RESEARCH ARTICLE

Synergistic Effect of L-cycloserine and Capecitabine on Human Colon Cancer Cell Line

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Received: 30th May, 2023; Revised: 16th July, 2023; Accepted: 14th August, 2023; Available Online: 25th September, 2023

ABSTRACT

Introduction: Colon cancer comes in second place in the list of cancers in developed countries. Drug resistance is one of the causes of colorectal cancer therapeutic failure that usually occurs in most patients in advanced stage of colon cancer. This study investigates the effect of adding L-cycloserine to capecitabine in the HCT-116 colon cancer cell line.

Materials and methods: We compared the combined L-cycloserine-capecitabine effect with each of the two drugs alone on the HCT-116 colon cancer cell line as a positive control group. The growth rate inhibition was examined by crystal violet assay.

Results: The growth inhibition effect of L-cycloserine-capecitabine, L-cycloserine and capecitabine on HCT-116 cells was assessed with crystal violet assay, different concentrations including 3.12, 6.25, 12.5, 25, 50, and 100 µg/mL were used for L-cycloserine-capecitabine combination, and for both L-cycloserine and capecitabine. After incubation for 24 hours, results appeared that the combination of L-cycloserine and capecitabine increased the effect of both drugs alone as seen by a significant increase ($p < 0.05$) of the growth inhibition percentages in the L-cycloserine-capecitabine combination as compared with the positive control groups.

Conclusion: From the result, we can conclude that the L-cycloserine combination with capecitabine has a synergistic effect for colon cancer treatment that could be a more effective regimen.

Keywords: L-cycloserine, Capecitabine, Colon Cancer**.**

International Journal of Drug Delivery Technology (2023); DOI: 10.25258/ijddt.13.3.34

How to cite this article: Sahib ZH, Almedeny SAR. Synergistic Effect of L-cycloserine and Capecitabine on Human Colon Cancer Cell Line. International Journal of Drug Delivery Technology. 2023;13(3):982-985.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Globally, colorectal cancer (CRC) is considered as part of the main reasons in deaths from cancer.[1] Until now, surgery and chemotherapy remain the main regimens in treating CRC. Although the survival rate has increased with chemotherapy, resistance occurs in most CRC patients, ultimately resulting in chemotherapy failure.[2]

Anticancer capecitabine is used in CRC. It is converted into 5-FU by many metabolic enzymes. It is one of antimetabolite chemotherapy that act by DNA damage. Simultaneously, capecitabine carries many side effects affecting bone marrow, liver, blood, and hair.[3] A new way is required in order to challenge CRC.[4] Drug combination therapies have become highly predominant to get rid of resistance and side effects of chemotherapy.[5] Capecitabine as an autophagy inhibitor was enrolled in a phase 2 clinical trial using the combination of hydroquinone (an antibiotic) in one of five current clinical trials on pancreatic cancer. The study results are extremely promising.[6]

Currently, antibiotics have been documented in cancer treatment. They can be used along with conventional cancer therapy, such as surgery, radiotherapy, targeted therapy and chemotherapy.[7] Antibiotics also enhance the body's immune function, enhance the effectiveness of treatment, and successfully prevent the metastasis and recurrence of cancer.[8] For example, ciprofloxacin can induce apoptosis and has an anti-proliferative effect by cell cycle process regulation.[9]

L-cycloserine (Seromycin) is an antibiotic and an inhibitor of GABA transaminase enzyme, used in the treatment of TB along with other anti-TB therapy especially for the active resistant type.[9] It is taken orally and the common adverse effects are allergic problems, drowsiness, convulsion and numbness. It is contraindicated in patients having epilepsy, depression and renal failure.[10]

In this study, the combination effect of capecitabine and L-cycloserine on HCT-116 colon cancer cells was assessed.

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MATERIALS AND METHODS

This study was carried in the College of Medicine, University of Babylon Postgraduate Cancer Research Lab, from December 2020 to May 2021.

Cell Lines

HCT-116 human colon cancer cell line used in the study was a Cancer Research Lab gift. It was maintained and cultivated in RPMI 1640 media with 100 U/mL penicillin, 100 mg/mL streptomycin, and 10% FBS. A monolayer culture was gained in a 5% CO_2 and 37℃ incubator.[12]

Drugs Stocks Preparation

Two-fold serial dilutions for L-cycloserine (Sigma Aldrich/ USA) and capecitabine (Sigma Aldrich, USA) was made at 100, 50, 25, 12.5, 6.25, and 3.12 µg/mL concentrations of each. The combination L-cycloserine-capecitabine was prepared from equal volumes of each drug concentration mixed together to get a combination carry a half dose of each drug.

Crystal Violet Assay

By using crystal violet assay, we assess the *in-vitro* cytotoxic effect of the L-cycloserine-capecitabine combination on HCT-116 cells.[13]

Cellular Handling

When 70 to 80% confluence growth of the HCT-116 cells was reached, trypsinization and counting of the cell were done, then returned in RPMI 1640 culture media in sterile 96 wells plates. In 200 µL of cell suspension was put in each well and incubated at 5% CO_2 incubator and 37℃ for 24 hours.

Cellular Drugs Exposure

After 24 hours of incubation, the RPMI media was expelled from the wells, then the sterile RPMI (serum-free medium) supplied with 100, 50, 25, 12.5, 6.25, and 3.12 µg/mL concentrations in three wells replicated of each concentration for all tested groups (L-cycloserine-Capecitabine combination, capecitabine group and L-cycloserine group). The RPMI-1640 medium was only used in the control group. Then, incubate for 24 hours with different treatments.

Staining with Crystal Violet Dye

With PBS buffer wells were washed 3 times, then 50 µL, 0.5% crystal violet stain solution was added to each well, then left for 20 minutes in 25℃. The stain was expelled from wells, tap water was added and poured gently, then the plate was left to be dried. Then, methanol as a fixative agent (200 µL) was poured to each well and the plates were gently rocked at room temperature. A reading of the absorbance for each well was done with an ELISA reader and from that, the percentages of growth inhibition in cancer cells were calculated.[14,15]

Statistical Analysis

By using SPSS version 23, statistical analysis was done. To compare the mean of the different groups, the analysis of variance ANOVA test was used. Results were expressed as mean ± SD with a 95% confidence interval, $p < 0.05$ was considered statistically significant.[16] The IC_{50} values were calculated from the dose-response curve for each group by using the excel sheet.[17]

RESULTS

L-cycloserine-Capecitabine Combination Effect on the HCT-116 Cells Growth

The growth inhibition percentages showed a dose-dependent manner in all treated groups. The L-cycloserine-capecitabine combination, capecitabine and L-cycloserine in all concentrations, produce significant ($p < 0.01$) growth inhibition in colon cancer cells in compare to the control group (except the concentrations of 3.12 µg/mL $p > 0.05$). Compared to capecitabine and L-cycloserine groups, the L-cycloserine-capecitabine combination in all tested concentrations shows highly significant ($p < 0.01$) growth percentage inhibition. The IC_{50} of L-cycloserine- Capecitabine combination was 22.3 µg/mL, of L-cycloserine was 24 µg/mL and of capecitabine was 24.7 µg/mL as presented in Table 1 and Figure 1.

DISCUSSION

Many antibiotics have anti-proliferative, pro-apoptotic, and anti-epithelial-mesenchymal-transition (EMT) effects. Therefore, antibiotics can help in the management of cancer.[17] They can inhibit the metastasis of cancer cells because of their EMT regulatory effects like salinomycin.[17] Antibiotics in general and to lesser extent some bactericidal can cause energy supply depletion and mitochondrial dysfunction in cancer cells by mitochondrial metabolism targeting effect.[18]

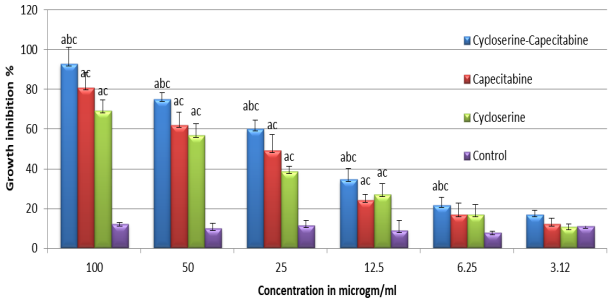
Current study results of L-cycloserine inhibition effect on colon cancer cell growth is agreed with Beuster *et al*. (2011), who investigated L-cycloserine and chloro-L-alanine two competitive inhibitors of L-alanine aminotransferase (ALAT).[20] ALAT inhibition resulted in reduced Lewis lung carcinoma cells LLC1 cells growth rates, suggesting that it can efficiently impair cancer growth by increasing mitochondrial oxidative metabolism and counteracting the Warburg effect it states that respiratory capacity impairment resulted in an inappropriate increase in glycolysis may be the cause of malignant growth.[19,20] That was beneficially exploited later on to increase the efficacy of the chemotherapeutic agents.[21] ALAT inhibition and D-glucose uptake impairment of LLC1 resulted in an initial energy deficit followed by an AMP-activated protein kinase activation, increased respiration rates, and mitochondrial production of reactive oxygen species. In addition to the phosphorylation alteration of p38, ERK (extracellular signal-regulated kinase 1/2), MAPK (mitogen-activated protein kinase 14), and Rb1 (retinoblastoma 1) proteins, on the other hand, a decreased expression of Cdk4 (cyclin-dependent kinase 4) and Cdc25a (cell division cycle 25 homolog A).[19] All these changes can result in L-cycloserine anti-proliferative effects.[20]

Also, the L-cycloserine inhibition effect is agreed with a study established by Cinatl *et al*. (1999), which showed that L-cycloserine had an anti-proliferative effect on human neuroblastoma and medulloblastoma cells and explained this by the suppression of ganglioside expression.[22]

Table 1: The growth inhibition percentage (Mean ± SD) of HCT-116 colon cancer cells with the calculated IC_{50} for different tested groups

Conc. in µg/mL	*The growth inhibition percentages in groups*			
	cycloserine-Capecitabine combination	*Cycloserine*	*Capecitabine*	*Control*
3.12	17.77 ± 2.4	11.51 ± 1.5	13.08 ± 3	11.05 ± 0.6
6.25	22.47 ± 4.2 [abc]	17.82 ± 5.2	17.67 ± 5.8	7.72 ± 1
12.5	35.49 ± 5.5 [abc]	27.89 ± 5.7 [ac]	25.16 ± 3 [ac]	9.03 ± 3.5
25	61.04 ± 4.6 [abc]	39.62 ± 2.6 [ac]	48.12 ± 8 [ac]	11.43 ± 1.5
50	76.14 ± 3 [abc]	57.72 ± 6 [ac]	62.94 ± 6.7 [ac]	9.93 ± 1.3
100	93.8 ± 8.3[abc]	68.33 ± 5.5 [ac]	82.75 ± 7.6 [ac]	12 ± 1
IC_{50} in µg / mL	22.3	24	24.7	

p-value <0.01 mean significant inhibition percentages from the control group. [b] mean significant inhibition percentages from treated groups. [c] mean significant inhibition percentages from other tested concentrations within the same group.



[a]Mean significant inhibition percentages for $p < 0.01$ from the control group. [b]mean significant inhibition percentages from treated groups. [c]mean significant inhibition percentages from other tested concentrations within same group.

Figure 1: The growth inhibition percentage (Mean ± SD) of HCT-116 colon cancer cells for different tested groups

According to our information, current study is the first one to examine the interaction effect between L-cycloserine and capecitabine when used in colon cancer cell line.

This study's combination of L-cycloserine and capecitabine indicates their synergistic effect. This may be due to the previous in vitro finding reported by Esner *et al.* (2017) who suggested a synergistic effect between antibiotics that can induce mitochondrial dysfunction and autophagy inhibitors resulting in targeting the cancer cell proliferation ability.[18]

So as the L-cycloserine can induce mitochondrial dysfunction and deplete the energy supply to cancer cells,[20] and capecitabine, considered one of autophagy inhibitor,[23,24] the synergistic interaction effect was recognized in this study.

CONCLUSION

The study showed that the combination of L-cycloserine with capecitabine has a synergistic effect for the colon cancer treatment that could be more effective regimen to be assessed in further clinical trial.

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