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The Anti-proliferative Effect of Caffeic Acid and Dactolisib on Human Cervical Carcinoma HeLa Cell Line

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ABSTRACT

Cervical carcinoma is a common gynecological cancer with high mortality rate among women worldwide. Caffeic acid exerts an antiproliferative effect against cervical carcinoma. Dactolisib is a dual PI3K and mTOR inhibitor that has a therapeutic potential for cervical carcinoma. This study aimed to reveal the anti-proliferative effect of combination treatment of caffeic acid and Dactolisib on cervical carcinoma HeLa cell line. Cytotoxicity of caffeic acid and Dactolisib on HeLa cell line was assessed by MTS assay. Colony formation of HeLa cells treated with caffeic acid and Dactolisib was determined by staining colonies with crystal violet and visualizing under light microscope. Dactolisib decreased cell proliferation of HeLa cells in time and dose dependent manner. 5 µM caffeic acid did not show any significant change in cell viability of HeLa cells. Combination treatment of 5 µM caffeic acid and 0.5 µM Dactolisib showed decrease in cell viability of HeLa cells when compared to Dactolisib treated cells. Combination of caffeic acid and Dactolisib decreased colony diameter of HeLa cells significantly when compared to control group. Caffeic acid and Dactolisib shows anti-proliferative effect on human cervical carcinoma HeLa cell line, so further studies should be performed to reveal the mechanism of action.

Keywords: Cervical carcinoma, Dactolisib, Caffeic acid, Anti-proliferative effect.

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Introduction

Cervical carcinoma, a common gynecological cancer, is the fourth most diagnosed cancer with high morbidity and mortality rate among women worldwide. Despite the successful smear screening and advanced treatment programs, each year new cervical carcinoma cases are being reported [1]. Current treatment modalities include radiotherapy, chemotherapy and surgery applied alone or in combination [2]. Platinum-based compounds are used as a first line chemotherapy to treat cervical carcinoma; however, drug resistance is seen in patients [3]. Thus, novel strategies such as natural anticancer compounds and conventional chemotherapeutics are used as combination cancer therapy to optimize the efficacy of chemotherapy regimens [4].

Caffeic acid (CA), a member of phenolic acid family of polyphenols, shows significant therapeutic and biological activities in disease treatment [5]. CA demonstrates anti-inflammatory, anti-bacterial, anti-oxidant and antiproliferative properties [6]. CA shows antitumor effect on hepatocarcinoma [7], melanoma [8], prostate [9], breast [10], ovarian [11] and cervical cancer [12]. CA can affect cancer cells alone or in combination with chemotherapeutic drug, to decrease the drugs dose and overcome resistance against those drugs [13]. Previous studies show that combination of cisplatin and CA significantly increased apoptosis in cervical carcinoma when compared to cisplatin administered alone [14]. Thus, new combination treatment strategies targeting key pathways is crucial for cervical carcinoma therapy.

The phosphatidylinositol 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/Akt/mTOR) is a key pathway that plays a role in wide range of normal cellular functions. In various cancers, somatic mutations in genes related with PI3K or mTOR pathway lead to constitutive activation of pathway which lead to dysregulation of tumor cell proliferation and cell survival [15]. Studies show that HPV oncogenes can activate the PI3K/Akt/mTOR signaling pathway to modulate tumor progression and initiation [16]. PI3K is amplified and activated in HPV-induced cervical cancers [17]. Dactolisib (NVP-BEZ235), an imidazo [4, 5-c] quinoline derivative, is a dual inhibitor of PI3K and mTOR kinases that is in phase I/II clinical trials for solid tumors. Dactolisib inhibits by binding to the ATP-binding cleft of PI3K and mTOR kinases [18]. Dactolisib suppressed HeLa cell invasiveness and metastasis by inhibiting the PI3K/Akt pathway. PI3K/Akt/mTOR signaling pathway activation promotes tumorigenesis of cervical carcinoma, thus inhibiting this pathway may be a strategy for targeting therapies [19]. Furthermore, a recent study shows that HeLa cell line was more sensitive to Dactolisib compared to other key pathway inhibitors, suggesting that this PI3K/mTOR inhibitor therapy may be a potential benefit to cervical cancer patient [20].

The purpose of this study was to identify the anti-proliferative effect of CA and Dactolisib on cervical carcinoma HeLa cell line. Cell viability of HeLa cell line was investigated under the treatment of CA, Dactolisib or their combination. The growth inhibition of HeLa cell line was assessed through colony formation assay. The present study suggests that combination therapy of CA and Dactolisib for treatment of cervical carcinoma may be a potential therapy. However, further studies are needed to reveal the mechanism of action of this combination therapy.

Material and Method

Reagents

Dactolisib was obtained by Selleck chemicals and dissolved in DMSO to obtain stock concentration of 2.12 mM. CA was purchased from Sigma-Aldrich and dissolved in DMSO to obtain stock concentration of 20 mM. The final concentration of DMSO was less than 0.1% in all cell cultures. MTS (3-(4,5-di-methyl-thiazol-2-yl)-5-(3-carboxy-methoxy-phenyl)-2-(4-sulfo-phenyl)-2H-tetrazolium) was purchased from Promega and crystal violet was obtained by Sigma-Aldrich.

Cell culture

The human cervical carcinoma HeLa cell lines were maintained in Dulbecco's Modified Eagle Medium (DMEM, Gibco) supplemented with 100 U/mL penicillin, 0.1 mg/mL streptomycin and amphotericin (1% PSA, Gibco) solution and 10% fetal bovine serum (FBS, Sigma-Aldrich). Cells were maintained in a humidified incubator with 5% CO_2 at 37 °C conditions.

Cell Viability Assay

Cell viability was determined using MTS colorimetric assay. In 96-well plates, HeLa cells were seeded with a density of 5 x 10^3 cells/well. After 24 h, cells were treated with Dactolisib, CA or their combination for 24 h and 48 h. After incubation period, cells were subjected to a mixture of MTS reagent and DMEM medium and the absorbance value was measured using a microplate reader (Bio-tek ELx800, USA) at 490 nm. Cell viability (%) was calculated by setting non-treated control cells to 100%.

Colony Forming Assay

HeLa cells were seeded at a density of 300 cells/ well in 6-well plates and incubated overnight to allow attachment of cells. The following day, HeLa cells were treated with Dactolisib, CA or combination of both and incubated for 14 days. Following incubation time, each well was fixed with 100% methanol at 4 °C for 20 minutes and stained with crystal violet for 5 minutes. The number of colonies were counted under an inverted light microscope at 4× magnification (DMi1, Leica Microsystems) combined with a digital camera.

Statistical Analysis

All experiments were performed (n=3) and results are expressed as the mean ± SD. Free trial version of GraphPad Prism 8.0 was used to draw graphics and t-test or two- way ANOVA was used as statistical analysis. $P < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***) and $p < 0.0001$ (****) were considered statistically significant.

Results

Combination treatment of CA and Dactolisib induces anti-proliferative effect in HeLa cells

Cell proliferation MTS assay was used to determine the cytotoxic effect of Dactolisib, CA or a combination of both on HeLa cell lines. Dactolisib demonstrated significant cytotoxicity on HeLa cell lines in time and dose-dependent manner. At 24 h, there was a significant decrease in cell viability of HeLa cells to 83.6%, 85.5%, 83.8%, 66.0% and 62.5% when treated with 0.25, 0.5, 1 ,2 and 4 µM Dactolisib, respectively. A decrease to 64.7%, 65.8%, 60.9%, 60.0%, and 57.4% was detected in HeLa cells when treated with 0.25, 0.5, 1, 2 and 4 µM Dactolisib, respectively at 48 h (Fig. 1).

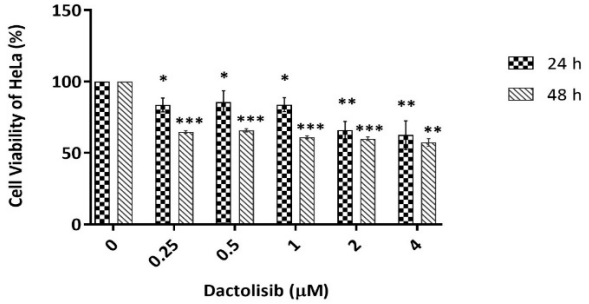

Figure 1. Cell viability of HeLa cells after exposure to 0.25 - 4 µM Dactolisib for 24 h and 48 h. The control group represents cells with no treatment. Data represents average of three independent experiments ± SD ($p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***)).

HeLa cells treated with 5 µM CA did not show any change ($p > 0.05$) in cell viability at 24h and 48h. CA demonstrated significant cytotoxicity on HeLa cell lines only at high dosage (40 µM). At 24 h, there was no significant change in cell viability of HeLa cells when treated with 5, 10 and 20 µM CA, showing a slight decrease of 3%, 2% and 7%, respectively. Similarly at 48 h, a slight ($p > 0.05$) decrease of 1% and 8% was detected when HeLa cells were treated with 10 and 20 µM CA, respectively. On the contrary, significant decrease in cell

viability of HeLa cells were detected when treated with 40 µM CA at 24h and 48 h (Fig. 2).

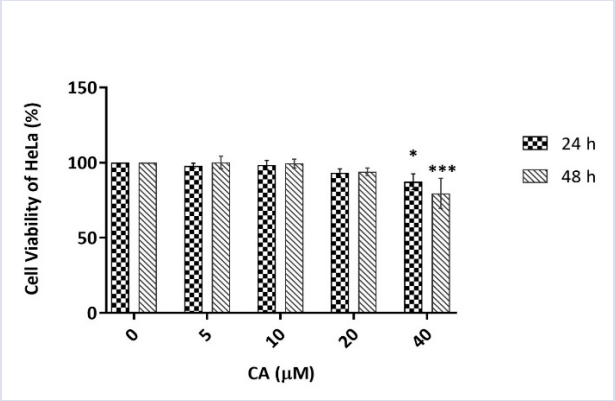

Figure 2. Cell viability of HeLa cells after exposure to 5- 40 µM CA for 24 h and 48 h. The control group represents cells with no treatment. Data represents average of three independent experiments ± SD ($p < 0.05$ (*) and $p < 0.001$ (***)).

As shown in Figure 3, cell viability of HeLa cells when treated with low doses of Dactolisib (0.25 µM and 0.5 µM) alone and in combination with CA was evaluated using MTS assay. There was no significant decrease in CA and control group at all time points. A significant decrease was observed in cell viability of HeLa cells treated with 0.25 µM Dactolisib alone and in combination (CA and Dactolisib) group when compared to CA group at all time points. No significant change ($p > 0.05$) was detected between 0.25 µM Dactolisib alone and in combination group (Figure 3A).

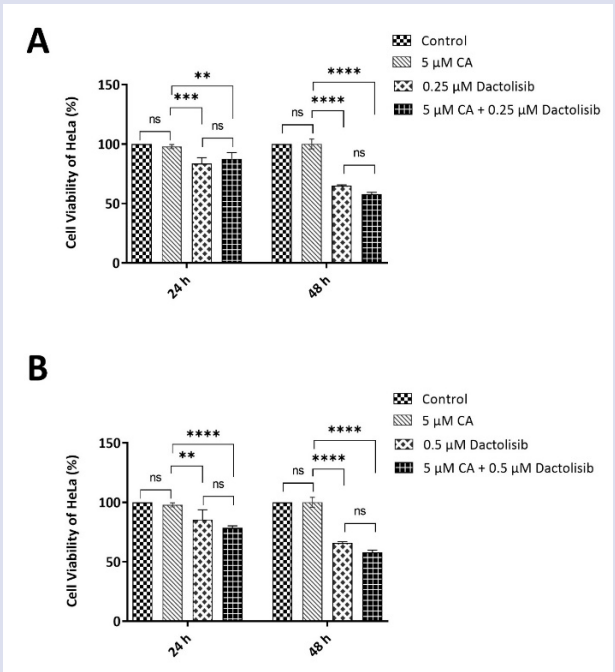

Figure 3. Cell viability of HeLa cells after exposure to (A) combination of 5 µM CA and 0.25 µM Dactolisib and (B) combination of 5 µM CA and 0.5 µM Dactolisib for 24 h and 48 h. The control group represents cells with no treatment. Data represents average of three independent experiments ± SD ($p < 0.01$ (**), $P < 0.001$ (***) and $P < 0.0001$ (****)).

Similarly, HeLa cells treated with 0.5 µM Dactolisib alone and in combination with CA showed significant decrease in cell viability when compared to CA group at 24 and 48 h. No significant change ($p > 0.05$) was detected between 0.5 µM Dactolisib alone and in combination with CA group (Figure 3B).

Combination Treatment of CA and Dactolisib Inhibits Growth Formation of HeLa Cells

Cell colony formation assay was used to determine the effect of CA and Dactolisib HeLa cells were incubated with Dactolisib, CA or combination of both for 14 days. The number of colonies for each group was counted. The well images and colonies under light microscope show that the number of colonies was reduced in Dactolisib alone and in combination with CA groups compared to control group (Fig. 4A-B). The colony diameters of HeLa cells were nearly 1.504, 0.213 and 0.127 mm for CA, Dactolisib and combination group, respectively. For control (un-treated) group the colony diameter was 1.719 mm. Thus, there was no significant change in colony diameter of HeLa cells when treated with CA. The colony diameter of HeLa cells decreased significantly when treated with Dactolisib alone and in combination with CA. This indicates that Dactolisib has inhibitory effect in HeLa cell formation (Fig. 4C).

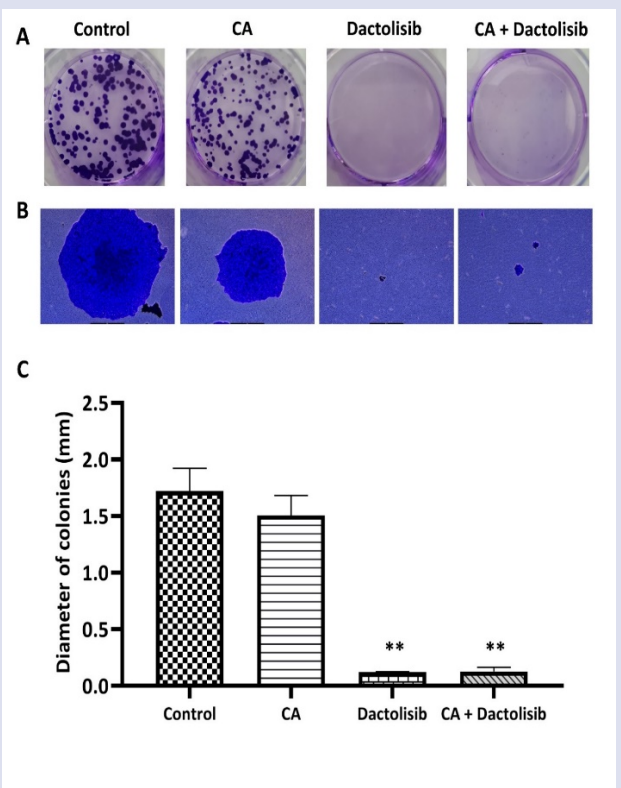

Figure 4. Colony formation of HeLa cells treated with 5 µM CA, 0.5 µM Dactolisib or a combination of both. (A) Representative images of colony formation in 6-well, (B) under light microscope and (C) the diameter of colonies observed in the HeLa cells after 14 days treatments with Dactolisib, CA or combination of both. Scale bar: 620 µm. $P < 0.01$ (**).

Discussion

Cervical carcinoma is one of the leading causes of cancer related deaths in females worldwide. Chemotherapy is the first line therapy in cervical carcinoma however, drug resistance is commonly seen [21]. PI3K/mTOR pathway is required for a wide range of normal cellular functions and is a major signaling pathway that modulate survival in cancer cells. Studies show that in various types of cancer, somatic mutations in genes related with PI3K or mTOR induces constitutive pathway activation; causing dysregulation of tumor cell growth, proliferation and apoptosis [15]. Activation of the PI3K/Akt/mTOR pathway promotes tumorigenesis of cervical carcinoma and this pathway may be a novel candidate for targeting this cancer [22]. Rapamycin is used by many researchers to inhibit the mTOR singaling pathway. This drug only inhibits mTOR complex (TORC) 1 causing it to induces Akt phosphorylation by feedback activation [23], which results in the reduction of the anti-cancer effect of rapamycin. Thus, more effective inhibitors targeting these pathways are needed.

Dactolisib is a dual PI3K and mTOR kinase inhibitor showing promise in preclinical mouse models for solid tumors [24]. A study conducted by Xie et. al., shows that Dactolisib has an anti-proliferative and anti-cancer effect on cervical carcinoma HeLa, SiHa and C33A cells and it has a potential in treatment of cervical carcinoma. 320 nM Dactolisib showed a significant decrease of cell viability in HeLa cells by reducing the cell viability to nearly 25% at 48 h using MTT assay [19]. In the current study, Dactolisib showed significant cytotoxicity on HeLa cell lines in time and dose-dependent manner (Figure 1). However, at highest dosage of 4 µM Dactolisib there was only decrease of HeLa cell viability to 57%. Our results show that low doses of Dactolisib did not have effective anti-proliferative effect on HeLa cells as literature. The final concentration of DMSO for both studies were stated as being less than 0.1%, so these differences could be due to different cell viability assays. Even though both MTT and MTS are tetrazolium based assays, they could show differences in cell viability [25].

Combination treatment of phytochemicals and chemotherapeutic act as a novel approach to cancer therapy as it increase the cancer treatment efficacy and reduce adverse side effects [26]. CA is a polyphenol pytochemical which shows antitumor effect on cervical carcinoma [12]. In the present study, (5- 20 µM) CA did have a significant effect on HeLa cells except when treated with 40 µM CA (Figure 2). Thus, low doses of CA could not be used as an effective anti-cancer agent, however potential synergism of CA combining with anticancer drug should be evaluated. The combination treatment of CA and Dactolisib exhibit anti-proliferative effect on cervical carcinoma HeLa cell line. CA administered alone on HeLa cells did not have a significant effect (nearly 99% viable cells). Dactolisib administered alone and in combination with CA showed nearly 7% more decrease in cell viability of HeLa cells (Figure 3). Studies show that synergistic effect was observed when CA was given in combination with other inhibitors suggesting a synergism [27]. Thus, CA could be a phytochemicals candidate for increasing efficacy of cancer treatment when administered with another drug.

Clonogenic cell survival assay determines the ability of a cell to proliferate and this assay shows antiproliferative effect of a drug. Dactolisib inhibits the colony formation of HeLa cells in 10 days, showing a dose dependent inhibition in HeLa cells [19]. In the present study, we have shown that Dactolisib alone or in combination with CA and Dactolisib inhibits the growth of colonies formed by HeLa cells at 14 days. The diameter of colonies significantly decreased in Dactolosib alone and in combination treated groups (Figure 4C). Interestingly, Dactolisib alone treated HeLa cells had slightly less colony number and diameter compared to combination group, but with no significant differences. Thus, these results confirm that CA and Dactolisib exert anti-proliferative effect on HeLa cells.

In conclusion, the PI3K/Akt/mTOR pathway is a key signaling pathway which promotes tumorigenesis of cervical carcinoma. Dactolisib is a potential anti-proliferative agent against cervical carcinoma. CA is a polyphenol that has an anticancer effect against cervical carcinoma. This is the first study in literature representing the anti-proliferative effect of combination treatment of CA and Dactolisib in cervical carcinoma. Dactolisib shows decrease of cell viability in HeLa cells in dose and time dependent manner, while CA did not show any significant differences in cell viability. The colony formation was inhibited significantly in HeLa cells when treated with CA and Dactolisib. Further studies are required to verify Dactolisib and CA to completely understand the anti-proliferative activity and mechanism of action in cervical carcinoma therapy.

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Conflict of interest

The author declares no conflict of interest.

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