Combinational Treatment Effect of Tetrahydrocurcumin and Celecoxib on Cervical Cancer Cell-Induced Tumor Growth and Tumor Angiogenesis in Nude Mice

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Background: *Tetrahydrocurcumin (THC) demonstrated an anti-cancer and anti-angiogenic effects in cervical cancer. Celecoxib, a selective cyclooxygenase-2 (COX-2) inhibitor, have also shown anticancer effect. However, the combinational treatment effect of THC and celecoxib on tumor growth and tumor angiogenesis, especially, using cervical cancer (CaSki)-implanted nude mice has yet not been reported.*
Objective: *To evaluate the combinational treatment effect of THC and celecoxib on tumor progression and tumor angiogenesis in cervical cancer (CaSki)-implanted nude mice.*
Material and Method: *CaSki cells were inoculated in mice to establish subcutaneous tumors. One month after inoculation, vehicle, THC100 mg/kg, Celecoxib100 mg/kg, or THC50 + Celecoxib50 mg/kg was orally administered every day for 28 consecutive days. The tumor volume was measured every 3-4 days. The microvascular density (MVD) was evaluated using the CD31 expression. VEGF, COX-2, and EGFR expression were also detected by immunohistochemistry.*
Results: *THC, celecoxib, and the combination treatments statistically retarded the tumor volume by 70.40, 65.11 and 77.04%, respectively. The MVD was significantly increased in CaSki + vehicle group, but THC, celecoxib, and the combination treatments markedly attenuated the MVD. VEGF, COX-2, and EGFR were up-regulated in CaSki + vehicle group; however, they were attenuated by THC, celecoxib, and the combination treatments.*
Conclusion: *The combinational treatment effect of THC and celecoxib causing inhibition of tumor growth and tumor angiogenesis via down-regulation of VEGF, COX-2 and EGFR expression. However, this combined treatment did not show the synergistic effect on inhibiting the tumor growth and tumor angiogenesis in cervical cancer (CaSki)-implanted nude mice model.*

Keywords: *CaSki cell, Combination treatment, Vascular endothelial growth factor (VEGF), Cyclooxygenase-2 (COX-2), Epidermal growth factor receptor (EGFR)*

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Cervical cancer is still associated with high mortality rates among woman worldwide[1]. Vascular endothelial growth factor (VEGF), a major mediator of tumor angiogenesis, promotes mobilization of endothelial progenitor cells, cell proliferation, migration, survival and vascular permeability[2]. VEGF was found to be over expressed in cervical cancer and associated with a poor prognosis[3,4]. In addition, cyclooxygenase-2 (COX-2) has role in the onset and progression of malignancies, including the cervical carcinoma, and is also considered as a marker of tumor aggressiveness. It can potentially predispose to cervical cancer by several mechanisms. An increased expression of COX-2 has been reported to inhibit apoptosis, suppress immune function, promote angiogenesis, and enhance the invasiveness of malignant cells[5]. Previous study found that EGF and TGF-α, ligands of EGFR, markedly induced COX-2 in a cervical carcinoma cell line,

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suggesting that deregulated signaling through EGFR is likely to account, at least in part, for increased expression of COX-2[6]. Moreover, Oh et al (2009) reported that COX-2-prostaglandinE2 (PGE2) pathway is also implicated in VEGF expression by HPV 16 E5[7]. Therefore, EGFR-COX-2-PGE2 pathway also plays an important role in tumor growth and angiogenesis in cervical cancer.

Tetrahydrocurcumin (THC) is an active metabolite of curcumin (CUR) in vivo. In the liver, CUR is reduced by endogenous reductase systems to hexahydrocurcumin, THC, and hexahydrocurcuminol, among of which THC has been demonstrated to be the major metabolite[8,9]. Therefore, THC might also play a crucial role in CUR-induced biological effects. It is notable that THC is stable in phosphate buffer and in saline at various pH values, which is quite different from CUR[10]. Recently, we have shown that THC has shown anti-tumor and anti-angiogenic activities in cervical cancer-implanted mice[11]. Moreover, we have shown that THC has more anti-angiogenic activity than CUR[12]. Particularly, THC is easily absorbed through the gastrointestinal tract, suggesting that THC is a preferred potential candidate for the development of anti-cancer agent.

Pharmacological agents that target inflammatory mediators such as prostaglandins (PGs) and cytokines are expected to have anticancer effects[13]. Accordingly, NSAIDs, which mainly block the cyclooxygenase-mediated production of PGs, have been used in both cancer chemotherapy and chemoprevention[14]. Moreover, blocking inflammation with NSAIDs is considered a successful way to prevent cancer because inflammation is involved in the overall process of carcinogenesis. Celecoxib is one of the most representative drugs of the selective cyclooxygenase-2 (COX-2) inhibitor, which is the most commonly prescribed in the treatment of chronic inflammatory diseases. The COX-2 has role in the onset and progression of malignancies, including the cervical carcinoma, and is also considered as a marker of tumor aggressiveness. It can potentially predispose to cervical cancer by several mechanisms. An increased expression of COX-2 has been reported to inhibit apoptosis, suppress immune function, promote angiogenesis, and enhance the invasiveness of malignant cells[5]. A relationship between COX-2, its synthesized product PGE2, and cervical cancer has previously been established[15]. Sales et al reported that COX-2, EP2, and EP4 expression and PGE2 synthesis are up-regulated in cervical cancer tissue and suggest the PGE2 may regulate neoplastic cell function in cervical carcinoma in an autocrine/paracrine manner via the EP2/EP4 receptors[15]. Celecoxib has also been actively studied as a chemopreventive agent in different types of cancer including colorectal, breast, and head and neck cancer[16]. Based on the well-established mechanism studies of chemopreventive agents reported to date, the combination chemoprevention method of using more than one drug with different mechanisms is expected to be a practical and meaningful strategy. Given the close relationship between inflammation and angiogenesis in cancer and their important respective roles in the early stages of carcinogenesis, the two processes could be an ideal target for cancer chemoprevention and the combined use of celecoxib with an angiogenesis inhibitor might be a successful regimen. Therefore, the present study was designed to determine the combinational treatment effect of THC and celecoxib on tumor progression and tumor angiogenesis in cervical cancer (CaSki)-implanted nude mice and to study the combinational treatment effect of THC and celecoxib on angiogenic biomarkers, COX-2, VEGF, and EGFR expressions.

Material and Method

Cell line and cell culture

Cervical cancer cells (CaSki), were purchased from the American Type Culture Collection. The cell lines were cultured in MEM medium supplemented with 10% fetal bovine serum. All cultures were maintained in an incubator at 37°C with 5% CO_2 in a humidified atmosphere.

CaSki-induced tumor mice

BALB/c-nude female mice weighing about 20-25 g were used. The animal experiments were conducted according to the guidelines on experimental animals of The National Research Council of Thailand (1999). According to the procedure reported previously[11], the mice were divided into 5 groups: 1) controls supplemented with corn oil (Control + vehicle; n = 6), 2) CaSki-implanted mice supplemented with corn oil (CaSki + vehicle; n = 6), 3) CaSki-implanted mice supplemented with THC (100 mg/kg) (CaSki + THC100, n = 6), 4) CaSki-implanted mice supplemented with celecoxib (100 mg/kg) (CaSki + Celecoxib100, n = 6) and 5) CaSki-implanted mice supplemented with THC (50 mg/kg) and Celecoxib (50 mg/kg)(CaSki + THC + Celecoxib, n = 6).

For the CaSki groups, a suspension of $10x10^6$ CaSki cells in 0.2 ml MEM[17] were subcutaneously

injected into the dorsa of mice at the proximal midline while control group was injected with MEM. The tumors were measured with Vernier calipers every 3-4 days by using the formula a^2 x b x 0.52 (where is the shortest and b is the longest diameter). When the tumor volume was 100-120 mm^3, mice were randomized. Then, the mice were daily supplemented with vehicle, THC, or combinational treatment 28 days.

The tumor volume at day n is expressed as Relative Tumor Volume (RTV) and calculated according to the following formula: $RTV = TV_n/TV_0$, where TV_n is the tumor volume at day n and TV_0 is the tumor volume at day 0.

Immunohistochemistry for CD31 expression and Microvessels density (MVD) determination

At the end of the experiment, the mice were sacrificed and the tumors were fixed in 10% formalin. Immunohistochemistry was performed using 5 mm thick paraffin sections. Paraffin sections were dewaxed and rehydrated through xylene and a graded alcohol series. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 15 min at room temperature. After washing in water, non-specific binding sites were blocked with 5% bovine serum in phosphate-buffered saline (PBS) for 30 min at room temperature. The tissue slide samples were incubated with primary monoclonal antibody CD31 (Thermo Fischer Scientific, UK) (1: 500) at 4°C overnight. The slide was then gently rinsed with PBS and developed by the Envision system/HRP (DAKO cytomation, USA) for 30 min and substrate-chromogen for 10 min at room temperature. The nuclei were counterstained with Mayer's hematoxylin.

To quantify angiogenesis, microvessel density (MVD) was assessed by immunostaining with the anti-CD31 antibody as previously described[18]. The sections were observed first under the low power (x40), then the most dense area of microvessel sections was selected and counted under the high power (x200, the surface area of every vision field being 0.4 mm^2).

Immunohistochemistry for VEGF, COX-2 and EGFR expression

Paraffin sections from dorsal skin tissue were dewaxed and rehydrated with xylene and a graded alcohol series. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 15 minute at room temperature. After washing in water, non-specific binding sites were blocked with 5% bovine serum, in phosphate-buffered saline (PBS) for 30 minute at room temperature. The tissue slide samples were incubated with primary monoclonal antibody VEGF (Thermo Fischer Scientific, UK) (1: 100) or COX-2 (Thermo Fischer Scientific, UK) (1: 50) or EGFR (VENTANA (ready to use), USA) at 4°C overnight. The slide was then gently rinsed with PBS and developed by the Envision system/HRP (DAKO cytomation, USA) for 30 minute and substrate-chromogen for 10 minute at room temperature. The nuclei were counterstained with Mayer's hematoxylin. The percentage area of VEGF, COX-2 and EGFR immuno reactivated proportionate to the total area were analyzed by Image J 1.38 software (National Institutes of Health, USA).

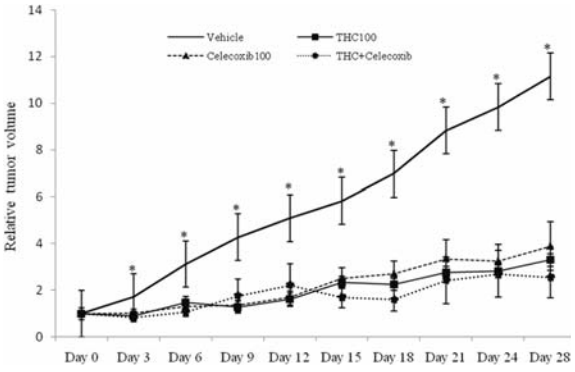
Statistical analysis

Data were expressed as means with standard error. SPSS 13 software was used for statistical analysis. Student's unpaired t-test was applied for comparison of the means of two groups (Control + vehicle and CaSki + vehicle groups), and analysis of variance was used for the means of multiple groups.

Results

Effects of THC, celecoxib, and their combined treatment on tumor growth

Cervical cancer (CaSki) cells were implanted in mice as described. When the tumors had attained a volume of 100-120 mm^3, therapy was started. Fig. 1 represents relative tumor volume. On day 3, all treated groups showed significantly decreased relative tumor volume as compared to CaSki + vehicle group ($p<0.05$). At the end of the experiment, THC, celecoxib, and their combined treatment dramatically retarded the growth of tumors by 70.40, 65.11 and 77.04%, respectively ($p<0.05$).



* $p<0.05$ vs. CaSki + THC100, CaSki + Celecoxib100, and CaSki + THC + Celecoxib

Fig. 1 Relative tumor volume (mean ± SEM).

Anti-angiogenic effect of THC, celecoxib, and their combined treatment

A detection of the CD31 antigen expression was used to determine the quantity of the angiogenesis process. CD31 primarily indicated the presence of endothelial cells in histological tissue sections. Fig. 2A shows representative immunostaining for CD31 in control (A), CaSki + vehicle (B), CaSki + THC100 (C), CaSki + celecoxib100 (D), and CaSki + THC + celecoxib (E) groups. In normal skin tissue, few CD31 expressions were detected adjacent to sweat glands, whereas they were highly expressed in CaSki-implanted tissues. However, all treatments attenuated the CD31 expression.

We measured the microvascular density (MVD), as determined by CD31 staining, to assess the quantity of the angiogenesis process. In Fig. 2B, the MVD in CaSki + vehicle group was significantly higher in the than in the control group (46.00±2.23 vs. 6.00±0.84; $p<0.001$). The MVD was significantly decreased in THC, celecoxib, and their combined treatment groups (13.00±0.85, 11.00±0.85 and 8.00±0.10, respectively). However, the reduction of the MVD in combined treatment group was not statistically different as compared with THC or celecoxib treatment alone.

Effects of THC, celecoxib, and their combined treatment on VEGF expression

Immunoreactivity for VEGF protein (shown in B), was presented diffusely in the cytosolic of cancer cells (Fig. 3A). Stronger VEGF expression was found in the CaSki + vehicle group than in the control group. Interestingly, our study demonstrated that all treatments attenuated VEGF expression. Fig. 3B show the expression ratio of VEGF. In the CaSki + vehicle group, VEGF (38.32±2.75%) expression were significantly higher than in the control group (5.33±0.32%) ($p<0.001$).

VEGF expression ratio in CaSki + THC100 (17.78±1.85%) and CaSki + celecoxib100 (16.25±1.78%), were significantly reduced as compared to CaSki + vehicle group ($p<0.001$). Furthermore, the expression ratio of VEGF in CaSki + THC + celecoxib group significantly reduced (16.67±1.91%) as compared to CaSki + vehicle group ($p<0.001$) but this reduction was not statistically different to THC or celecoxib monotherapy.

Effects of THC, celecoxib, and their combined treatment on COX-2 expression

Fig. 4A shows microscopic images of immunohistochemical stained sections for COX-2 expression. Stronger COX-2 expression was found in the CaSki + vehicle group than in the control group. THC, celecoxib, and their combined treatment attenuated COX-2 expression.

Fig. 4B shows the expression ratio of COX-2. The expression ratio of COX-2 was significantly

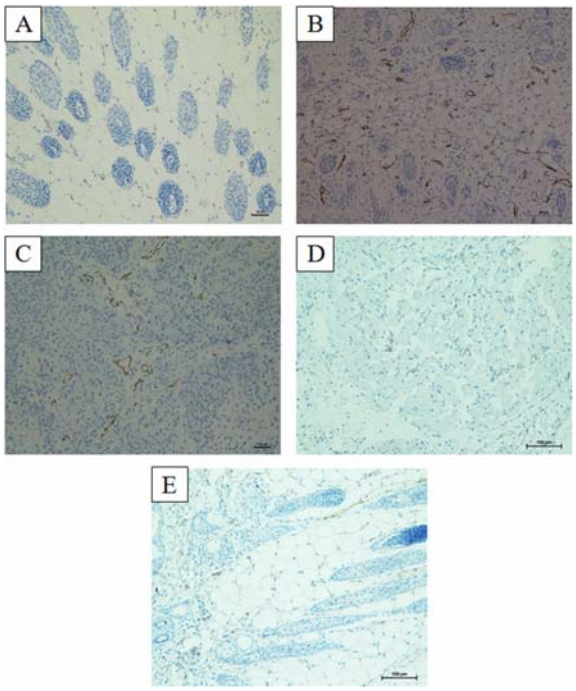
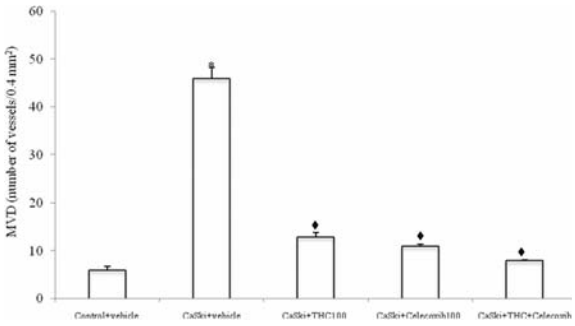


Fig. 2A CD31 expression in Control + vehicle group (A), CaSki + vehicle group (B), CaSki + THC100 (C), CaSki + Celecoxib100 (D), and CaSki + THC + Celecoxib (E).



* $p<0.001$ vs. Control + vehicle group, ♦ $p<0.001$ vs. CaSki + vehicle group

Fig. 2B Microvascular density (MVD) (number of capillary/0.4 mm^2) (mean ± SE).

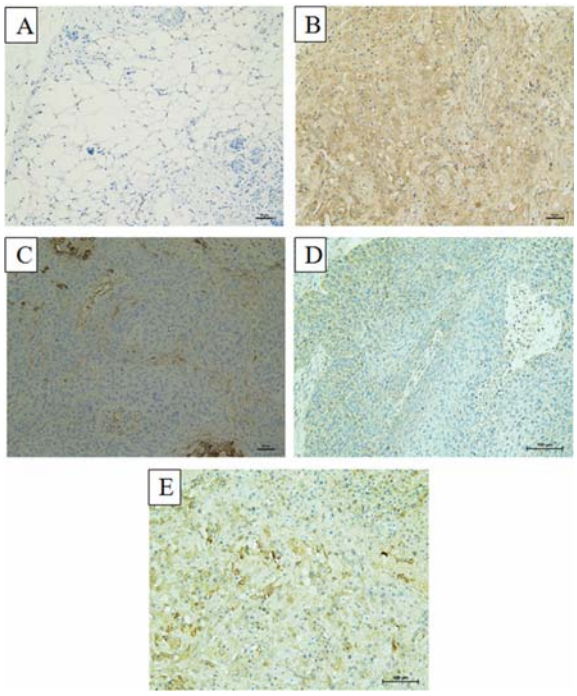
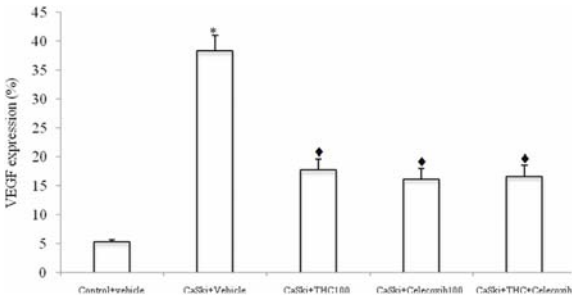


Fig. 3A VEGF expression in in Control + vehicle group (A), CaSki + vehicle group (B), CaSki + THC100 (C), CaSki + Celecoxib100 (D), and CaSki + THC + Celecoxib (E), 200x.



* p<0.001 vs. Control + vehicle group, ♦ p< 0.001 vs. CaSki + vehicle group

Fig. 3B VEGF expression (%) (mean ± SE).

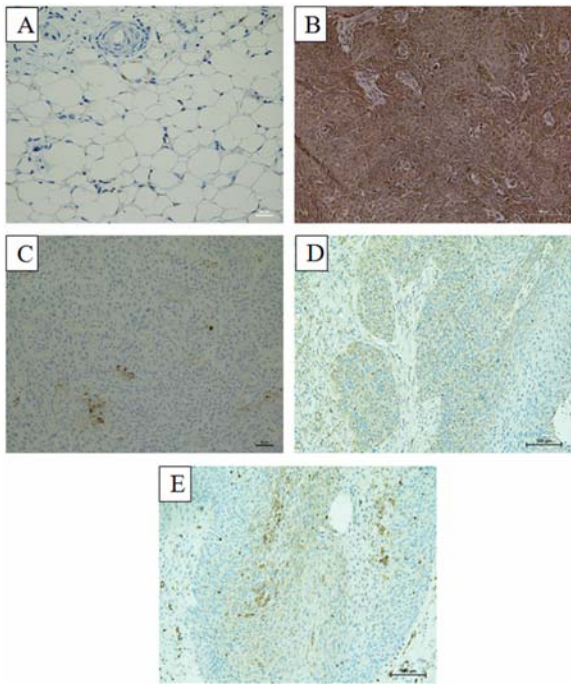
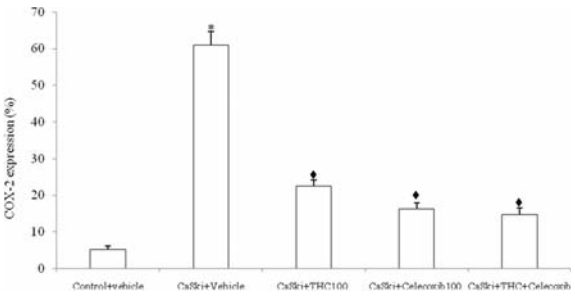


Fig. 4A COX-2 expression in Control + vehicle group (A), CaSki + vehicle group (B), CaSki + THC100 (C), CaSki + Celecoxib100 (D), and CaSki + THC + Celecoxib (E), 200x.



* p<0.001 vs. Control + vehicle group, ♦ p<0.001 vs. CaSki + vehicle group

Fig. 4B COX-2 expression (%) (mean ± SE).

increased in the CaSki + vehicle group (60.98±3.75%) as compared to the control + vehicle group (5.25±0.85%) (p<0.001). Interestingly, the expression ratio of COX-2 was significantly reduced in THC, celecoxib, and their combined treatment groups (22.53±1.54%; 16.25±1.67%; 14.67±1.82%) (p<0.001). However, the expression ratio of COX-2 in combined treatment did not reach significant levels as compared with THC or celecoxib monotherapy.

Effects of THC, celecoxib, and their combined treatment on EGFR expression

Fig. 5A shows microscopic images of immune histochemical stained sections for EGFR expression. The EGFR staining pattern was predominantly membrane with occasional cytoplasmic positivity. EGFR was over expressed in CaSki + vehicle group; however, it was attenuated after treatment with all treated groups.

Fig. 5B shows expression ratio of EGFR. The

EGFR expression significantly increased in the CaSki + vehicle group (95.84±2.55%) as compared to the control + vehicle group (5.19±0.32%) (p<0.001), but EFGR expression decreased after treatments with THC, celecoxib, and their combined (33.68±2.76%; 32.12±1.32%; 29.02±1.58%, respectively) (p<0.001). Again, the expression ratio of EGFR in combined treatment did not reach significant levels as compared with THC or celecoxibmono therapy.

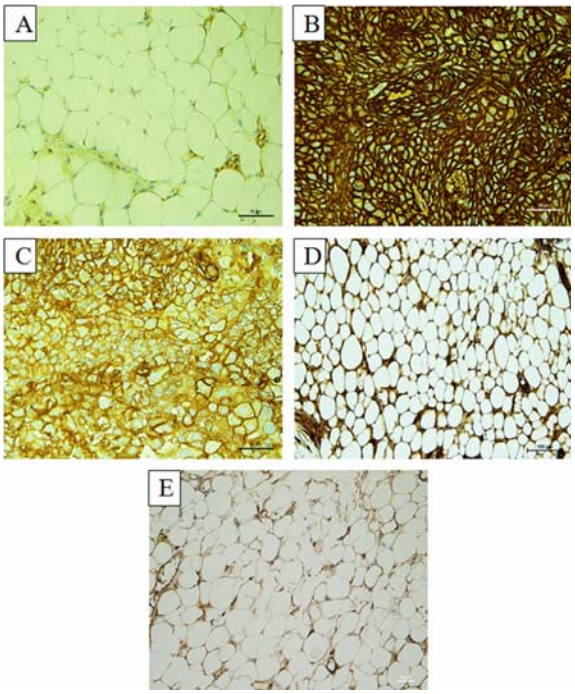
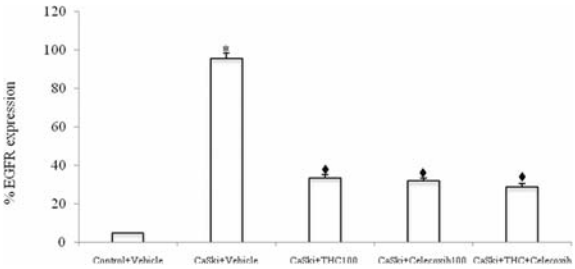


Fig. 5A EGFR expression in Control + vehicle group (A), CaSki + vehicle group (B), CaSki + THC100 (C), CaSki + Celecoxib100 (D), and CaSki + THC + Celecoxib (E), 400x.



* p<0.001 vs. Control + vehicle group, ♦ p<0.001 vs. CaSki + vehicle

Fig. 5B EGFR expression (%) (mean ± SE).

Discussion

The new therapeutic strategies in order to obtain better results in patients with cervical cancer continue to be made. One of these domains of interest is represented by targeted therapies. Thus, even in an incipient phase of the clinical research process, the combination treatment seems to represent a new, feasible and promising approach in several types of cancer, including the cervical cancer. In the present study, we evaluate the combinational treatment effect of THC and celecoxib on tumor progression and tumor angiogenesis in cervical cancer (CaSki)-implanted nude mice. In addition, we studied the combinational treatment effect of THC and celecoxib on angiogenic biomarkers, COX-2, vascular endothelial growth factor (VEGF), and the epidermal growth factor receptor (EGFR). We demonstrated that all treatments (THC alone, celecoxib alone and their combined treatments) markedly reduced tumor growth (reduced relative tumor volume) and tumor angiogenesis (reduced MVD) in CaSki-implanted mice model.

VEGF and COX-2 plays a pivotal role in the control of angiogenesis as well as tumor growth and metastasis in cervical cancer[19,20]. VEGF plays a crucial role in normal and pathologic angiogenesis, triggering multiple signaling networks that result in endothelial cell survival, migration, proliferation, differentiation, and vascular permeability[2]. In addition, an increased expression of COX-2 has been reported to inhibit apoptosis, suppress immune function, promote angiogenesis, and enhance the invasiveness of malignant cells[5]. In the present study, we showed that strong expression of VEGF and COX-2 were demonstrated in CaSki-implanted mice. These results were similar to our previous finding that VEGF and COX-2 played an important role in tumor biological behavior and neovascularization in cervical cancer-implanted mice models[21].

Furthermore, we demonstrated that strong expression of EGFR was found in CaSki-implanted mice and that VEGF and COX-2 were strongly positively related. We confirmed that over expression of the angiogenic biomarkers, VEGF and COX-2 might be mediated by the induction of EGFR signaling pathway. The EGFR signal pathway plays an important role in various tumorigenic processes, including cell cycle progression, angiogenesis, and metastasis, as well as protection from apoptosis. Recently reported VEGF COX-2 and EGFR were an important tissue biomarkers of cervical cancer[21,22]. These data suggest that EGFR, COX-2 and VEGF expressions are important biomarkers

in cervical cancer tumor angiogenesis and tumor growth. In our study, we have clearly demonstrated that the treatments with THC, celecoxib and their combination attenuated VEGF, COX-2 and EGFR expression. However, the down regulation of VEGF, COX-2 and EGFR expression in combined treatment group did not reach significant levels as compared with their treatment alone. This result suggests that combined treatment did not show the synergistic effect on inhibiting the tumor growth and tumor angiogenesis in cervical cancer (CaSki)-implanted nude mice model. This is possibly due to both THC and celecoxib inhibit tumor growth and tumor angiogenesis via the same pathway, which may be regarded as high-dose monotherapy.

Conclusion

The evidence presented in this in vivo study gives new insight into the combinational treatment effect of THC and celecoxib on tumor progression and tumor angiogenesis, especially, using cervical cancer (CaSki)-implanted nude mice. Our findings indicate that the combinational treatment effect of THC and celecoxib caused the inhibition of tumor growth and tumor angiogenesis via down-regulation of VEGF, COX-2 and EGFR expression. However, this combined treatment did not show a synergistic effect on inhibiting the tumor growth and tumor angiogenesis in cervical cancer (CaSki)-implanted nude mice model.

What is already known on this topic?

Anti-cancer and anti-angiogenic effects of THC have been reported. Celecoxib, a selective COX-2 inhibitor, have also shown anti-cancer effects.

What this study adds?

The evidence presented in this in vivo study gives new insight into the combinational treatment effect of THC and celecoxib on tumor progression and tumor angiogenesis, especially, using cervical cancer (CaSki)-implanted nude mice. Our findings indicate that the combinational treatment effect of THC and celecoxib cause inhibition of tumor growth and tumor angiogenesis via down-regulation of VEGF, COX-2 and EGFR expression. However, this combined treatment did not show a synergistic effect on inhibiting the tumor growth and tumor angiogenesis in cervical cancer (CaSki)-implanted nude mice model.

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Author contributions

Yoysungnoen B designed research, performed research, data analysis and wrote the paper; Bhattarakosol P prepared the cervical cancer cell; Changtam C prepared THC, and Suthiluk Patumraj designed research.

Potential conflicts of interest

None.

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ผลการรักษาร่วมของเตทตราไฮโดรเคอร์คูมินและซิลิคอกซิบต่อการเจริญของก้อนมะเร็งและการสร้างหลอดเลือดใหม่ในก้อนมะเร็งในหนูไร้ขนที่ถูกเหนี่ยวนำให้เป็นมะเร็งด้วยเซลล์มะเร็งปากมดลูก

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ภูมิหลัง: *เตทตราไฮโดรเคอร์คูมิน (THC) มีฤทธิ์ต้านมะเร็งและต้านการสร้างหลอดเลือดใหม่ในมะเร็งปากมดลูกซิลิคอกซิบ (Celecoxib) เป็นยาที่ออกฤทธิ์ยับยั้งการทำงานเฉพาะ cyclooxygenase-2 (COX-2) ก็มีฤทธิ์ยับยั้งมะเร็ง อย่างไรก็ตามฤทธิ์การรักษาร่วมของ THC และ celecoxib ต่อการเจริญของก้อนมะเร็งและการสร้างหลอดเลือดใหม่ในก้อนมะเร็งในหนูไร้ขนที่ได้รับการปลูกถ่ายเซลล์มะเร็งปากมดลูก (CaSki) ยังไม่มีรายงาน*

วัตถุประสงค์: *เพื่อประเมินผลการรักษาร่วมของเตตราไฮโดรเคอร์คูมินกับซีลิคอกซิบต่อการเจริญของก้อนมะเร็งและการสร้างหลอดเลือดใหม่ในหนูไร้ขนที่ถูกเหนี่ยวนำด้วยมะเร็งปากมดลูก*

วัสดุและวิธีการ: *เซลล์ CaSki ถูกปลูกถ่ายเพื่อทำให้เป็นมะเร็งที่ชั้นใต้ผิวหนังของหนูไร้ขน หนึ่งเดือนหลังการปลูกถ่ายเซลล์มะเร็ง สัตว์ทดลองถูกป้อนด้วยน้ำมันข้าวโพด (Vehicle), THC 100 mg/kg, Celecoxib 100 mg/kg, หรือ THC50 + Celecoxib 50 mg/kg นาน 28 วัน หลังจากนั้นวัดขนาดก้อนมะเร็งเพื่อหาค่า tumor volume ทุก 3-4 วัน ค่าความหนาแน่นของหลอดเลือดขนาดเล็ก (Microvascular density, MVD) ถูกประเมินจากการแสดงออก ของ CD31 นอกจากนี้วัดการแสดงออกของ VEGF, COX-2, และ EGFR ด้วยวิธี immunohistochemistry*

ผลการศึกษา: *THC, celecoxib, และการรักษาร่วมสามารถลดขนาดของก้อนมะเร็งอย่างมีนัยสำคัญทางสถิติได้ 70.40, 65.11 and 77.04% ตามลำดับ ค่า MVD เพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติในกลุ่ม CaSki + vehicle group แต่การรักษาด้วย THC, celecoxib และการรักษาร่วมสามารถลด MVD ได้อย่างชัดเจน การแสดงออกของ VEGF, COX-2 และ EGFR เพิ่มขึ้นในกลุ่ม CaSki + vehicle อย่างไรก็ตามการรักษาด้วย THC, celecoxib และการรักษาร่วมสามารถลดการแสดงออกของ VEGF, COX-2 และ EGFR ได้*

สรุป: *การรักษาร่วมของ THC กับ celecoxib สามารถยับยั้งการเจริญของก้อนมะเร็งและยับยั้งการสร้างหลอดเลือดใหม่ในก้อนมะเร็งโดยผ่านการยับยั้งการแสดงออกของ VEGF, COX-2 และ EGFR อย่างไรก็ตามการรักษาร่วมนี้ไม่แสดงผลการเสริมฤทธิ์กัน (synergistic effect) ในการยับยั้งการเจริญของก้อนมะเร็งและยับยั้งการสร้างหลอดเลือดใหม่ในก้อนมะเร็งในหนูไร้ขนที่ได้รับการปลูกถ่ายเซลล์มะเร็งปากมดลูก*