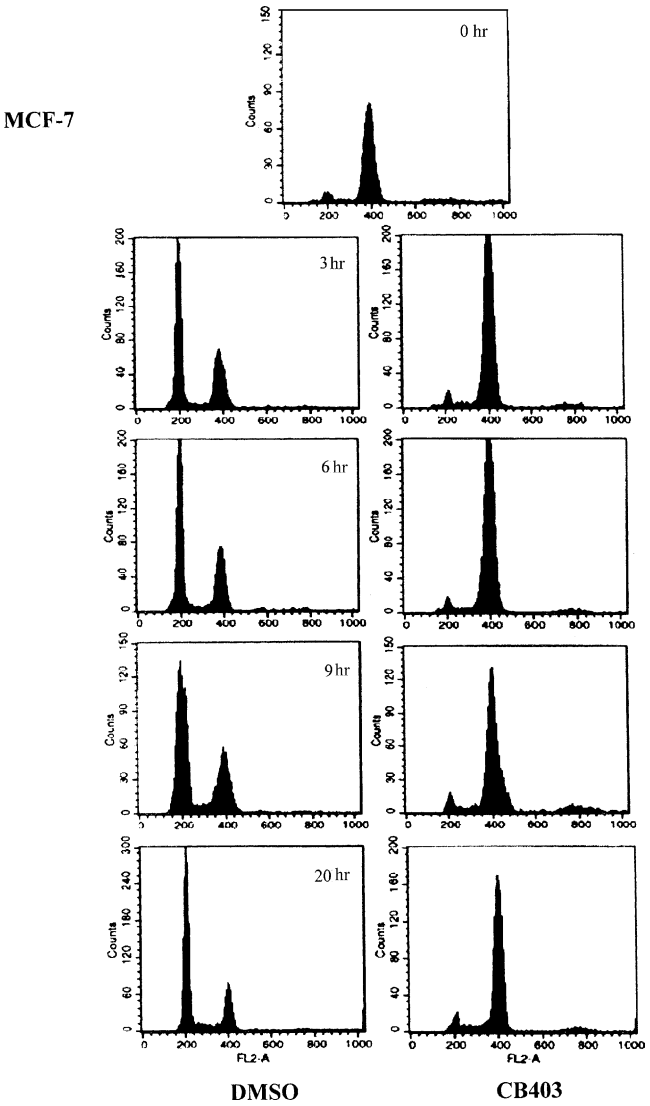

Fig. 5. Cell cycle distribution of M-phase synchronized MCF-7 cells in the presence and absence of CB403. MCF-7 cells were treated with 500 ng/mL of nocodazole for synchronizing in mitosis, as described in Section 2. The mitotic cells were replated in T25 flasks in medium containing 0.1% DMSO or CB403 (5 μg/mL). At the indicated time points after nocodazole release, cells were subjected to FACScalibur analysis to determine the distribution of cells through the G_1, S, and G_2/M phases. Experiments were performed at least three times with consistent and repeatable results.

3.4. Effects of CB403 on CDK and cyclin expression

To characterize the molecular events involved in the effect of CB403 on cell cycle progression, the amounts of CDK, cyclin, CDKIs, and Rb protein were measured during the course of G_1/S progression in thymidine-synchronized MCF-7 cells. Analysis of the cell extracts by immunoblotting (Fig. 6) demonstrated that important "engine" components for progression through the G_1 phase of the cell cycle (including CDK2, CDK4, CDK6, $p21^{Waf1/Cip1}$, $p27^{Kip1}$, and cyclin D1) were not altered by CB403 treatment. Phosphorylation of the pRb was analyzed to measure CDK4(6) activities. Although the amount of pRb varied during the course of G_1/S progression in thymidine-synchronized MCF-7 cells, the phosphorylation patterns of pRb were identical in the treated and untreated cells. These results indicated that CB403 did not modify CDK4(6) activities. Subsequently, the expression levels of cyclins A and E were analyzed in CB403-treated MCF-7 cells. A-type cyclins

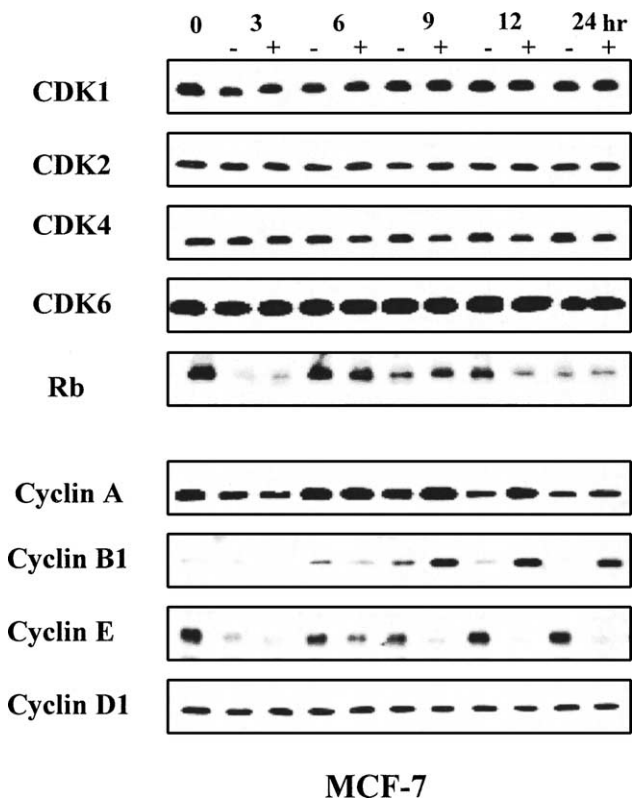

Fig. 6. Effects of CB403 on the expression of cell cycle proteins. MCF-7 cells were synchronized at the G_1/S phase boundary by 2 mM thymidine. Then the cells were released from growth arrest with fresh medium containing DMSO (0.1%) or CB403 (5 μg/mL). Cells were harvested at the indicated time points, and lysates were prepared, as described in Section 2. The amount of cell cycle proteins was determined by western blot analysis using specific antibodies. The same cell extracts were quantified using the Bradford reagent (Bio-Rad) and used for the analysis of each cell cycle protein. Equal sample loadings were confirmed by staining the membrane with Ponceau S. Experiments were performed two times with consistent results.

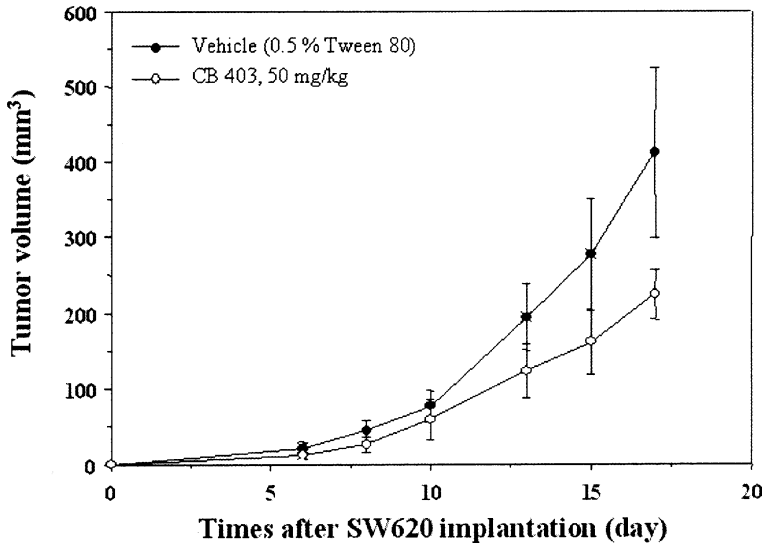

Fig. 7. Change in tumor volume of SW620 xenografted nude mice treated with CB403. For the evaluation of *in vivo* antitumor activity of CB403, SW620 cells (0.3 mL of 3×10^7 cells/mL) were implanted subcutaneously into the right flank of nude mice on day 0. Tumor volumes were estimated by the formula length (mm) × width (mm) × height (mm)/2. The compound was dissolved in 0.5% Tween 80 and was administered i.p. at concentrations of 50 mg/kg one time per day from day 1 to day 17. The amount of the dosage was 0.2 mL/20 g body weight of the animals. The results (means ± SD) were obtained from one assay using 16 mice (8 mice for vehicle and 8 mice for CB403).

are involved in the regulation of S phase and G_2/M phase transition, and E-type cyclins are involved in the regulation of the S phase. As shown in Fig. 6, a little increase of cyclin A was evident after 12 hr of treatment. After 12 hr, the cyclin E level was reduced markedly in the CB403-treated cells.

Western blot analysis with a cyclin B1 antibody showed elevated levels of cyclin B1 protein in CB403-treated cells. Cyclin B1 was undetectable in cells in G_1/S transition. After 12 and 24 hr of CB403 treatment, the cells exhibited maximal cyclin B1 protein accumulation, corresponding to their accumulation in the G_2/M phase.

3.5. *In vivo effect of CB403 on tumor cell growth*

SW620 and MDA-MB-231 tumor xenograft models of nude mice were used to investigate the inhibitory activity of CB403 on tumor growth. MCF-7 cell lines were not used for the *in vivo* test because the cells did not display tumorigenicity in nude mice. SW620 and MDA-MB-231 cells were implanted subcutaneously into the right flank of nude mice on day 0, and the compound was administered i.p. at a concentration of 50 mg/kg per day for 17 days. To determine the toxicity of the compound, the body weight of the tumor-bearing animals was measured. On day 17 the mice were killed, and the tumors were weighed [11]. Seventeen days after implantation, tumor volume was decreased by 45.6% as compared to control SW620 mice (Fig. 7), and tumor weights were also decreased (by 25.5%) compared with those of the control mice (data not shown). In nude mice injected with MDA-MB-231 cells, tumor volumes and weights were decreased 36.2 and 16.2%, respectively (data not shown). Body weight loss was not observed in mice implanted with SW620 and MDA-MB-231 cells.

4. Discussion

Cinnamaldehyde has been shown to have antitumor activity [12]. However, the mechanism causing this activity is still being debated. Previously, we reported that cinnamaldehyde derivatives inhibit FPTase activity, which may (at least in part) [5,8] explain the mechanism by which cinnamaldehydes exhibit their antitumor activity. As shown in Table 1, the growth of 20 different cell lines was inhibited by treatment with CB403 for 48 hr. CB403-treated cells had a GI_{50} value of 1.6 to 10.7 μg/mL, depending on the cell line used.

In our previous experiments [9], we determined that CB403 inhibited CDK4/cyclin D1 kinase activity *in vitro* with an IC_{50} value of 5 μg/mL and showed very weak inhibitory activity for CDK2/cyclin E ($IC_{50} > 30$ μg/mL) and for CDK1/cyclin B1 ($IC_{50} > 50$ μg/mL). Because CDK4/cyclin D1 is involved in the G_1/S transition, CB403-treated cells were expected to arrest in the G_1 phase. Contrary to expectations, when CB403 was tested for a cell cycle effect, treated cells were arrested in the G_2/M phase (both MCF-7 and SW620 cells), as shown in Figs. 2 and 3. To determine whether CB403-treated cells were blocked in the G_2 phase or in the M phase, we investigated whether CB403-treated cells could progress from mitotic arrest. By replating cells with fresh medium, nocodazole-induced M-phase cells completed mitosis and progressed to the G_1 phase. However, cells replated with CB403-containing medium remained in the M phase,

confirming the anti-mitotic activity of CB403 (Fig. 5). These results indicate that CB403 inhibits the proliferation of MCF-7 and SW620 cells via arrest in the M but not the G_2 phase of the cell cycle. However, CB403 did not arrest non-tumorigenic NIH3T3 cells in mitosis. Currently, it is not clear why CB403 has an inhibitory effect on SW620 and MCF-7 cells but not on NIH3T3 cells. It will be very interesting to characterize the mechanism by which CB403 distinguishes between cancer cells and non-cancerous cells.

Progression of the cell cycle is mediated by activation of the CDKs. Activation of a CDK requires binding to a specific cyclin. Originally, cyclins were named because of their fluctuating levels through the cell cycle. Therefore, improper degradation or accumulation of cyclins causes a disaster in cell cycle progression. In vertebrate cells, the G_2/M transition is triggered by regulation of the CDC2/cyclin B1 complex that promotes the breakdown of the nuclear membrane, chromatin condensation, and microtubule spindle formation [13]. The mitotic process is completed by destruction of cyclin B1, and then inactivation of CDC2/cyclin B1 kinase. Western blot analysis with an antibody directed against cyclin B1 showed elevated levels of cyclin B1 in CB403-treated cells. The levels reached a maximum after 12–24 hr, time points corresponding to an increase in the cellular DNA content to 4N. Given that degradation of cyclin B1 is a prerequisite for progression from metaphase to anaphase, this accumulation of cyclin B1 in CB403-treated cells caused a block in the cell cycle before the onset of anaphase. Therefore, these results indicate that the mitotic arrest induced by CB403 in MCF-7 and SW620 cells is associated with a marked abnormal accumulation of cyclin B1 and cyclin B1-dependent kinase activity.

It has been proposed that the degradation of cyclin B1 is regulated by either a modification of the substrates or by activation of the mechanism by which ubiquitin is produced [14,15]. Then how can CB403 cause accumulation of cyclin B1? Because cyclin B1 is degraded by the ubiquitin pathway, it is possible that CB403 blocked ubiquitin-dependent proteolysis. However, if this is true, CB403 should block the entire cell cycle because cyclins A, D, and E are also degraded through the ubiquitin pathway. However, this was not the case because CB403-treated cells had less cyclin E than untreated control cells (Fig. 6). Therefore, CB403 may not affect the ubiquitin-dependent proteolysis machinery itself. Another possibility is that CB403 induces cyclin B1 expression. To comment further on this, we need more time to collect information involving the cyclin B1 promoter and its regulatory component.

When the cell cycle profile of MCF-7 and SW620 cells treated with CB403 for 48 hr was analyzed, the extent of M-phase arrest was found to be reduced. The cell cycle profile of cells treated with CB403 for 48 hr was similar to that of control cells, implying that M-phase arrest by CB403 is transient (Figs. 2 and 3). This interpretation was supported by a cell adhesion assay. After 48 hr, CB403-treated cells could re-attach to the bottom of the culture flasks. Therefore, one possible explanation is that CB403 is unstable if the cells are incubated for more than 48 hr. Currently, we are designing more stable compounds *in vivo*.

It was also observed that CB403 treatment caused cells to float on the culture medium, resulting in an increase in the number of detached cells and a decrease in the number of attached cells. There are several possible explanations for this phenotype. One is that the accumulation of cyclin B1 and the consequent activation of CDC2/cyclin B1 are responsible for this phenotype. As cells enter mitosis, phosphorylation of structural proteins by CDC2/cyclin B1 reorganizes the cytoskeleton, i.e. the microtubule network, actin filaments, or nuclear lamina. For example, phosphorylation of caldesmon, an actin-binding protein, by cyclin B/CDC2 induces microfilament weakening, causing weak cell–extracellular matrix interactions and rounding of the cells [16]. The second possibility is inhibition of integrin activation by CB403 through R-ras protein [17]. Because cinnamaldehyde derivatives display an inhibitory activity against FPTase, CB403 may block R-ras function. The third possibility is that CB403 may affect either the dissembly or assembly of tubulin. By inhibiting microtubule functions, CB403 may cause cell rounding and detachment. In addition, malfunction of microtubules may arrest the cell cycle at the M phase, causing accumulation of cyclin B1. If this is true, accumulation of cyclin B1 is not the cause but a consequence of CB403 action. To solve this question, it is important to identify the direct target protein of CB403 *in vivo*. Currently, the mechanism of CB403-induced cell detachment is under investigation.

SW620 tumor xenograft models of nude mice were used to investigate the inhibitory activity on tumor growth of CB403, which was administered i.p. at concentrations of 50 mg/kg from day 1 to day 17. CB403 inhibited the growth of tumors as shown in Fig. 7, and also significantly reduced the weight of tumors excised on the final day (data not shown). A promising aspect of CB403 is its apparent lack of obvious toxicity in tumor-bearing nude mice.

In summary, the cinnamaldehyde derivative CB403 exerts cytostatic effects by inducing mitotic arrest in cancer cells. The growth inhibitory and antitumor activities of CB403 in cell culture and in animal studies support its promise as an antitumor compound.

Acknowledgments

This research was supported by a grant from the Center for Biological Modulators of the 21st Century Frontier Research Program and by the Ministry of Science & Technology (99-I-01-04-A-009). It was also supported, in part, by grants from the Good Health R&D Program (01-PJ2-PG4-J201PT01-0008), Ministry of Health and Welfare of Korea.

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