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IJBLS 2023; 2(2):189-195

International Journal of BioLife Sciences

Original paper

Enhanced Synergistic Effects of AgCl Nanoparticles and 5FU on in vitro Colon Cancer Cells

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Received: *25 September 2023* ***Revised***: *2 October 2023* ***Accepted***: *9 October 2023*

Abstract

Background and aim: Numerous studies have established the cytotoxic potential of nanoparticles and chemotherapy drugs against cancer cells. However, the specific impact of co-administering silver chloride (AgCl) nanoparticles and 5-fluorouracil (5FU) on colon cancer cells remains less elucidated. This study aims to investigate the individual cytotoxic effects of silver chloride nanoparticles and 5FU on colon cancer cells, as well as to explore the synergistic cytotoxic effects resulting from their combined treatment.

Materials and methods: In this experimental research, colon cancer cells were categorized into control groups (no treatment), those treated with AgCl nanoparticles, those treated with 5FU, and those subjected to the combined treatment of AgCl nanoparticles and 5FU. Cell viability was assessed at 24 hours post-treatment using the MTT assay. The data were subjected to statistical analysis using one-way analysis of variance.

Results: The viability of colon cancer cells significantly decreased following 24 hours of AgCl, 5FU, and combined AgCl nanoparticles and 5FU treatment. The IC50 value in the AgCl-treated group was lower compared to the other groups, and the IC50 value for the group treated with AgCl + 5FU was lower than that for the group treated with 5FU alone.

Conclusion: The outcomes of this investigation underscore the potent cytotoxic synergy of co-administered silver chloride nanoparticles and 5FU against colon cancer cells compared to 5FU.

Keywords: *Silver chloride nanoparticles, 5FU, Colon cancer*

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Introduction

Silver chloride nanoparticles (AgCl nanoparticles) are nanoscale particles composed of silver and chlorine, possessing unique properties attributable to their small size and high surface area-to-volume ratio. These nanoparticles find applications in materials science and medicine due to their distinct characteristics. AgCl nanoparticles may exhibit antimicrobial properties, catalytic activity, and optical features, depending on factors such as size, shape, and synthesis method. Additionally, they have been investigated for their potential anticancer effects, where their properties may be leveraged for targeted therapies or imaging applications in the field of oncology. The controlled manipulation of AgCl nanoparticles allows for the customization of their properties, rendering them valuable for a wide range of technological and scientific endeavors [1], [2], [3], [4].

5-Fluorouracil, abbreviated as 5-FU, is a chemotherapy drug categorized as an antimetabolite. It is a pyrimidine analog that disrupts nucleic acid synthesis, particularly RNA and DNA. Widely employed in treating cancers like colorectal, breast, and gastrointestinal cancers, 5-FU inhibits the enzyme thymidylate synthase, crucial for thymidine synthesis in DNA. This interference hinders cancer cell replication, leading to cell death. The synergistic effects of nanoparticles, such as silver nanoparticles, and the chemotherapy drug 5-Fluorouracil (5FU) in anticancer applications involve a complementary enhancement of therapeutic efficacy. Nanoparticles can serve as drug carriers, delivering 5FU directly to cancer cells with precision. The small size and high surface area of nanoparticles facilitate cellular uptake and distribution of the drug. Additionally, nanoparticles may exhibit inherent cytotoxic effects, contributing to the overall anticancer activity [5], [6], [7], [8], [9].

In the case of 5FU, it is a pyrimidine analog that interferes with DNA and RNA synthesis, disrupting cancer cell replication. When combined with nanoparticles, the drug's delivery to tumor sites is improved, ensuring a more targeted and efficient approach. Furthermore, the inherent properties of certain nanoparticles, such as their ability to induce reactive oxygen species or interact with cellular membranes, can potentiate the cytotoxic effects of 5FU [10].

The synergistic combination enhances the overall anticancer effects while potentially reducing the dosage of 5FU needed, minimizing adverse side effects. Moreover, the combination may overcome resistance mechanisms that cancer cells can develop against individual therapies. This synergistic approach reflects the growing interest in combining nanotechnology with traditional chemotherapy for more effective and targeted cancer treatment strategies.

The study aims to bridge a gap in current knowledge by investigating the combined impact of AgCl NPs and 5FU on colon cancer cells in vitro. Understanding the synergistic effects of these two components is crucial for optimizing treatment strategies. The gap in the current research landscape centers on the need for comprehensive insights into the interaction between AgCl NPs and 5FU specifically in the context of colon cancer cells. This research is essential for advancing our understanding of novel therapeutic approaches and addressing the need for more targeted and efficient treatments for colon cancer.

Material and Methods

Human colon cancer cell lines (HT-29) were obtained from Pasteur Institute (Tehran, Iran) and cultured in appropriate media supplemented with fetal bovine serum and antibiotics. AgCl nanoparticles were produced using an environmentally friendly green synthesis method, ensuring precise control over both size and dispersion. 5FU was dissolved in sterile dimethyl sulfoxide (DMSO) to prepare stock solutions of varying concentrations. Experimental groups, including control, AgCl nanoparticles alone, 5FU alone, and combinations of AgCl nanoparticles and 5FU

at effective ratio, were designed. The viability of colon cancer cells was assessed using MTT assays after treatment with AgCl nanoparticles and 5FU, with absorbance or fluorescence measured using a microplate reader. The data underwent analysis for group comparison through one-way analysis of variance between subjects (ANOVA), followed by the Tukey post hoc test. Significance for differences between groups and hours was determined at $p < 0.05$.

Results

The results of the MTT test indicated that in the SW480 cell line, no significant differences were observed in the viability between the 1.5625 and 3.125 µg/ml AgCl groups when compared to the control group ($P > 0.05$). However, a significant decrease in viability was noted in the SW480 cell line compared to the control group at concentrations of 6.25, 12.5, 25, and 50 µg/ml of AgCl ($p < 0.001$) (Figure 1). The determination of the IC50, representing the half-maximal inhibitory concentration, was conducted, and the calculated value for AgCl was found to be 16.2 µg/ml.

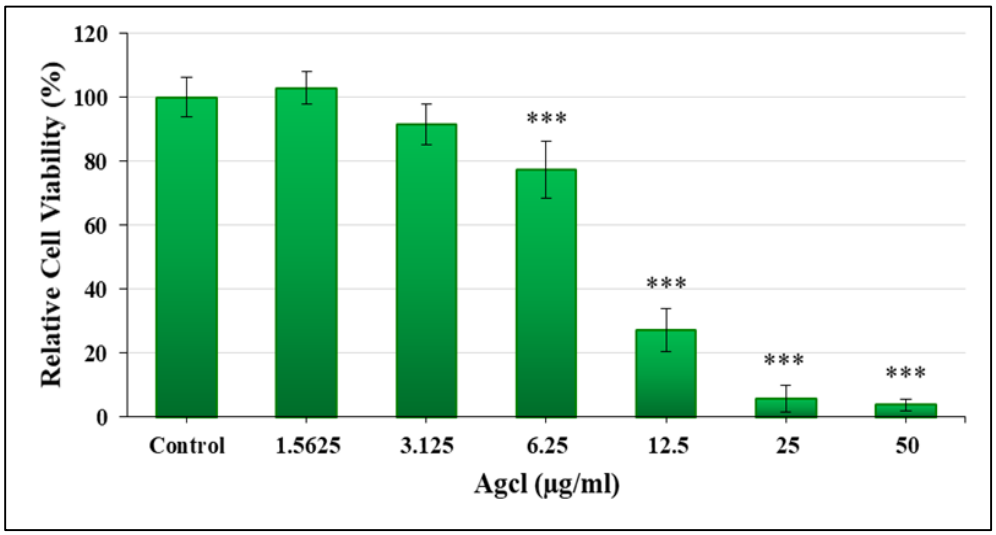

Figure 1. Viability of SW480 cells exposed to varying doses of AgCl nanoparticle (µg/ml). * indicates significant difference compared with control group (***: p<0.001).

The results from the MTT test revealed that in the SW480 cell line, there were no significant differences in viability between the 3.90625 and 7.8125 µg/ml of 5FU groups when compared to the control group ($P > 0.05$). However, a significant reduction in viability was observed in the SW480 cell line compared to the control group at concentrations of 15.625, 31.25, 62.5, 125, 250, and 500 µg/ml of 5FU ($P < 0.001$) (Figure 2). The IC50 value for 5FU was found to be 100.35 µg/ml.

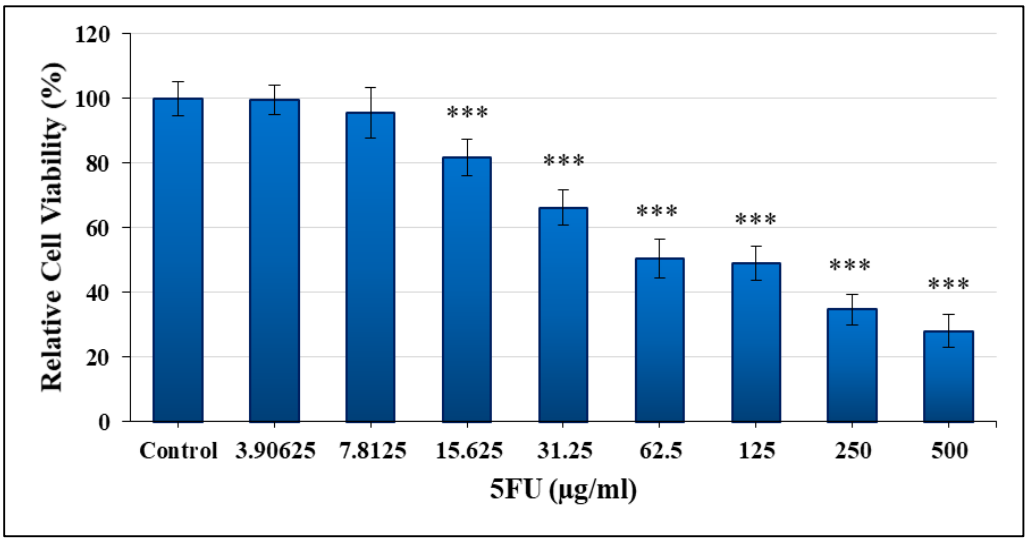

Figure 2. Viability of SW480 cells exposed to varying doses of 5FU (µg/ml). * indicates significant difference compared with control group (***: p<0.001).

The MTT test results demonstrated that in the SW480 cell line, there were no significant differences in viability between the 3.90625 and 7.8125 µg/ml of 5FU+AgCl groups when compared to the control group (P > 0.05). However, a significant reduction in viability was observed in the SW480 cell line compared to the control group at concentrations of 15.625, 31.25, 62.5, 125, 250, and 500 µg/ml of 5FU+AgCl (P < 0.001) (Figure 3). The calculation of IC50 revealed that the IC50 for 5FU and AgCl was 62.2 µg/ml.

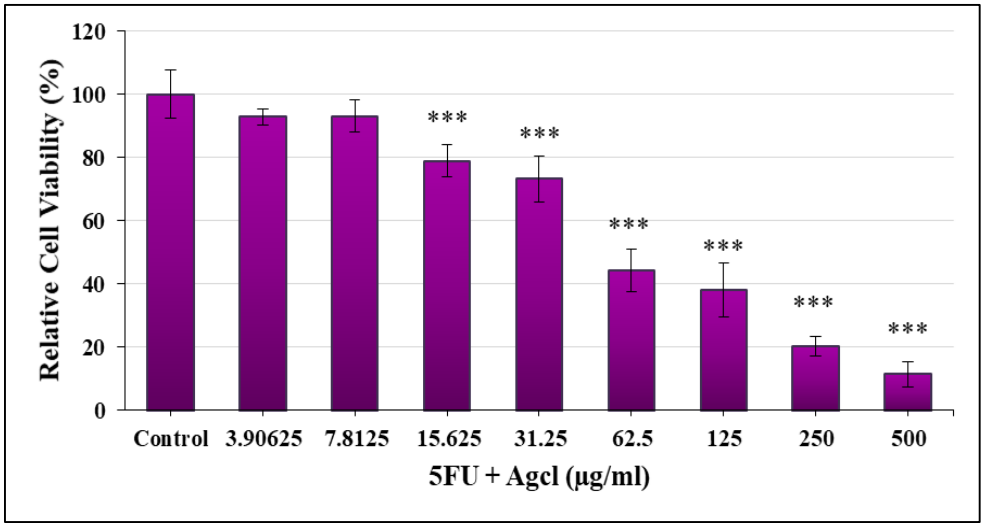

Figure 3. Viability of SW480 cells exposed to varying doses of 5FU in combination with AgCl nanoparticle (µg/ml). * indicates significant difference compared with control group (***: p<0.001).

Discussion

Our findings indicated that the IC50 value of AgCl was lower than that of 5FU and the combination of 5FU and AgCl. However, it was noteworthy that the IC50 value for the combination of 5FU and AgCl was lower compared to 5FU alone, implying a significant synergistic anticancer effect between 5FU and AgCl nanoparticles against colon cancer cells when contrasted with 5FU alone.

This synergy was not observed when comparing the combination with AgCl alone.

Previous studies on silver nanoparticles have established their cytotoxic potential against various cancer cell types, owing to their ability to interact with cellular components, including DNA, proteins, and cell membranes. In the context of colon cancer, the current research aligns with a growing body of evidence suggesting that silver nanoparticles, and specifically AgCl nanoparticles, can exert potent anticancer effects [11], [12], [13].

The unique mechanisms by which AgCl nanoparticles induce cytotoxicity, such as the generation of reactive oxygen species (ROS) and interference with cellular signaling pathways, underscore their potential as a targeted therapeutic approach. Importantly, the selectivity of AgCl nanoparticles towards cancer cells, while sparing normal cells, is a critical aspect that enhances their therapeutic profile [14], [15], [16], [17].

The exploration of the synergistic effects of AgCl nanoparticles and 5FU against colon cancer cells builds upon a foundation of background studies that have individually investigated the anticancer potential of both entities [7], [18].

Background studies on AgCl nanoparticles have highlighted their intrinsic cytotoxicity against various cancer cell types. The small size and unique physicochemical properties of AgCl nanoparticles enable them to penetrate cell membranes and induce oxidative stress, disrupting essential cellular processes. Research has demonstrated that AgCl nanoparticles can generate reactive oxygen species and interfere with signaling pathways, contributing to their cytotoxic effects on cancer cells while sparing normal cells [14], [15]. On the other hand, 5FU, a widely used chemotherapeutic agent, operates by inhibiting nucleic acid synthesis, particularly interfering with DNA replication and repair processes in rapidly dividing cells. Numerous studies have underscored the efficacy of 5FU in suppressing cancer cell proliferation, making it a key component in colon cancer treatment regimens [10]. The rationale behind investigating the synergistic effects of AgCl nanoparticles and 5FU stems from the potential complementarity of their mechanisms of action. AgCl nanoparticles may enhance the cellular uptake of 5FU or potentiate its cytotoxic effects through additional pathways, leading to a more robust anticancer response. Previous research on combination therapies involving nanoparticles and chemotherapeutic agents has demonstrated enhanced efficacy compared to individual treatments.

Conclusion

While these background studies provide a strong basis for exploring the synergistic effects, the specific molecular interactions and pathways involved in the combined action of AgCl nanoparticles and 5FU against colon cancer cells remain an area of active investigation. Understanding these interactions will be crucial for optimizing the therapeutic potential of the combination and advancing its translational application in colon cancer treatment. The emerging trend of combining nanoparticles with traditional chemotherapeutic agents represents a promising strategy in the ongoing pursuit of more effective and targeted cancer therapies.

Acknowledgment

The authors express their gratitude to the staff of Javid Biotechnology for their assistance and support.

Conflict of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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