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ORIGINAL ARTICLE

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Antiproliferation and radiosensitization of caffeic acid phenethyl ester on human medulloblastoma cells

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Abstract *Purpose*: To investigate antiproliferative and radiosensitizing effects of caffeic acid phenethyl ester (CAPE) on medulloblastoma (MB) Daoy cells. *Methods and materials*: Daoy cells were treated with CAPE in different concentrations and assessed for cell viability, apoptosis, cell cycles, cyclin B1 expressions, radiosensitization and chemosensitization. Human astroglia SVGp12 cells were treated with CAPE to present the possible protection or complication effects in normal tissues. *Results*: CAPE inhibited the growth of Daoy cells in a time- and concentration-dependent manner in MTT and Trypan blue exclusion assays. Flow cytometry revealed that CAPE significantly decreased G2/M fraction, and increased the S phase fraction. Western blot demonstrated a down-regulated cyclin B1 protein expression. Pretreatment with CAPE markedly decreased the viability of irradiated Daoy cells. The sensitizer enhancement ratios (SERs) were increased in CAPE-treated Daoy cells. CAPE in doxorubicin and cisplatin did not show chemosensitizing effect. *Conclusions*: These findings demonstrate the antiproliferative and radiosensitizing effects of CAPE for Daoy cells, which might bring improvement to the treatment of MB. For clinical application, in vivo models are expected.

Keywords Caffeic acid phenethyl ester (CAPE) · Medulloblastoma · Antiproliferation · Radiosensitization · Radiotherapy · Propolis

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Introduction

Medulloblastoma (MB) is a malignant cerebellum tumor predominantly found in children [1]. Standard treatments of MB involve surgical resection, postoperative radiotherapy with frequent combination of chemotherapy. For MB children, craniospinal axis radiotherapy can improve survival and reduce recurrence and metastases [2, 3]. However, radiotherapy may cause endocrine imbalance (lack of somatotropic hormone and thyroid hormone) and have considerable effects on children's growth, brain development, and behaviors [4, 5]. Current studies on the nature of MB and new treatments are limited. To improve therapeutic outcome and reduce toxicity of current treatment strategies, new and novel therapeutic agents are needed for MB patients.

Caffeic acid phenethyl ester (CAPE), a phenolic compound and a structural derivative of flavonoids, is an active ingredient of honeybee propolis [6]. Existing

studies show that CAPE has a broad spectrum of biological activities including antioxidant [7–9], anti-inflammatory [10–14], antiviral action [15], prevention of re-perfusion injury [16, 17], anticancer [18, 19], and antifibrotic effects [20]. Isolated from propolis by Dr. Dezider Grunberger in 1985, CAPE is found to have cytotoxicity to transformed melanoma and human breast cancer cells in rats, but not to normal cells [6]. Similar effects are found in human tumor cells [21]. Pharmacological activities of CAPE in anticancer effects include antiproliferation, cell apoptosis induced by antioxidation, and increase of radiosensitivity [13, 21, 22]. Based on these findings, CAPE might be a potential agent for antiproliferation and radiosensitization in MB.

In this study, we investigated the antiproliferative and radiosensitizing effects of CAPE on human Daoy cells and human astroglia SVGp12 cells in vitro.

Methods and materials

Chemicals

CAPE is a biologically active ingredient of honeybee propolis. It was purchased from SIGMA C8221 (Steinheim, Germany).

Cell line and cell culture condition

Human medulloblastoma Daoy cell line and Human astroglia SVGp12 were obtained from ATCC. Both cells were maintained in Eagle's Minimum Essential Medium (EMEM) in a 37°C incubator containing 5% CO_2 and 95% air.

Cell growth analysis

The cells were seeded in the petri dishes. Various concentrations of CAPE (0.3, 1, 3, 10, 30 μM) were added to half of the dishes. Absolute ethanol (0.1% v/v) was added to the other half as the untreated control. The cells were collected for analysis at different time intervals.

Cell viability test

The cells were seeded at the density of $2–3\times10^3$ cells/well in 96-well flasks for 18 h. They were then treated with various concentrations of CAPE (0.01, 0.1, 1, 10, 100 μM) for different time intervals (24, 48, and 72 h) and collected for analysis. For the assay, both the treated and untreated cells were incubated with 100 μl MTT (tetrazolium compounds) for 4 h, lysed with 100 μl SDS buffer, and the color crystals were solubilized with an ELISA reader at a wavelength of 570 nm.

Measurements of apoptosis by morphology, TUNEL assay, and DNA fragmentation assay

After the treatment with 0.3–30 μM CAPE for 24 h, the cells were collected for analysis with morphology, TUNEL assay, and DNA fragmentation, respectively. Cells treated with cycloheximide (CHX) were used as positive control for TUNEL assay and DNA fragmentation.

To assess apoptosis, cells were stained with hematoxylin–eosin (HE) stain and observed under inverted microscope before and after treatment with CAPE. The density, shape, cell membrane, and other changes in morphology before and after treatment were photographed for comparison.

For TUNEL assay, cells were seeded on the microslides and fixed with 4% paraformaldehyde. TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP–biotin nick end labeling) (TUNEL Apoptosis Detection Kit, Upstate, NY, USA) stain was done according to the manufacturer's instructions. The cells were then observed under a fluorescence microscope.

For DNA fragmentation assay, the collected cells were analyzed with Apoptotic DNA Ladder Detection Kit (Biovision K120-50, CA, USA) according to the manufacturer's instructions. The dead cells treated with 10 μM CAPE were collected at different time intervals (0, 6, 12, 18, 24, 30, 36, 42 and 48 h) for DNA fragmentation analysis.

Cell cycle analysis

Flow cytometric analysis was performed to determine cell cycle changes after treatment with CAPE for 24 h. The cells were washed with PBS and collected with trypsinization. Solution A (50 μg/ml propidium iodide [PI], 3% polyethyleneglycol 6000, 0.1%Triton X-100, 180 U/ml RNase, 4 mM citrate buffer pH7.2) was used to stain total cellular DNA at 4°C for 30 min. Solution B (50 μg/ml PI, 3% polyethyleneglycol 6000, 0.1% Triton X-100, 0.4 M NaCl pH7.2) was then added at 4°C for 10 min before analyzation by a flow cytometer.

For 5-bromo-2′-deoxy-uridine (BrdU) incorporation assay, 10 μM BrdU was added to the treated cells for 4 h. The cells were washed with PBS and incubated with 100 μl of 4 mM citrate buffer and 10 μl BrdU conjugated FITC (Chemicon, Temecula, CA, USA) at 4°C for 30 min. They were then added with 20 μl PI (1 mg/ml) and 100 μl solution B at 4°C for 10 min before analyzation by a flow cytometer.

Western blot analysis

After treatment with different concentrations of CAPE (0.3, 1, 3, 10, and 30 μM) for 24 h, cells were washed with PBS and lysed with extraction buffer (50 mM Tris pH8.0, 0.12 M NaCl, 0.5% NP-40, 1x protease inhibitor cocktail, 4°C). Cellular debris was cleared by centrifugation (500*g*,

10 min, 4°C) and the protein concentration was assessed with Bradford reagent (Biorad). An equal amount of protein was subjected to SDS-polyacrylamide gel electrophoresis and transferred onto a nitrocellulose (NC) membrane (PerkinElmer, Boston, USA). The membranes were probed with mouse anti-human cyclin B1 monoclonal antibodies (Upstate, NY, USA) and HRP-conjugated secondary antibody (Chemicon, Temecula, CA, USA). The proteins were visualized by the use of ECL (Electro-Chemical Luminescence) in a dark room.

Radiosensitization of human MB cells: clonogenic assay

The cells were seeded at the density of 1×10^2 cells/well in 6-well dishes for 18 h. They were then treated with 0–30 μM CAPE for 24 h. After CAPE was removed, irradiation was performed by blood product irradiation facilities (J-L-Shepherd & Associates, San Fernando, CA, USA) at various doses (0, 2, 4, 6, 8 Gy) with the dose rate of 5.34 Gy/min. After incubation at 37°C, 5% CO_2 incubator for 14 days, the number of cell colonies (cells > 50) was counted. The surviving fraction was calculated as such: average number of colonies/number of seeded cells × plating efficiency (PE). Radiation doses 2 and 4 Gy were used in the following radiosensitivity experiments. Sensitizer enhancement ratio (SER) was calculated as follows: radiation dose for radiotherapy only/radiation dose required for CAPE plus radiotherapy, at 37% surviving fraction.

Chemosensitization of human MB cells: clonogenic assay

The cells were spread evenly on 6-well petri dishes with a concentration of 1×10^2 cells/well for 18 h. Then the cells were treated in two different ways: (1) The cells were treated simultaneously with 0.1 μM CAPE and different concentrations of cisplatin or doxorubicin for 24 h; (2) The cells were treated first with 0.1 μM CAPE for 24 h and then with different concentrations of cisplatin or doxorubicin for another 24 h. They were then washed with PBS twice and cultured in a 37°C incubator for 14 days. Afterward, the cells were washed with PBS twice and dyed with 1% crystal violet (containing 30% mathanol) for 20 s. Cell growth can be calculated by counting the cell colonies.

Statistics

All data were presented as mean ± standard deviation (SD). One- or two-way ANOVA was employed to determine whether the results had statistical significance. Significant differences were considered at $P < 0.05$. The statistics software used in this study was Sigma Stat 3.0.1 (SPSS Inc., USA).

Results

Effect of CAPE on cells viability of Daoy cells

Daoy cells were treated separately with 0.01, 0.1, 1, 10, and 100 μM CAPE and observed at an interval of 24, 48, and 72 h followed by MTT assay for cell viability. The cells treated with 0.01 and 0.1 μM CAPE showed no significant difference compared with the control group. There was a significant decrease of cell viabilities in those treated with 1, 10, and 100 μM CAPE ($P < 0.05$). Cell viabilities were 58.7, 38.9, and 21.6% after 24 h; 63.8, 24.7, and 22.3% after 48 h; 25.1, 19.0, and 14.3% after 72 h, respectively (Fig. 1).

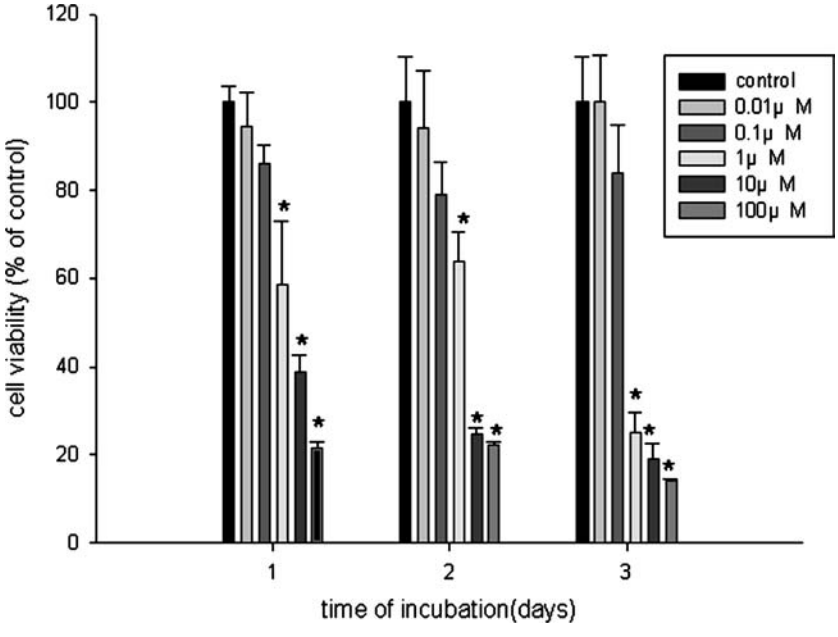

Fig. 1 Cell viability of Daoy cells treated with 0–100 μM CAPE for 24, 48, and 72 h and analyzed with MTT assay. The control was cells treated with 0.1% ethanol ($n = 6$). *$P < 0.05$

Daoy cells were treated separately with 0.3, 1, 3, 10, and 30 μM CAPE for 24 h, stained with trypan blue, and observed under microscope for cell number counting. The cells treated with 0.3, 1, 3 μM CAPE showed no significant difference from the control group, given the increase of time and concentration. However, cell growth of those treated with 10 and 30 μM CAPE were considerably inhibited with statistical significance ($P < 0.05$). The rate of inhibition was 24.7 and 14.4%, respectively as opposed to 100% of the control group.

These results indicate that CAPE is capable of inhibiting growth in Daoy cells.

Apoptosis assessed by morphology, TUNEL assay, and DNA fragmentation assay

Daoy cells were treated separately with 0.3, 1, 3, 10, and 30 μM CAPE for 24 h and observed under inverted microscope with and without HE stain. As the concentration increased, the density of the cells diminished compared with the control group. However, there was no distinct difference in cell morphology. The cells showed no distinctive apoptotic signs such as membrane shrinking or apoptosis bodies (data not shown).

Daoy cells were treated with 0.3, 1, 3, 10, and 30 μM CAPE for 24 h and stained with TUNEL assay. Both the control group and the experimental group showed a small number of TUNEL-positive cells. However, the cells treated with CHX contained more TUNEL-positive cells. In TUNEL assay, no significant apoptosis occurred in Daoy cells treated with CAPE. Moreover, no apoptosis was found among dead cells collected for TUNEL assay.

Daoy cells were treated with 10 μM CAPE for 24, 30, 36, 42, and 48 h. Then the DNAs were extracted to Agarose gel for assessment. No apoptotic DNA fragmentation was found. The same results appeared in the cases with 0.3, 1, 3, 10, and 30 μM CAPE treatments. The cells treated with CHX, however, showed signs of DNA fragmentation (data not shown). It is then indicated that CAPE does not induce apoptotic DNA fragmentation in Daoy cells.

Effect of CAPE on Daoy cell cycles

Daoy cells were treated with 0.3, 1, 3, 10, and 30 μM CAPE for 24 h and analyzed with flow cytometry for cell cycle change, BrdU incorporation assay, and Western blot. Compared with the control group, S phase cell ratio of the experimental group had statistically significant difference ($P < 0.05$). In 10 μM CAPE treatment, the S phase cell ratio rose from 32.3 to 74.3%. As for the G2/M cell ratio, those with 3, 10, and 30 μM CAPE treatments showed statistically significant difference ($P < 0.05$). In 10 μM CAPE treatment, the G2/M cell ratio dropped from 15.4 to 10.1%. In 30 μM CAPE treatment, S phase cell ratio dropped to 58.2% with

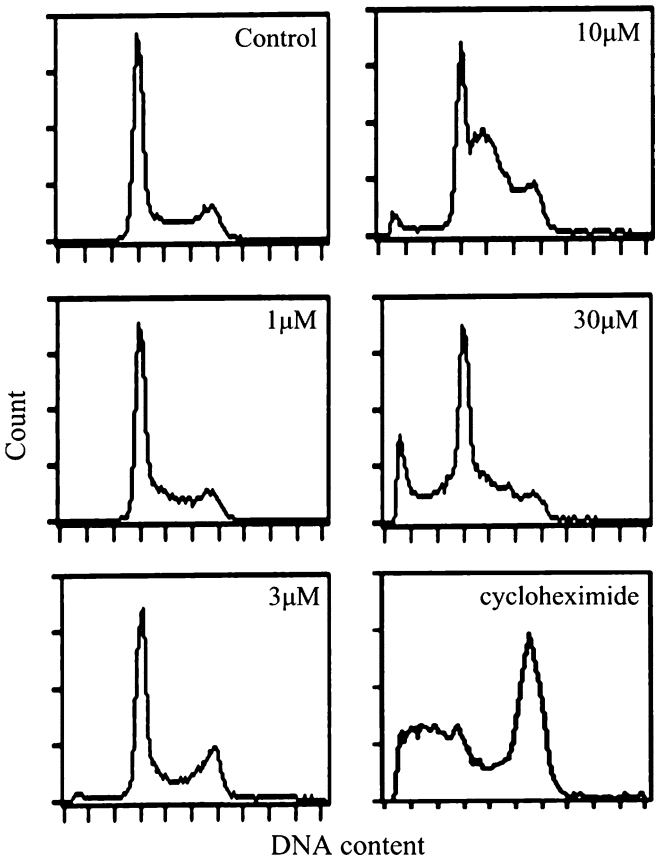

Fig. 2 Cell cycle of Daoy cells treated with 0–30 μM CAPE for 24 h and analyzed with flow cytometry after PI stain. The control was cells treated with cycloheximide for 24 h ($n = 4$)

increasing cell fragmentation. (Fig. 2; Table 1) Hence, no apoptosis was found in cell cycles analysis for CAPE treated Daoy cells. Meanwhile, in those treated with CHX, sub-G1 peak which indicated apoptosis was observed.

In BrdU incorporation assay, the portion of S phase increased as the CAPE concentration increased. Compared with the control group, those with 3, 10, and 30 μM CAPE treatments indicated statistically significant difference ($P < 0.05$). In 10 μM CAPE treatment, S phase rose from 26.7 to 38.7%. However, the portion of G2/M phase dropped with inverse proportion to CAPE concentration. Compared with the control group, those of 1, 3, 10, and 30 μM CAPE treatments showed statistically significant difference ($P < 0.05$). In 10 μM CAPE treatment, G2/M phase decreased from 26.9 to 15.5%. These results indicate that the antiproliferative effect of CAPE on Daoy cells can be explained by cell cycles arrest at S phase and the delay of cell progression to G2/M phase.

Cyclin B1 expressions of CAPE-treated Daoy cells were assessed with Western blot. Cyclin B1 expressions decreased as CAPE concentration increased. The results showed significant statistical difference ($P < 0.05$) at 10 μM and 30 μM concentration, with cyclin B1 expressions at 47.3 and 49.7%, respectively (Fig. 3;

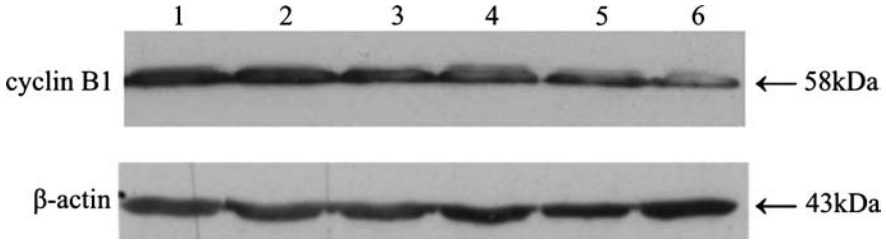

Fig. 3 Cell cyclin B1 protein expression of Daoy cells treated with 0–30 μM CAPE for 24 h and analyzed with Western blot. Lane 1 = control; Lane 2 = 0.3 μM; Lane 3 = 1 μM; Lane 4 = 3 μM; Lane 5 = 10 μM; Lane 6 = 30 μM

Table 1). Cyclin B/Cdc2 kinase activity drives the G2/M phase transition.

Effect of CAPE on radiosensitivity of Daoy cells

Daoy cells were treated with 0.1–10 μM CAPE for 24 h, then were exposed to different radiation with 0, 2, 4, 6, and 8 Gy separately and incubated for 14 days. The control group received no radiation and the plating efficiency is 57%. Cell viabilities were 100, 88.5, 56.1, 24.7, and 0%, respectively. The results showed statistically significant difference ($P < 0.05$) compared with the control group. We can find the survival fraction of the CAPE-treated MB cells decreased and with concentration-dependent effect. The SERs of Daoy cells after 0.1, 0.3, 1, 3 μM CAPE treated were 1.19, 1.32, 1.50, and 2.04, respectively (Fig. 4). After normalizing the antiproliferative effect of CAPE, the results still showed statistically significant difference ($P < 0.05$).

The effect of CAPE on chemosensitivity of human MB cells

Doxorubicin

When the cells were given 0.0003–0.01 μM doxorubicin, no obvious effect on cell growth was found as the concentration increased from 0.0003 to 0.003 μM, comparing to the control group. When the concentration increased up to 0.01 μM, cell growth was obviously inhibited, reducing cell viability from the untreated 100 to 41.5%. When the cells were given 0.1 μM CAPE and 0.01 μM doxorubicin simultaneously, cell viability was reduced from the untreated 100 to 69.8%. For cells treated with 0.1 μM CAPE for 24 h then added with 0.01 μM doxorubicin, cell viability was reduced from the untreated 100 to 58.5%. Compared with the control group, the results in all three groups showed statistically significant differences ($P < 0.05$). Therefore, 0.01 μM doxorubicin was sufficient to inhibit cell viability. The addition of 1 μM CAPE simultaneously or 24 h prior to the treatment of doxorubicin did not show chemosensitizing effect.

Cisplatin

When the cells were given 0.01–0.3 μM cisplatin, no obvious effect on cell growth was found as the concentration increased from 0.01 to 0.1 μM, comparing to the control group. When the concentration was up to 0.3 μM, cell growth was obviously inhibited, reducing cell viability from the untreated 100 to 27.6%. When the cells were given 0.1 μM CAPE and 0.3 μM cisplatin simultaneously, cell viability was reduced from the untreated 100 to 24.9%. For cells treated with 0.1 μM CAPE for 24 h then added with 0.3 μM cisplatin, cell viability was reduced from the untreated 100 to 55.2%. Compared with the control group, the results in all three groups showed statistically significant differences ($P < 0.05$). Therefore, 0.3 μM cisplatin was sufficient to inhibit cell growth. The addition of 0.1 μM CAPE simultaneously or 24 h prior to the treatment of cisplatin did not show chemosensitizing effect.

Effect of CAPE on SVGp12 cells viability

SVGp12 cells were treated with 0.03, 1, 3, 10, and 30 μM CAPE for 24, 48, and 72 h and assessed with MTT assay. While no effect on cell viability was observed at 0.3 μM, considerable difference was found at 1, 3, 10, and 30 μM concentration. The cell viabilities after 24 h were 91.3, 86.8, 85.5, and 80.0%; those after 48 h were 91.7, 86.4, 82.4, and 75.8%; and those after 72 h were 88.5, 76.4, 65.2, and 63.0%, respectively (Fig. 5).

Table 1 The effect of CAPE on Daoy cell cycle and cyclin B1 expression

	G0-G1 (%)	S phase (%)	G2-M (%)	Cyclin B1 (%)
Control	52.38 ± 1.18	32.30 ± 0.53	15.43 ± 0.67	100.00 ± 10.77
0.3 μM CAPE	48.40 ± 0.47*	37.00 ± 0.41*	14.60 ± 0.48	95.70 ± 10.60
1 μM CAPE	41.98 ± 0.72*	45.10 ± 0.87*	12.93 ± 0.42	93.84 ± 3.57
3 μM CAPE	40.45 ± 0.52*	42.65 ± 0.80*	16.90 ± 0.51*	74.20 ± 7.65
10 μM CAPE	15.43 ± 1.70*	74.33 ± 1.51*	10.05 ± 0.41*	47.33 ± 7.13*
30 μM CAPE	31.75 ± 1.41*	58.23 ± 1.39*	10.05 ± 0.80*	49.68 ± 5.83*

The control was 0.1% (v/v) 100% ethanol ($n = 4$)*CAPE* (Caffeic acid phenethyl Ester)* $P < 0.05$

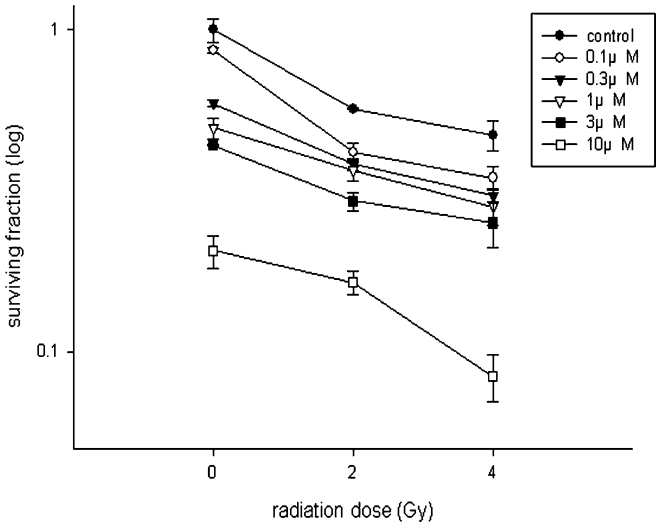

Fig. 4 Radiosensitization of Daoy cells treated with 0.1–10 μM CAPE for 24 h and exposed to 0–4 Gy radiation for colony counting. The control was cells without the treatment of CAPE and radiation. ($n=4$) *filled circle* control; *open circle* 0.1 μM; *filled inverted triangle* 0.3 μM ; *open inverted triangle* 1 μM ; *filled square* 3 μM ; *open square* 10 μM

Therefore, CAPE was also capable of inhibiting SVGp12 cell growth.

Discussion

Medulloblastoma is a malignant tumor predominantly found in children with poor prognosis. There have been ongoing attempts to identify effective antiproliferation agents for the treatment of MBs [23–25]. CAPE has been found to have anticancer effects including antiproliferation, cell apoptosis induced by antioxidation, and increase of radiosensitivity in some tumor cell lines [13, 21, 22]. Based on these findings, CAPE might be a potential agent for antiproliferation and radiosensitization in MB. In our lab, the pathological characteristics of Daoy cells transfected on nude mice were most similar to that of human MB (data not shown). So, we chose Daoy cells as an MB model to evaluate the possible antiproliferation and radiosensitization effects of CAPE. We have shown the antiproliferative effects of CAPE in MB cell, consistent with those described in some other malignant tumors [22, 26, 27]. CAPE inhibited Daoy cells growth through cell cycle arrest, not through apoptosis. We also demonstrated for the first time that radiosensitizing effects could be achieved by CAPE in MB cells.

The results indicated that CAPE could inhibit the proliferation of Daoy cells by cell cycle arrest. No increase of apoptotic cells induced by CAPE treatment was observed. Apoptosis has been shown to be a major pathway leading to cell death upon exposure to chemotherapeutic agents, but this phenomenon is not necessarily valid for solid tumor and senescence may be sufficient to sensitize cancer cells to chemotherapy [28]. We found cell cycles arrest at S phase and the delay of cell progression to G2/M phase in Daoy cells treated with CAPE. CAPE was proven to inhibit DNA, RNA, and protein synthesization [18, 20, 29, 31]. Accumulation of S phase is crucial to the inhibition of DNA synthesization [30]. CAPE might affect the MB cells by inhibiting DNA synthesization, delaying cell cycle to enter G2/M phase and thereby resulted in cell cycle arrest.

Our study also found CAPE could downregulate Cyclin B1 expression. Cyclin B1 is the regulatory subunit of M-phase promoting factor, and proper regulation of

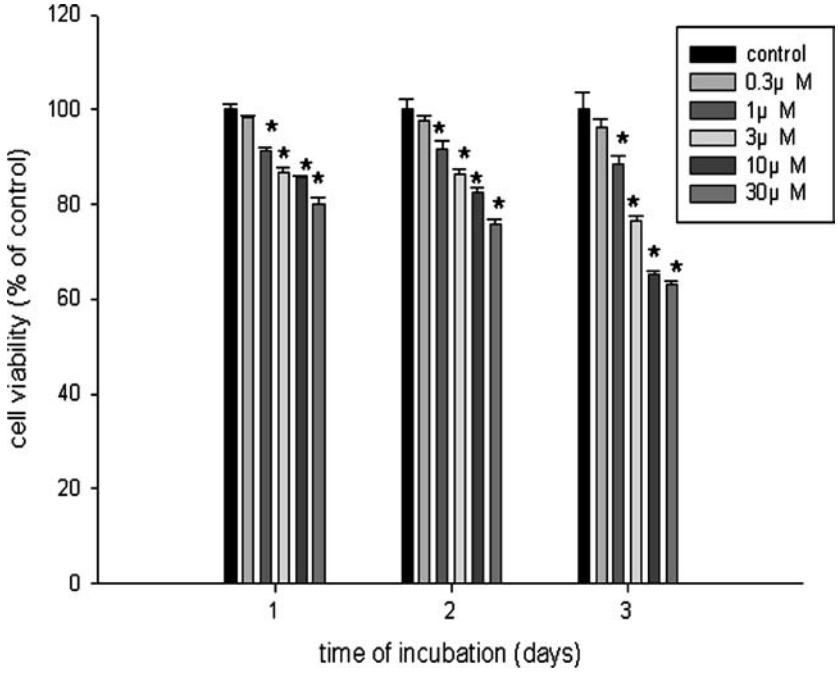

Fig. 5 Cell viability of SVGp12 cells treated with 0–30 μM CAPE for 24, 48, 72 h and analyzed with MTT assay. The control was 0.1% (v/v) 100% ethanol ($n=6$). *$P<0.05$

cyclin B1 is essential for the initiation of mitosis. Increasing evidence indicates that the deregulation of cyclin B1 is involved in neoplastic transformation, suggesting the suppression of cyclin B1 could be an attractive strategy for antiproliferative therapy [31]. The loss of cyclin B1 resulted in the downregulation of cyclin A and Cdk2, S-phase delay and eventually led to cell apoptosis and the decrease of cell viability and proliferation [32]. As MB is relatively resistant to radiotherapy, it is important to study how to increase the radiosensitivity of MB with radiation sensitizing agents. Our study showed CAPE had radiosensitizing effects for Daoy cells. In general, cell survival data showed that cells were most sensitive to irradiation during mitosis and in G2, less sensitive in G1, and least sensitive during the latter part of the S phase [33, 34]. CAPE inhibits cell-cycle progression by arresting cells in the S phase, so the phenomenon of radiosensitization cannot be adequately explained by the phase of cell-cycle arrest. Chen's study suggested that the effects of CAPE on radiosensitization are associated with glutathione depletion that occurred shortly after treatments [22]. In his study, CAPE inhibited intracellular H_2O_2 production and the GSH level of A549 cells in a very rapid and profound manner. Since GSH is a thiol antioxidant, the depleting intracellular stores of GSH by CAPE can render cells more susceptible to oxidative stress-induced apoptosis.

In the study of drugs for brain tumor, brain blood barrier (BBB) limited the potential clinical application in most drugs. According to the studies of ischemia-reperfusion injuries on spinal cord and brain functions in vivo, intraperitoneal injection of CAPE in rats before ischemia-reperfusion could reduce damages to the spinal cord and the brain [17, 35, 36]. It was derived that CAPE could reach the brain via BBB. This suggested the potential of CAPE in clinical application for MB treatment. However, it required further studies on cerebral blood and cerebrospinal fluid in vivo.

Postoperative treatments of MB usually include craniospinal radiotherapy combined with chemotherapy to reduce radiotherapy dose and optimize treatment results. Chemotherapy is usually used in bimodality treatment. Chemotherapeutic agents most commonly used in MB, including CCNU, vincristine, and cisplatin, usually have myelosuppression effects, resulting in anemia leukocytopenia and thrombocytopenia [37, 38]. Our study showed that CAPE had no chemosensitization on cisplatin and doxorubicin. Since chemotherapy alone is not sufficient in the treatments of MB, the absence of chemosensitization of CAPE in MB does not affect its potential in clinical application [2]. On the other hand, no chemosensitization implies that CAPE might not aggravate the side effects of chemotherapy. However, this requires further investigation.

In this series, we used SVGp12 cells to present the possible protection or complication effects in normal tissues. The SVGp12 cell line was established by transfecting cultured human fetal glial cells from brain material dissected from 8- to 12-week-old embryos with DNA from an ori-mutant of SV40. No radiosensitizing effect was found. Although cytotoxicity was found in SVGp12 cells, the effect was mild compared with that of Daoy cells. This implies the clinical potential for CAPE as a radiosensitizer in MB treatment without increasing normal tissue complication by radiation. It was also reported that CAPE had no toxicity to kidney, liver, bone marrow, and other normal cells [5, 39, 40]. Cytotoxicity in normal tissues by CAPE has been found in other series. Lee et al. [19] reported that CAPE had considerable cytotoxicity to oral submucosal fibroblast (OSF). Besides inhibiting tumor cell growth [41, 42], CAPE also inhibits the growth of liver stellate cells [20]. CAPE was found to have cytotoxicity on oncogene-transformed fibroblast cell and TPA induced metastasis epidermal cell in mice [43]. However, the mechanism of cytotoxicity of CAPE to normal tissues requires further investigation.

We conclude that CAPE had antiproliferative and radiosensitizing effects for Daoy cells, which might bring improvement to the treatment of MB. For clinical application, in vivo models are expected.

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