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Original paper

Exploring the Impact of 5FU and Environmentally-Synthesized Silver Chloride Nanoparticles on Gastric Cancer Cells

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Abstract

Background and aim: Extensive research has highlighted the potential cytotoxic effects of nanoparticles and chemotherapy agents on cancer cells. However, the synergistic effects arising from the simultaneous utilization of silver chloride nanoparticles and 5FU in the treatment of gastric cancer cells remain insufficiently understood. In consideration of this knowledge gap, the current study endeavors to elucidate the cytotoxic outcomes of silver chloride nanoparticles and 5FU on gastric cancer cells, while also delving into the combined impact of these therapeutic interventions.

Materials and methods: In this experimental study, gastric cancer cells were categorized into distinct groups: control (untreated), treated with silver chloride nanoparticles alone, treated with 5FU alone, and subjected to the combined treatment of silver chloride nanoparticles and 5FU. Cell viability was assessed using the MTT method at 24 and 48-hour intervals post-treatment. The gathered data were subjected to analysis via one-way analysis of variance.

Results: The study reveals enhanced cytotoxic effects with the combined treatment of silver chloride nanoparticles and 5FU on AGS cancer cells compared to 5FU alone. Lower concentrations of 5FU exhibit limited impact on cell viability, whereas the addition of silver chloride nanoparticles amplifies the reduction in viability, indicating a potential synergy.

Conclusion: The findings of this investigation emphasize that the concurrent application of silver chloride nanoparticles exerts heightened cytotoxic effects on gastric cancer cells in comparison to the use of 5FU alone.

Keywords: *Silver chloride nanoparticle, 5FU, Gastric cancer*

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Introduction

Gastric cancer, also known as stomach cancer, is a formidable global health challenge characterized by the malignant growth of cells within the stomach lining. Despite significant advancements in medical science, it continues to be associated with high mortality rates. This malignancy poses a substantial global healthcare burden. Gastric cancer is a pervasive global health concern. It ranks as the fifth most common cancer worldwide and stands as the third leading cause of cancer-related death globally. The complications arising from gastric cancer are multifaceted and may include metastasis to nearby lymph nodes and distant organs, gastrointestinal bleeding, obstructions within the digestive tract, and cachexia. Treatment for gastric cancer typically involves a multidisciplinary approach tailored to the disease's stage and individual patient factors. Common modalities include surgery, chemotherapy, radiation therapy, targeted therapy, and immunotherapy. In 2022, gastric cancer garnered significant attention as it was incorporated into the updated EU recommendations for targeted cancer screening. This development underscores the growing recognition of gastric cancer's global burden and the need for improved screening and early detection strategies [1], [2], [3].

Gastric cancer, characterized by its rapid progression and limited treatment options, necessitates a multifaceted therapeutic approach. 5FU, a mainstay in cancer chemotherapy, operates as an antimetabolite by disrupting DNA synthesis and repair processes. However, its clinical utility is hampered by systemic toxicity and the development of drug resistance [4], [5], [6].

Conversely, silver chloride nanoparticles (AgCl NPs), synthesized through environmentally friendly methods, have emerged as intriguing nanomaterials due to their unique physicochemical properties. These nanoparticles inherently possess antimicrobial attributes and offer controlled release capabilities, making them promising candidates for drug delivery systems. AgCl NPs have garnered substantial interest in recent years for their potential as novel anticancer agents. These nanoparticles, with their unique physicochemical properties, hold promise in the field of oncology due to their ability to inhibit cancer cell growth and induce apoptosis while sparing healthy cells. The exploration of AgCl NPs as a cutting-edge approach to cancer treatment represents an exciting frontier in the ongoing quest to develop more effective and targeted therapies for malignancies. This short introduction provides a glimpse into the burgeoning research and applications of AgCl NPs in the realm of cancer therapy [7], [8].

By harnessing the cytotoxic potential of 5FU in conjunction with the versatility of AgCl NPs, a novel and synergistic approach to gastric cancer therapy is being explored. Finding innovative and targeted approaches to combat this aggressive malignancy is of paramount importance. In this pursuit, the combination of 5FU, a conventional chemotherapeutic agent, with environmentally-synthesized AgCl NPs has emerged as a potential therapeutic strategy worth exploring. This research endeavor focuses on investigating the synergistic effects of 5FU and AgCl NPs on gastric cancer cells.

Material and Methods

- *Cell Culture and Treatment*

Gastric cancer cells were procured from the cellular and molecular bank of the Pasteur Institute of Iran. Cells were cultured and maintained in sterile and standard conditions within nitrogen tanks. The gastric cancer cell line was categorized into three groups: a control group (no exposure to drugs or silver chloride nanoparticles), a group treated with varying concentrations of 5FU, and a group treated with distinct concentrations of silver chloride nanoparticles in combination with 5FU.

- *Synthesis of Silver Chloride Nanoparticles*

Silver chloride nanoparticles were synthesized using a green method involving *Onopordum acanthium* plant extract. The procedure encompassed macerating plant materials, followed by filtration and concentration of the extract. A solution of silver nitrate was combined with the plant extract, resulting in the reduction of silver ions and the formation of silver nanoparticles, as indicated by a change in solution color. The synthesized silver chloride nanoparticles were integrated into the experimental setup for combined treatment with 5FU.

- *Preparation of 5FU*

5FU was dissolved in PBS solvent and sterilized using a syringe filter. The desired concentration of 5FU was then prepared for use in the experimental treatments.

- *MTT Assay for Cytotoxicity Assessment*

To assess the cytotoxic effects of the treatments, the MTT assay was employed. Multiple concentrations of the drugs and combinations were added to cell culture wells, ensuring adequate culture medium and a minimum of six replicates. Plates were incubated for 24 hours. At the end of the incubation period, MTT solution was added, and after a subsequent 4-hour interval, DMSO solution was introduced to dissolve the formazan crystals. Absorbance measurements were taken at a wavelength of 571 nm to determine cell viability percentages.

- *Statistical Analysis*

Data analysis was performed using SPSS 20 software. The distribution of data was assessed using the Kolmogorov-Smirnov test. Upon confirming data normality, one-way analysis of variance (ANOVA) and Tukey's post hoc test were applied for inter-group comparisons. Additionally, the independent t-test was employed for comparisons between different time points. Statistical significance was set at $P < 0.05$ for inter-group differences.

Results

The MTT test outcomes revealed that viability within the AGS cell line showed no significant differences when subjected to concentrations of 3.90625, 7.8125, and 15.625 µg/ml of 5FU, in comparison to the control group ($P>0.05$). Conversely, a notable decline in viability was observed in the AGS cell line at concentrations of 31.25, 62.5, 125, 250, and 500 µg/ml of 5FU, with a significant decrease relative to the control group ($P<0.001$). This pattern is visually depicted in Figure 1.

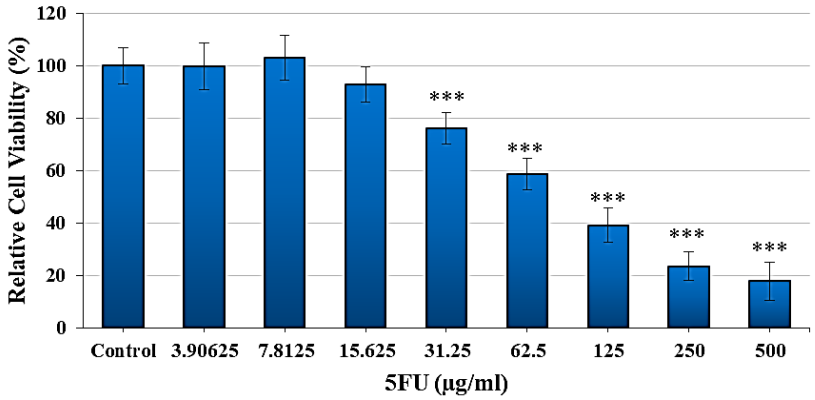

Figure 1. Viability of AGS cells exposed to doses of 5FU (µg/ml). Significant difference compared to the control group (***: $P<0.001$).

The MTT test outcomes for the AGS cell line exposed to the 5FU+AgCl NPs treatment exhibited no discernible differences in viability at a concentration of 3.90625 µg/ml when compared to the control group ($P>0.05$). However, a substantial reduction in viability was observed across concentrations of 7.8125, 15.625, 31.25, 62.5, 125, 250, and 500 µg/ml within the 5FU+ AgCl NPs treatment group, showcasing significant decreases relative to the control group ($P<0.001$). This trend is visually depicted in Figure 2.

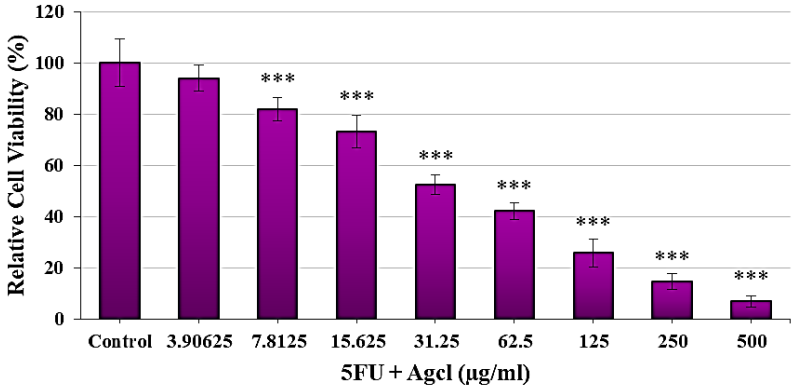

Figure 2. Viability of AGS cells exposed to doses of 5FU+ AgCl NPs (µg/ml). Significant difference compared to the control group (***: $P<0.001$).

Comparing the two settings of results, it is evident that while the viability of AGS cells remains relatively unaffected at lower concentrations (3.90625, 7.8125, and 15.625 µg/ml) of 5FU when compared to the control group, the introduction of silver chloride nanoparticles in the 5FU+ AgCl NPs treatment significantly enhances the reduction in cell viability at these concentrations. Notably, the combined treatment consistently leads to substantially lowered viability across a broader range of concentrations (7.8125 to 500 µg/ml) in comparison to 5FU alone, showcasing the potential synergistic cytotoxic effects of silver chloride nanoparticles and 5FU on AGS cancer cells.

Discussion

Nanoparticles exhibit multifaceted effects in anticancer therapy, primarily through enhanced drug delivery and targeted actions [9], [10], [11]. Their diminutive size allows for efficient encapsulation and controlled release of chemotherapeutic agents, amplifying drug bioavailability at tumor sites while minimizing systemic toxicity [12], [13]. Functionalized nanoparticles can selectively bind to cancer cell receptors, enabling precise drug delivery and sparing healthy tissues [14], [15]. Furthermore, certain nanoparticles generate localized hyperthermia upon exposure to external stimuli, leading to cancer cell death [16]. Additionally, nanoparticles can sensitize cancer cells to radiation therapy, augmenting the therapeutic effects of ionizing radiation [17]. These collective effects position nanoparticles as versatile tools in the fight against cancer, offering improved drug delivery strategies and enhanced therapeutic outcomes.

AgNPs have garnered significant attention for their potential effects on gastric cancer cells. AgNPs exhibit distinct anticancer properties, including the induction of apoptosis and cell cycle arrest in gastric cancer cells [18]. These nanoparticles can interfere with various cellular processes, such as DNA replication and repair, leading to genomic instability and cell death [19]. AgNPs also exert

their effects through the generation of reactive oxygen species (ROS), causing oxidative stress and subsequent damage to cellular components [20]. Furthermore, the small size and high surface area of AgNPs facilitate their cellular uptake, enhancing their bioavailability and cytotoxicity [21], [22]. 5FU is a widely used chemotherapeutic agent with well-established effects on gastric cancer cells. 5FU exerts its cytotoxicity by inhibiting thymidylate synthase, a crucial enzyme involved in DNA synthesis, thereby disrupting DNA replication and leading to cell cycle arrest. Additionally, 5FU incorporates into RNA and DNA molecules, further interfering with nucleic acid metabolism. Its incorporation into RNA can impair protein synthesis and disrupt essential cellular processes [23], [24]. Furthermore, 5FU induces apoptosis in gastric cancer cells by activating pro-apoptotic pathways and inhibiting anti-apoptotic signals. While 5FU has demonstrated efficacy in gastric cancer treatment, its use is often part of combination therapies to enhance its anticancer effects and reduce the risk of drug resistance [25], [26]. We have previously shown that AgCl NPs have cytotoxic effects on gastric cancer cells [27]. Current study demonstrates an enhanced cytotoxic effect when utilizing a combination of silver chloride nanoparticles and 5FU on AGS cancer cells, surpassing the impact of 5FU alone. Lower concentrations of 5FU show limited effects on cell viability, but the addition of silver chloride nanoparticles significantly amplifies the reduction in viability, suggesting a potential synergistic effect. Previous research has also reported synergistic interactions between nanoparticles and 5FU on cancer cells, such as the study on Fe3O4 nanoparticles in combination with 5-FU showing superior antitumor effects in a colon cancer cell model [28]. Additionally, in vitro experiments using biosynthesized Vv-AgNPs in conjunction with 5-FU have revealed synergistic cytotoxic, antiproliferative, apoptotic, and oxidative effects against the HT-29 cell line [29]. Furthermore, the evaluation of the chemo-preventive effects of Camellia sinensis silver nanoparticles and their synergistic effects with 5-fluorouracil in colorectal cancer-induced rats has shown promising results [30].

The combination of 5FU with AgNPs represents a promising approach in tackling gastric cancer. 5FU, a well-established chemotherapeutic agent, disrupts DNA synthesis and induces apoptosis in cancer cells. When combined with AgNPs, which exhibit inherent cytotoxic properties, synergistic effects can be observed. AgNPs augment the anticancer potential of 5FU by enhancing cellular uptake and facilitating drug delivery. Moreover, AgNPs generate reactive oxygen species (ROS) that further induce oxidative stress and DNA damage in gastric cancer cells, enhancing 5FU's cytotoxicity. This combination therapy not only targets multiple cellular pathways but also holds the potential to overcome drug resistance mechanisms, making it a compelling strategy for improving the treatment outcomes of gastric cancer. However, further research is essential to optimize the dosing, timing, and safety of this combination therapy for clinical applications.

Conclusion

In conclusion, the MTT test outcomes highlight distinct cytotoxic impacts between 5FU and the combined treatment of silver chloride nanoparticles and 5FU on AGS cancer cells. While lower concentrations of 5FU show minimal effects on cell viability compared to the control group, the introduction of silver chloride nanoparticles alongside 5FU significantly enhances the reduction in cell viability, suggesting a potential synergistic effect. Across a broader concentration range, the combined treatment consistently induces notably lower cell viability than 5FU alone. These findings underscore the potential of silver chloride nanoparticles to augment the cytotoxic efficacy of 5FU in treating AGS cancer cells, motivating further exploration for improved gastric cancer treatment strategies.

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Conflict of interests
The authors declare that there are no competing interests.

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