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Amlodipine Inhibits Cell Proliferation and Induces Cell Cycle Arrest in Colorectal Cancer Cells

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ABSTRACT

Amlodipine, a dihydropyridine Ca^{2+} channel blocker, was previously shown to exhibit antitumor effects on different human cancer cells both in vitro and in vivo through the inhibition of Ca^{2+} cell entry. However, according to our current knowledge, amlodipine antitumor effect has not been previously examined on colorectal cancer cells (CRC). In this study, the effects of amlodipine on CRC cell proliferation, cell cycle and apoptosis were examined. In two different cell lines, treatment of CRC cells with 50 μM of amlodipine resulted in a significant reduction in cell viability compared to cells treated with dimethyl sulfoxide (DMSO) (with IC50 values of 27.17 μM for HCT116 cells and 37.69 μM for SW480 cells). Flow cytometric analysis using propidium iodide revealed that treatment with amlodipine (50 μM for 48 hours) induced G1 phase cell accumulation in both cell lines compared to DMSO-treated cells. However, treatment with amlodipine (50 μM for 48 hours) did not induce cellular apoptosis in CRC cells. Our findings showed that amlodipine has significant antiproliferative effect on CRC cells, where G1 cell cycle arrest is partially responsible for this growth inhibitory action.

Keywords: Amlodipine, Colorectal cancer, Antiproliferative effect, cell cycle arrest.

1. INTRODUCTION

Colorectal cancer (CRC) is one of the most commonly diagnosed cancers worldwide.[1,2] In Jordan, it is the most common type of cancer among males and the second after breast cancer in females.[3-5] Surgery, radiotherapy and chemotherapy are the modalities of choice for most cases, with certain targeted therapies recommended in the current guidelines.[6-9] However, unfortunately chemotherapeutic resistance is still a considerable challenge that has not improved in the last few decades. Moreover, combination chemotherapies usually add more adverse effects that eventually result in higher burden in overall healthcare costs, thus highlighting the need for new agents that might increase the sensitivity of cancer cells to chemotherapy.[6-9]

Calcium channel blockers (CCBs) are antihypertensive agents that inhibit Ca^{2+} influx via their interaction with the dominant L-type Ca^{2+} channels in cardiac and smooth muscles.[10,11] This group has been used for the treatment of hypertension, angina pectoris and ventricular tachyarrhythmia.[10,11] Interestingly, CCBs have been shown to have pleiotropic effects in both vascular and nonvascular tissues through its interaction with other cellular structures in addition to Ca^{2+} channels.[10,11] One established effect of CCBs is an antitumor effect previously demonstrated on tumor cell lines such as human breast, brain and prostate cancer cells.[12-16] For instance, verapamil, a nondihydropyridine CCB, has been shown to inhibit cell proliferation in prostate cancer and in lymphocytic leukemia.[13,14] Dihydropyridine (DHP) derivatives have also been shown to have antitumor effects in several cancers such as leukemia, epidermoid carcinoma and breast cancer, inhibiting cell proliferation and the cell cycle, inducing apoptosis and increasing the sensitivity of cancer cells to chemotherapy.[15-20] For instance, the DHP

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CCB amlodipine has been shown to inhibit cell growth both in vitro and in vivo, suppress cell invasion and enhance chemotherapeutic activity in breast cancer cells.[16-18] However, there is no study that has examined the effect of a DHP CCB in colorectal cancer. Therefore, we aimed at investigating the possible effects of amlodipine on colorectal cancer cell growth, the cell cycle and apoptosis.

General Experimental

Cell Lines and Tissue Cultures

The colorectal cancer cell lines used were HCT116 and SW480 (University of Newcastle, Australia). Cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS) (Hyclone, Logan, UT), 1% penicillin/streptomycin and 1% glutamine under a humidified atmosphere containing 5% CO_2 at 37°C.

Cell Viability Assay

Cell viability was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT) colorimetric assay.[19] HCT116 or SW480 cells (1×10^4 cells/well) were plated in a 96-well plate in triplicate for each condition. Cells were treated with dimethyl sulfoxide (DMSO) as the control group or with different concentrations of amlodipine (Tocris Bioscience) as the experimental groups for 48 hours. Cell viability was assessed by adding 10 mL of filter-sterilized MTT solution at 0.5 mg/mL to each well. Following 4 hours of incubation, the media was removed, and the blue formazan crystals trapped in the cells were dissolved in DMSO. Absorbance was measured at 540 nm using a microplate spectrophotometer. The results were expressed as a percentage of viable amlodipine-treated cells relative to DMSO-treated cells.

Flow Cytometric Analysis of Deoxyribonucleic Acid (DNA) Content

The propidium iodide (PI) flow cytometric assay was used for the evaluation of apoptosis and cell cycle analysis.[30] HCT116 and SW480 cells (1.5×10^5 cells/well) were plated in 24-well plates and incubated overnight.[30] On the next day, cells were treated with DMSO or 50 μM of amlodipine. After 24 or 48 hours of treatment, cells were harvested. First, media were removed and placed in falcon tubes. Each well was then washed by 1 mL of phosphate-buffered saline (PBS), which was then added to the media in the falcon tubes. The tubes were then centrifuged (at 2000 rpm, at 24°C for 5 minutes), and liquid content was discarded. PI stain (750 μL) was added to each well and incubated for 20 to 30 minutes. After incubation, well contents were mixed thoroughly using micropipettes and placed into the corresponding falcon tubes, which were then sent to the flow cytometry lab for analysis.

Statistical analysis

Statistical significance was detected using the one-way ANOVA. Multiple group comparisons were analyzed with Tukey's multiple comparison tests. Data were analyzed using the SPSS program. Data were expressed as a mean ± standard deviation. A p-value of less than 0.05 was considered statistically significant.

Results

Antiproliferative Effect of Amlodipine on CRC Cells

The biological activity of amlodipine was examined using the MTT assay. As shown in Figure 1, the viability of HCT116 and SW480 cells was significantly decreased by 70% and 60%, respectively, after treatment with 50 μM of amlodipine compared to the control treatment with DMSO. The IC50 values were 27.17 μM for HCT116 cells and 37.69 μM for SW480 cells.

Although the antiproliferative effect of amlodipine was dose-dependent and significant at concentrations of 10, 25 and 50 μM in HCT116 cells, the effect was significant only at a concentration of 50 μM in SW480 cells.

Flow Cytometric Analysis of the Effect of Amlodipine on Apoptosis and the Cell Cycle

Since amlodipine treatment resulted in growth suppression of CRC cells, we further investigated the

underlying molecular mechanism related to this antiproliferative effect by analyzing the cell cycle and apoptosis following treatment with amlodipine. We performed flow cytometry analysis of the cell cycle in DMSO- and amlodipine-treated HCT116 and SW480 cells. Cell cycle kinetics showed that treatment with 50 µM of amlodipine for 48 hours resulted in a significantly increased percentage of 13% and 17% in HCT116 and SW480 cells at the G0/G1 phase, respectively (Figures 2, 3 and 4).

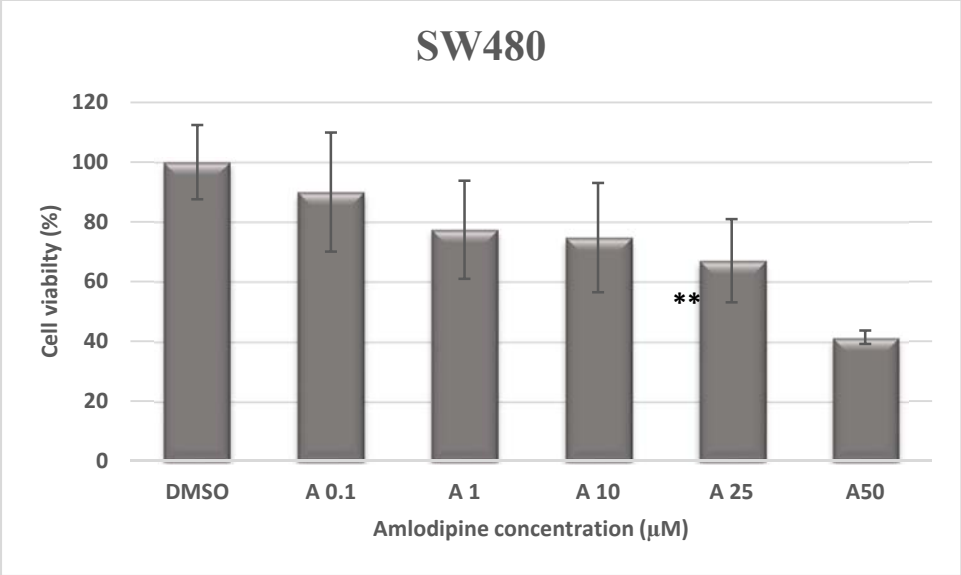

Figure 1: MTT cell proliferation assay to evaluate the antiproliferative effect of amlodipine on (A) HCT116 and (B) SW480 cells. Cells were plated in a 96-well plate, then treated with amlodipine (0-50 µM) for 48 hours, after which an MTT assay was performed. Concentrations of amlodipine that induced significant ($p<0.05$) antiproliferative effects on HCT116 (Figure 1A) were as follows: 10 µM ($p=0.0225$), 25 µM ($p=0.0418$) and 50 µM ($p <0.0001$), with IC50 equal to 27.17 µM. However, for SW480 (Figure 1B), only 50 µM of amlodipine ($p=.0004$) induced a significant reduction in viable cells, with IC50 equal to 37.69 µM.). For SW480, reductions associated with 10 µM ($p=0.1258$) and 25 µM ($p=0.0574$) were insignificant.* indicates a significant difference between the experimental group as compared to the control group (treatment with DMSO) with p value of <0.05. ** indicates p value <0.01

We then explored whether amlodipine-mediated cell cycle arrest could cause HCT116 and SW480 cellular apoptosis. We found that amlodipine did not induce cellular apoptosis at a concentration of 50 µM in either HCT116 or SW480 cells after 24- or 48-hour treatments (Figures 2 and 5).

Discussion

CCBs have an established antitumor effect in several cancer types. In the present study, the antiproliferative effect of amlodipine in CRC cells was demonstrated for the first time. Under our conditions, amlodipine reduced HCT116 and SW480 CRC cell viability at least by 60%, which was associated with cell cycle arrest at the G1 phase. Our findings were consistent with previous studies related to either CCBs in general or specifically amlodipine in other types of human cancer cell lines as described below.

In breast cancer, amlodipine has been found to significantly inhibit cell proliferation of HT-39 human breast cancer cells in vitro as well as in vivo with athymic mice.[21] In addition, amlodipine has been shown to induce a remarkable reduction of viable MDA-MB-231, MCF-7 and MCF-7/ADR breast cancer cells.[15,17,18]. Moreover, amlodipine has been found to inhibit the proliferation of chronic myeloid leukemia (CML) cells and promyelocytic leukemia (PML) cells. Among different CCBs, amlodipine

has been shown to be the most potent inhibitor of the proliferation of human epidermoid carcinoma A431 cells in vitro and in vivo.[19,20]

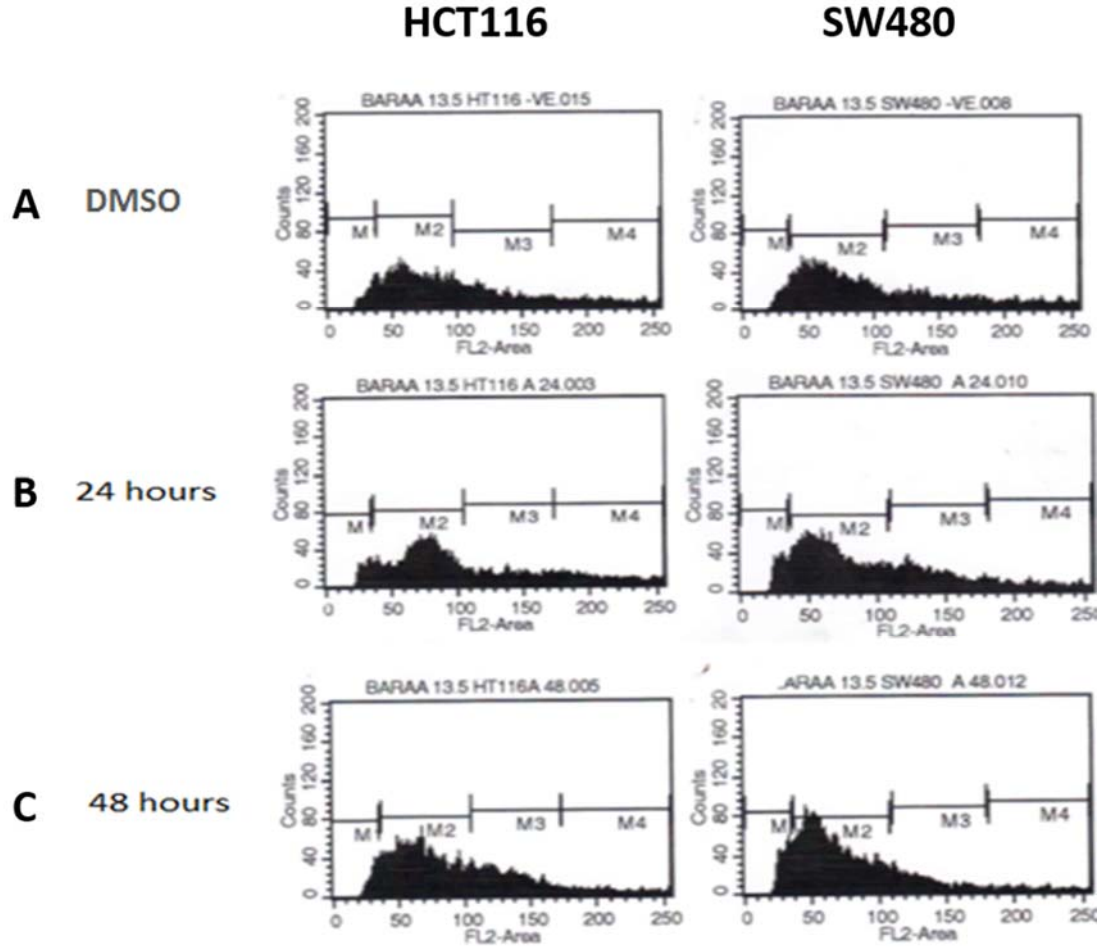

Figure 2: Flow cytometric analysis of cell counts vs. DNA content of HCT116 and SW480 cells after 24 hours and 48 hours of incubation with 50 µM of amlodipine (experimental group) or DMSO (control group). Here, M1 denotes apoptotic cells, M2 the G0/G1 phase, M3 the S phase and M4 the G2 phase.

In this study, flow cytometric analysis of DNA content in amlodipine-treated cells revealed that the antiproliferative effect of amlodipine was not due to the induction of apoptosis. Conversely, amlodipine induced G1 cell cycle arrest in HTC116 and SW480 CRC cell lines after 48 hours of treatment. This cytostatic effect of amlodipine has also been observed in epidermoid carcinoma A431 cells.[22]

Intracellular Ca^{+2} concentrations are controlled by entry through the plasma membrane and Ca^{+2} release from sarco/endoplasmic reticulum stores. Typical amlodipine action is exerted by the inhibition of Ca^{+2} entry through L-type calcium channels. This type of channel is found to be expressed in CRC cells, and their numbers increase with disease progression.[23] Since amlodipine blunts Ca^{+2} release from intracellular stores,[19] amlodipine treatment

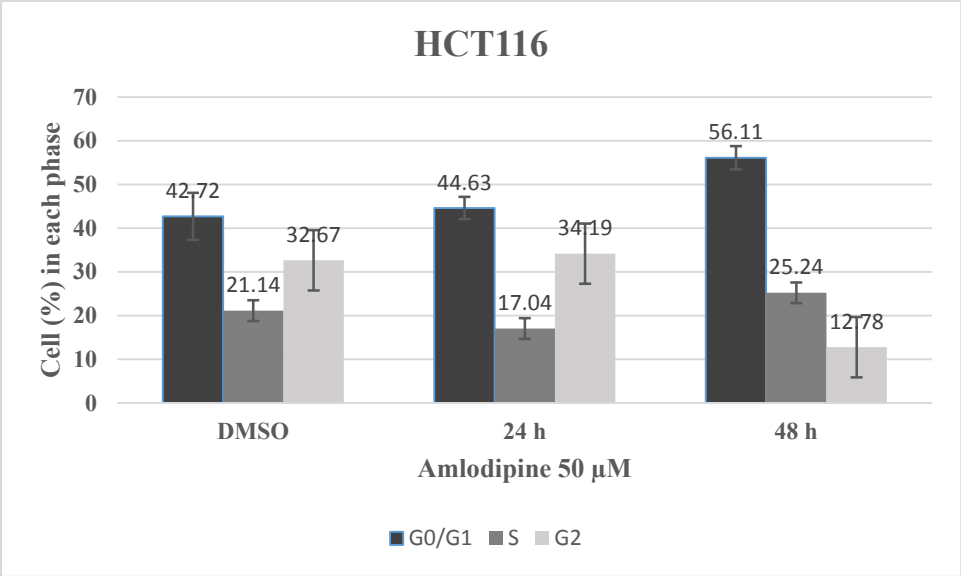

Figure 3: Effects of amlodipine on the cell cycle of HCT116 cells. Cells were cultured in a 24-well plate and treated with 50 µM of amlodipine. After the indicated time of incubation, cells were collected, and PI flow cytometry was used to analyze the cell cycle distribution. Amlodipine did not cause cell cycle arrest at any phase after 24 hours. However, after 48 hours of amlodipine treatment, the number of cells arrested in the G1 phase was 13% higher than that in the control group (DMSO).

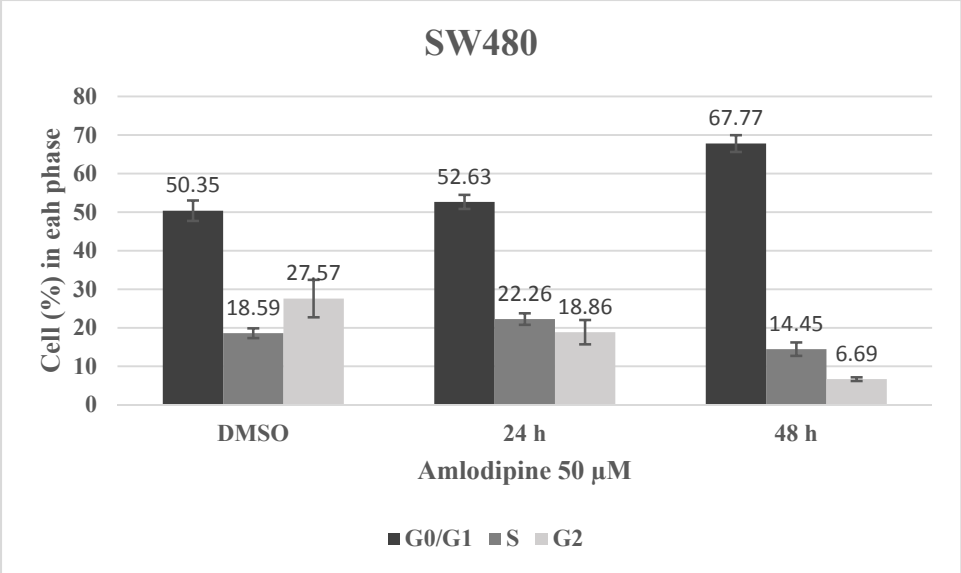

Figure 4: Effects of amlodipine on the cell cycle of SW480 cells. Cells were cultured in a 24-well plate and treated with 50 µM of amlodipine. After the indicated time of incubation, cells were collected, and PI flow cytometry was used to analyze the cell cycle distribution. Amlodipine did not cause cell cycle arrest at any phase after 24 hours of treatment ($p \geq 0.05$). However, after 48 hours of amlodipine treatment, the number of cells arrested in the G1 phase was 17% higher than that in the control group (DMSO)

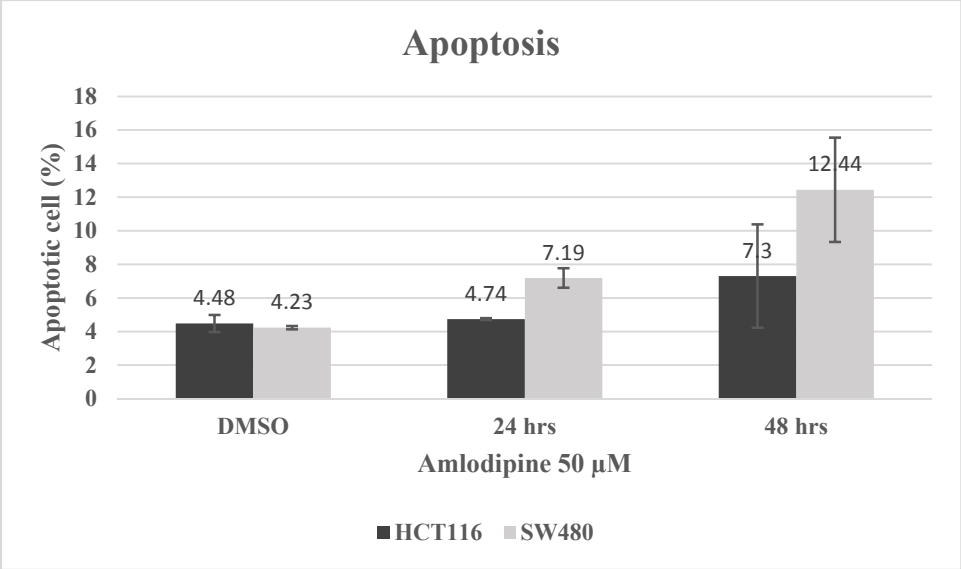

Figure 5: Flow cytometric analysis of apoptotic cells after treatment with amlodipine. This figure depics apoptotic cell percentages of HCT116 and SW480 cells in the presence of 50 µM of amlodipine after incubation for 24 hours and 48 hours, with the DMSO group being incubated for 48 hours. The percentages of apoptotic cells were measured using flow cytometry with PI staining. Amlodipine induced an insignificantly increased percentage of apoptotic cells as compared to cells in the DMSO group ($p \geq 0.05$).

decreases intracellular Ca^{+2} concentrations. Specific Ca^{+2} concentrations in both the extracellular and intracellular environments are critical for progression through the cell cycle, particularly the G1 phase. Since specific Ca^{+2} levels are required for the expression of G1 cyclin genes,[23-26] depletion of endoplasmic reticulum Ca^{+2} stores may lead to cell cycle arrest. However, cell cycle analysis of HTC116 and SW480 cells cannot be used to explain entirely the significant antiproliferative effects of amlodipine. This indicates that alternative signaling pathways leading to cell death might be involved. Therefore, the exact molecular mechanisms that contribute to amlodipine cytotoxicity in CRC require further investigation.

In conclusion, our study elucidates the ability of amlodipine to suppress the growth of aggressive CRC cells. This modulatory effect of amlodipine may be due to inhibition of calcium channels as well as suppression of intracellular calcium release which may be involved in the pathogenesis of CRC. In addition, our findings of cell cycle arrest of CRC cells may partially explain the antiproliferative effect of amlodipine.

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تقييم الدور المثبط لنمو الخلايا لعقار الاملوديبين على خلايا سرطان القولون والمستقيم

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ملخص

يعد عقار أملوديبين من حاصرات قنوات الكالسيوم الدايهيدروبيريديه، وقد تبين أنّ له تأثيراً على مختلف الخلايا السرطانية البشرية على حد سواء في المختبر وفي الجسم الحي، من خلال تثبيط دخول الخلايا الكالسيوم ومع ذلك، لم يتم فحص تأثير مضاد الأورام أملوديبين من قبل على خلايا سرطان القولون والمستقيم. في هذه الدراسة، تم فحص تأثير أملوديبين على تكاثر الخلايا، ودورة الخلية وموت الخلايا المبرمج على خلايا سرطان القولون والمستقيم.

في اثنين من انواع الخلايا المختلفة، أدى العلاج ب 50 ميكرومولار أملوديبين الى انخفاض كبير في نمو الخلية بالمقارنة مع الخلايا غير المعالجة (كانت قيم IC50 27.17 ميكرومولار للخلايا HCT116 و37.69 ميكرومولار لخلايا SW480). كشف تحليل التدفق الخلوي باستخدام يوديد بروبيديوم أن العلاج مع أملوديبين (50 ميكرومولار، لمدة 48 ساعة) يسبب تراكم الخلايا المرحلة G1 مقارنة مع الخلايا غيرالمعالجة في كلا النوعين من الخلايا. ومع ذلك، فإن العلاج مع أملوديبين (50 ميكرومولار لمدة 48 ساعة) لم يحفز موت الخلايا المبرمج الخلوي في خلايا سرطان القولون والمستقيم. وأظهرت نتائج البحث أن أملوديبين له تأثير كبير على نمو خلايا سرطان القولون والمستقيم، لأنّ تثبيط دورة الخلية في G1 هو المسؤول جزئيا عن هذا العمل المثبط للنمو.

الكلمات الدالة: عقار الأملوديبين، تقييم دور المثبط، خلايا سرطان القولون والمستقيم.

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