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Original Article

Evaluation of the Synergistic Effects of Gold Nanoparticles and Hyperthermia on Chronic Myeloid Leukemia (CML) Cell Line

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Abstract

Introduction: Leukemia is one of the types of cancer that its most common treatments, including radiation and chemotherapy, are associated with many problems and side effects such as drug resistance. Therefore, researchers are looking for multi-factor combined therapies with fewer side effects and more anti-cancer effects to treat leukemia. The aim of this study was to analyze the combination effect of gold nanoparticles (Au NPs) and hyperthermia on the proliferation of chronic myeloid leukemia cells.

Materials and Methods: To study the nanoparticle absorption by K562 cancer cells, various amounts of nanoparticles were used. The MTT and apoptosis assays were used to evaluate the anti-proliferation properties of NPs (20, 40, and 80 µg/ml) and hyperthermal (41 °C for an hour) on cancer cells.

Results: Our results revealed a significant decrease in the proliferation index (43 ± 3.311, $p<0.01$) and an increase in apoptosis (54 ± 3.605, $p<0.01$) of K562 cells in the group receiving a combination of 80 µg/ml of Au NPs and hyperthermia compared to the control and other treatment groups.

Conclusions: According to the results, the combination of Au NPs and hyperthermia could significantly suppress the proliferation and induce apoptosis in K562 cells in comparison with Au NPs or hyperthermia, alone. Therefore, it confirms that the application of several anti-cancer strategies using NPs and hyperthermia is a useful strategy in cancer treatment.

Keywords: Gold Nanoparticles, Hyperthermia, Chronic Myeloid Leukemia (CML), Apoptosis, Proliferation
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Introduction

Leukemia is a type of cancer that may lead to the irregular growth of white blood cells in the bone marrow and the lymphatic system. leukemia is a disruptive mechanism in which blood cell development is out of balance.[1] The Bilateral transformation between the ABL gene for chromosome 9 and the BCR gene for chromosome 22 in pluripotent stem cells develop the BCR-ABL fusion gene which is one of the most well-known causes of leukemia.[2] This transformation produces a 210 kDa protein ABL-P210BCR which is a permanently active tyrosine kinase and is recognized in more than 95% of the CML patients as Philadelphia chromosome. This protein triggers myeloid cell proliferation and interferences with programmed cell death.[3] K562 cell line originating from a 53-year-old female patient with blast myelogenic leukemia, is the first human myelogenous leukemia cell line.[4,5] Many drugs are used in CML chemotherapy to induce apoptosis and to inhibit the growth of cancerous cells. However, the side effects and the resistance to anti-apoptotic drugs are the most important obstacles in CML chemotherapy limiting their application in cancer treatment.[6] Efforts are proceeding for the development and application of medications without lower side effects and resistance.[7] Nanotechnology, used in the treatment and detection of human tumors, has given rise to a modern specialty within oncology named nano-oncology.[8] Nanoparticles have increasingly emerged to picture tumors, highlighted biomolecules and biomarkers of cancer, and they are also served as a vehicle for drug delivery. Therapeutic enzymes, in particular, are even more stable when carrying within polymer nanoparticles because of their strengthened shape, which protects them from high temperatures, pH, enzymes, and other molecules that could damage their structure.[9] Gold

nanoparticles are unique carriers, as they are thin, biocompatible, and non-toxic. They are also useful in medical applications such as biosensors due to their usage in different residential properties,[10] drug delivery,[11] chemotherapy,[12] and radiation therapy.[13] To increase the effectiveness of nanoparticles, they are often combined with other anti-cancer agents. One of the known causes of hyperthermia is used in combination with other cancer treatment methods such as chemotherapy and radiation therapy.[14] Many clinical studies have been performed on the combination of hyperthermia and radiation therapy or chemotherapy on various types of cancers such as sarcoma, melanoma, head and neck cancer, rectum, liver, cervix, mesothelium, breast, bladder, lung, brain, etc.[15] Many of these studies showed the hyperthermia's effect on the reduction of tumor size as a treatment.[16] The minimum temperature for the treatment of hyperthermia is usually around 39.5-40.5 °C. With advances in the tools and methods of hyperthermia, today, about 50% of deep tumors can be heated to 42 °C in certain areas. However, reaching a specific temperature in different tumors with clinical applications is still unclear.[17] This study aimed to examine the combined effect of gold nanoparticles (Au NPs) and hyperthermia (H) on K562 cells proliferation and apoptosis as a model of CML.

Materials and Methods

Reagents

Dioxin, dimethyl sulfoxide (DMSO) and 3-[4,5-dimethylthiazol-2-yl]-2,5- diphenyl tetrazolium bromide (MTT) were obtained from Sigma-Aldrich (St. Louis, USA). RPMI 1640 and fetal calf serum were bought from GIBCO/Life Technologies Inc. (New York, USA). Au NP was purchased from US Research Nanomaterials, Inc (USA).

Experimental Design and Cell Culture

The K562 cell line (Human myeloid leukemia cell line) was received from the National Cell Bank of Pasteur Institute of Iran, Tehran, Iran. The cells were cultured in RPMI 1640 medium supplemented with streptomycin (100 mg/ml), penicillin G (100 units/ml) and 10% fetal bovine serum (Sigma, St. Louis, MO, USA) (FBS, Invitrogen). Cells were incubated at 37 °C in a humidified atmosphere comprising of 5% CO_2. The K562 cells were cultured and randomly assigned to nine groups; one negative control (NC; untreated cells), seven treatment groups, and one healthy control group utilizing doxorubicin at 800 nM. The features of the treatment and control categories are summarized in Table 1. Treatment categories were split between those receiving a specific agent and those receiving a combination of agents.

Table 1. The Research Groups' Characteristics

Groups	Characteristics
1	NC
2	PC
3	H
4	NP 20 µg/ml
5	NP 40 µg/ml
6	NP 80 µg/ml
7	NP 20 µg/ml+H
8	NP 40 µg/ml+H
9	NP 80 µg/ml+H

NC: Negative control; PC: Positive control; NP: Nanoparticle; H: Hyperthermia.

Identification and Formulation of Au NPs

The Transmission Electron Microscopy (TEM) image and the Au NPs characteristics including X-ray diffraction pattern by US Research Nanomaterials, Inc Company have been illustrated in Figure 1.

Measurement of Au NP Uptake

K562 cells were suspended in RPMI to a final concentration of 1×10^5 cells/well and incubated separately for 24 h with serial concentrations of Au NPs (20, 40, and 80 g/ml) in 5% CO_2 incubator. Then, the treated cells were washed three times with PBS to wash non-absorbed nanoparticles. Cells were then digested overnight at room temperature with 0.3 ml of 3% HCl in distilled HNO_3. After adding 5 µl of 5-ppm indium (as an internal reference) and 5 ml of matrix solution (2% HCl and 2% HNO_3), the Au NP content of each sample was determined and compared to the standard curve using an atomic absorption spectrometer (wavelength 280 nm, Model 603, Perkins Elmer, Waltham, MA).

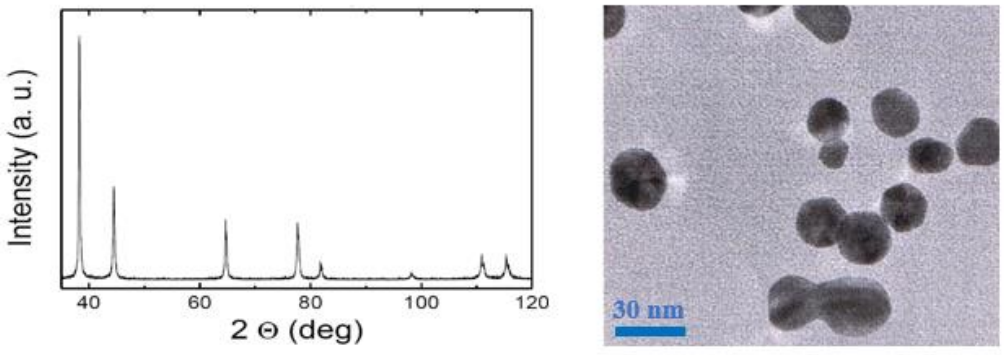

Figure 1. X-ray Diffractogram and Transmission Electron Microscopy (TEM) Image of Au Nanoparticles (Image taking from US Research Nanomaterials, Inc Company web).

Hyperthermic Exposure

K562 cells were seeded onto 96-well plates containing RPMI-1640 (200 ml) with or without Au NPs (20, 40, and 80 µg/ml). After that, cells were incubated overnight at 37 °C. The next day, the cells were transferred to an incubator at 41 °C (SB-14012, Germany) for 1 h

MTT Assay

The cytotoxicity of Au NPs in the treated groups was measured using the MTT assay. The K562 cells (1×10^5 cells/well) were seeded on a 96-well plate in serum-free media. Cells were distributed in the groups and incubated for 24 h in 5% CO_2 at 37 °C. Then, the cells were incubated with MTT dye (Sigma, USA) for 4 h at 37 °C to purple formazan substance formed. After 4 h, DMSO was added and the absorbance (optical density, OD) was measured at 570 nm using a 96-well microplate reader (Bio-Rad, Hercules, CA). All experiments were run in triplicates and cell viability was calculated as the percentage of viable cells in groups divided into the control cells. The IC_{50} values were estimated from the results of the MTT test described as the drug concentrations that reduced absorbance to 50% of control values.

Evaluation of K562 Cell Line Apoptosis

To investigate the effect of NPs and hyperthermia on the cells, apoptosis assay was applied. Briefly, 1 mg/ml propidium iodide and 1 mg/ml acridine orange were added to 10 ml of PBS to make the staining solution. K562 cell suspensions were combined 1:1 with the staining solution. Using a fluorescent microscope, the number of apoptotic cells was counted in five random fields and the number of the apoptotic cells was calculated.

Statistical Analysis

A one-way ANOVA test was used to evaluate the differences between study groups and the data was interpreted as mean ± Standard Deviation (SD). A Tukey multiple post-hoc tests were also used to identify major variations. The *p*-value<0.05 was considered statistically significant.

Results

Measurement Cellular Uptake of NPs

Based on the current standard curve (Figure 2), K562 cells were treated with Au NPs at concentrations of 0, 20, 40, and 80 µg/ml.

Measurement of IC_{50}

The IC_{50} values of the Au NPs for K562 cell lines were 81.62 µg/ml according to the normal curve (Figure 3).

Cell Cytotoxicity of NPs

As shown in Figure 4, MTT was used to assess the in vitro cytotoxicity of Au NPs and various treatment groups of K562 cells. The results were compared with the negative control and normal Doxorubicin groups. Au NPs 80 µg /ml exhibited the highest cytotoxicity as compared to the control and other categories. The findings of cytotoxicity demonstrated an increase in the efficacy of NPs combined with the Hyperthermia group.

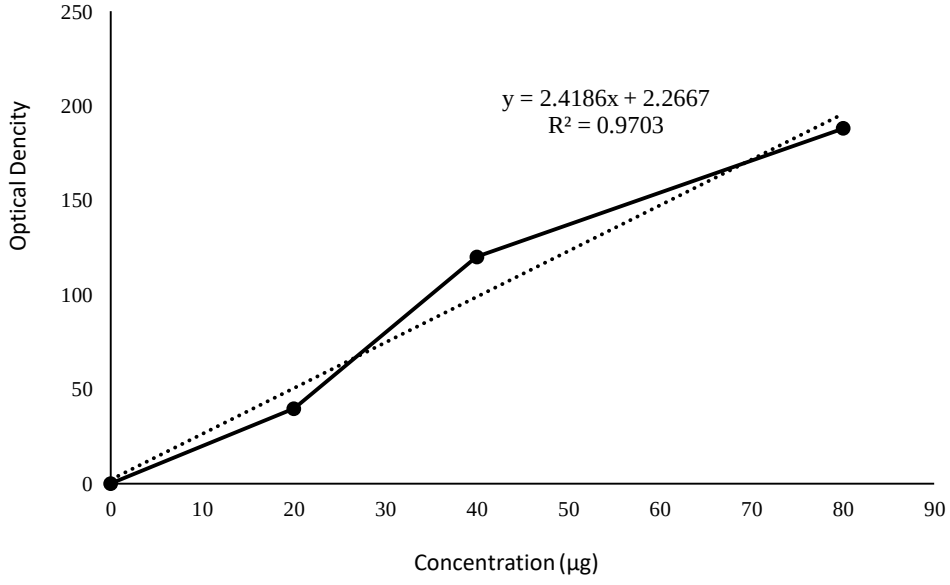

Figure 2. Standard Curve of Cellular Uptake of Au NPs into the K562 Cells at 0, 20, 40, 80 µg/mL Concentrations.

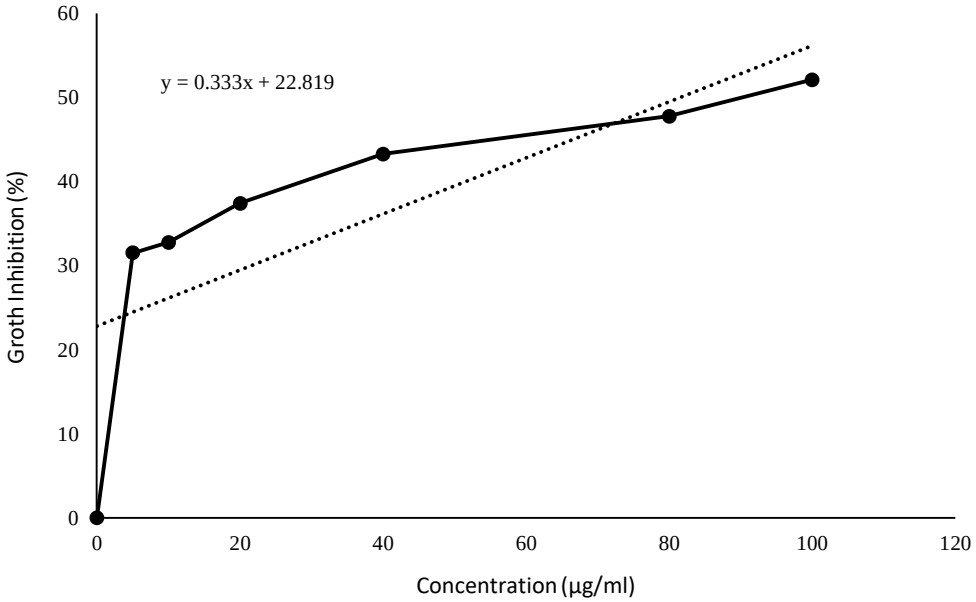

Figure 3. The Impact of Au NPs on the K562 Cell Line. The IC50 Was Determined Using Linear Regression.

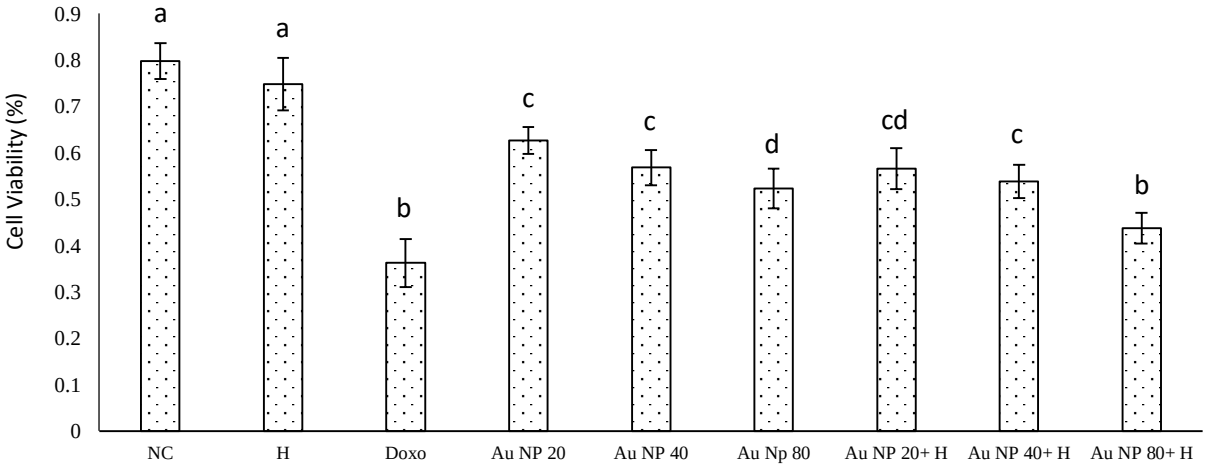

Figure 4. Au NPs and Hyperthermia Effect on the Viability of K562 Cells (NC: Negative control; PC: positive control; NP: nanoparticle; H: hyperthermia). Significant statistical variations between groups are denoted by a separate superscript letter (p<0.05) in each index.

Apoptosis Assay

Figure 5 shows that the viable cells are green with diffused chromatin, apoptotic cells are in red with condensed chromatin and necrotic cells are also red without condensed chromatin.

As shown in Figure 6, the effect of cytomorphological Au NPs on K562 cells at different concentrations (20, 40, and 80 µg/ml) is intracellular suicide, manifested by cell shrinkage, coiling, oxidative stress, and biochemical reactions resulting in apoptosis. The findings demonstrate an elevated degree of apoptosis in K562 cell lines as a consequence of a growing trend in Au NPs concentrations and hyperthermia, as found in cytotoxicity outcomes. Au NP 80 µg/ml had the greatest inhibitory influence on K562 cell lines, while the H group had the least cytotoxic effect on K562 cell lines. Acridine orange/propidium iodide was used to determine the longevity of K562 cells. The cell lines were categorized according to the morphology and color of their chromatin.

Discussion

Cancer is one of the most important diseases and the second leading cause of death worldwide. Leukemia accounts for 4% of all malignancies and is ranked fifth in mortality.[1] At present, drugs used to treat leukemia are often ineffective and the death of patients eventually happens due to drug resistance. Therefore, extensive global studies are underway to find treatment strategies focusing on the simultaneous use

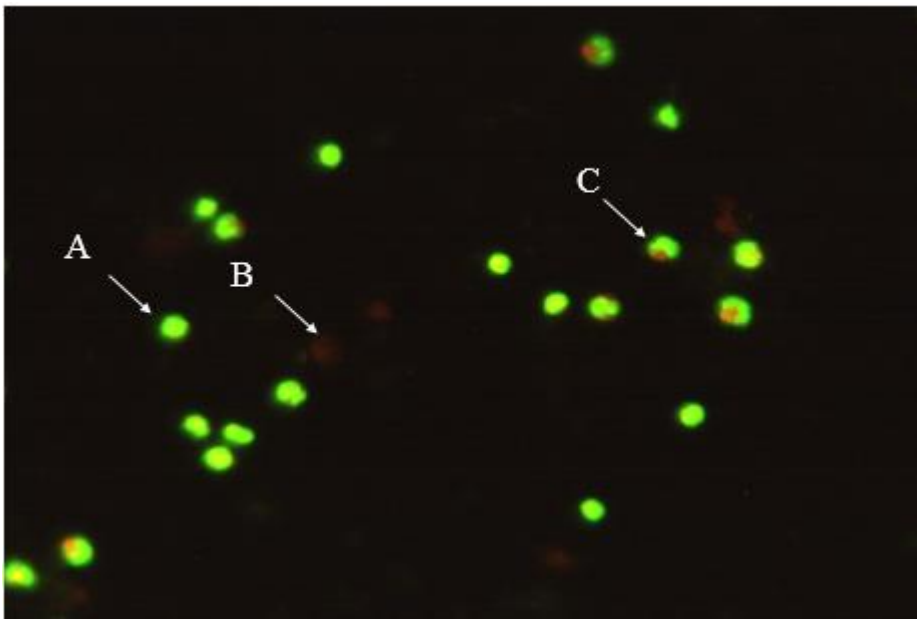

Figure 5: Propidium iodide/Acridine Orange Staining Was Used to Determine the Percent of Apoptosis in K562 Cells. A) Green cells with chromatin diffused are alive, B) Red cells without chromatin condensate are necrotic and C) condensed chromatin cells are apoptotic.

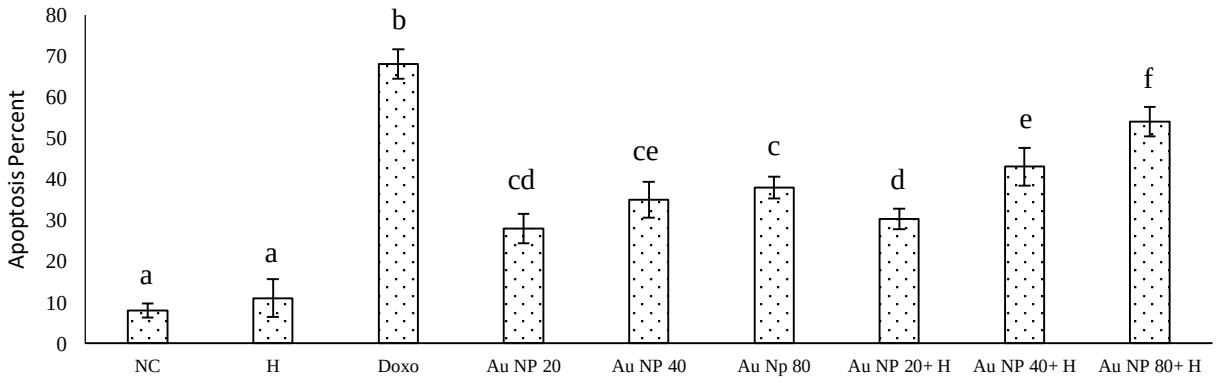

Figure 6: Au NPs and Hyperthermia Decrease the Cellular Apoptosis Caused by the Treatment Combination on K562 Cells. (NC: Negative control; PC: positive control; NP: nanoparticle; H: hyperthermia). Significant statistical variations between groups are denoted by a separate superscript letter (p<0.05) in each index.

of several agents with anti-cancer properties.[3] Nanotechnology is one of the most effective and less risky solutions that its benefits are more than chemotherapy and radiotherapy.[10] Gold nanoparticles have great potential for diagnosis, curing, and treatment of cancer. High bioavailability, selective accumulation in cancer cells and low toxicity are among the advantages of these particles. Today, researchers are applying gold nanoparticles as a contrast enhancer for Raman imaging and X-ray imaging.[18] Hyperthermia, as an adjunct therapy to cancer, has made significant progress in the last 40 years.[19] The objective of this analysis was to investigate the combined effects of gold nanoparticles (Au NPs) and hyperthermeia (H) on the proliferation and apoptosis of K562 cells as a model of chronic myeloid leukemia. In the presence of gold nanoparticles, thermotherapy led to an increase in apoptosis and a decline in survival of K562 cells. MR, et al. also reported that gold nanoparticles in a dose-dependent manner reduce the proliferation and increase the apoptosis of the HepG2 liver cancer cell line. Also, these researchers reported that gold nanoparticles selectively have anti-cancer effects since these nanoparticles had no toxic effect on the Vero cell line.[20] Jabbari et al. showed that calcium carbonate nanoparticles in combination with hyperthermia and radiotherapy reduce proliferation and increase apoptosis in MCF-7 cancer cells. Also, it has been reported that this combination therapy activated the mitochondrial pathway of apoptosis in cancer cells.[21] Esmaeili Govarchin Ghaleh et al. reported that the use of a combination of multifactorial therapy including hyperthermia, copper nanoparticles and radiotherapy in the treatment of breast cancer, activates the

mitochondrial pathway of apoptosis by increasing caspase-9 expression, reducing cancer cell proliferation, and increasing the production of reactive oxygen species.[19] Azemati et al. proved that the effectiveness of drugs delivered through nanoparticles in combination with hypertension and radiotherapy is significantly higher than drugs that have been studied independently.[22] Hainfeld et al. revealed that gold nanoparticles combined with hyperthermia and radiotherapy reduce the amount of required radiation. In addition to increasing the effectiveness of radiotherapy, this also reduces the side effects of radiotherapy.[23] Moradi et al. showed that the cell viability of the Y79 cells for a 0.5 to 11 min hyperthermia time, compared with the control group, was 93 to 27% and 95 to 32% with and without Au-NPs, respectively. These results suggest that hyperthermia and gold nanoparticles make cells more sensitive to treatment.[24]

The results of our study in parallel with all studies in this field showed that the use of gold nanoparticles in combination with hypertension can be used as a new treatment method and confirm their effectiveness in the treatment of leukemia.

Conclusion

Cancer is one of the most serious health problems in today's society and many extensive efforts have been done in process to combat it. So, especially in the last two decades, scientists have been trying to come up with equally clever solutions to fight against cancer. Targeting cancer cells with a mixture of anti-cancer treatments of gold nanoparticles and hypertension is a great way to create an intelligent protection. According to the current study's findings, a mixture of Au NPs and hyperthermia has the potential to inhibit the proliferation and induce apoptosis in K562 cells more effectively than their sole application. Therefore, it seems that the combined use of several anti-cancer strategies as a new method of cancer treatment can be considered.

Authors' Contributions

The conceptualization was done by MG and HEG. The formal analysis and interpretation were done by MG and HEG. The resource and writing-original draft preparation were carried out by MG, HEG and SMY. The writing review and editing were performed by MKh. The supervision was done by SMY. The whole manuscript was read and approved by all the authors.

Funding and Ethics Approval

This study was funded by the Student Research Committee, Shahid Beheshti University of Medical Sciences, Tehran, Iran (project NO. 1398/9789). The experimental procedures of this study were supervised by the Ethical Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran (ethical code: IR.SBMU.RETECH.REC.1398.260).

Conflict of Interest Disclosures

The authors declare that they have no conflicts interest.

Acknowledgment

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References

1. Hallek M. Chronic lymphocytic leukemia: 2020 update on diagnosis, risk stratification and treatment. Am J Hematol. 2019;94(11):1266-87. doi:10.1002/ajh.25595
2. Short NJ, Konopleva M, Kadia TM, Borthakur G, Ravandi F, DiNardo CD, et al. Advances in the Treatment of Acute Myeloid Leukemia: New Drugs and New ChallengesAdvances in AML Therapeutics. Cancer Discov. 2020;10(4):506-25. doi:10.1158/2159-8290.CD-19-1011
3. DiNardo CD, Wei AH. How I treat acute myeloid leukemia in the era of new drugs. Blood. 2020;135(2):85-96. doi:10.1182/blood.2019001239
4. Yen JH, Lin CY, Chuang CH, Chin HK, Wu MJ, Chen PY. Nobiletin promotes megakaryocytic differentiation through the MAPK/ERK-dependent EGR1 expression and exerts anti-leukemic effects in human chronic myeloid leukemia (CML) K562 cells. Cells. 2020;9(4):877. doi:10.3390/cells9040877
5. Yoshimura A, Asahina Y, Chang LY, Angata T, Tanaka H, Kitajima K, et al. Identification and functional characterization of a Siglec-7 counter-receptor on K562 cells. J Biol Chem. 2021;296:10477. doi:10.1016/j.jbc.2021.100477
6. Diaz-Santa J, Rodriguez-Romanos R, Osca G, Pratcorona M, Garrido A, Coll R, et al. UGT1A1 genotype influences clinical outcome in patients with intermediate-risk acute myeloid leukemia treated with cytarabine-based chemotherapy. Leukemia. 2020;34(11):2925-33. doi:10.1038/s41375-020-0784-2
7. Alkhouli M, Laflouf M, Alhaddad M. Efficacy of aloe-vera use for prevention of chemotherapy-induced oral mucositis in children with acute lymphoblastic leukemia: a randomized controlled clinical trial. Compr Child Adolesc Nurs. 2021;44(1):49-62. doi:10.1080/24694193.2020.1727065
8. Shen J, Lu Z, Wang J, Zhang T, Yang J, Li Y, et al. Advances of nanoparticles for leukemia treatment. ACS Biomater Sci Eng. 2020;6(12):6478-89. doi:10.1021/acsbiomaterials.0c01040
9. Bhattacharjee R, Victor O, Florence S. Application of nano based drug delivery channel against leukemia chemotherapeutic resistance. Glob J Cancer Ther. 2021;7(1):001-9. doi:10.17352/2581-5407.000036
10. Cerrato-Alvarez M, Bernalte E, Bernalte-Garcна MJ, Pinilla-Gil E. Fast and direct amperometric analysis of polyphenols in beers using tyrosinase-modified screen-printed gold nanoparticles biosensors. Talanta. 2019;193:93-9. doi:10.1016/j.talanta.2018.09.093
11. Kong FY, Zhang JW, Li RF, Wang ZX, Wang WJ, Wang W. Unique roles of gold nanoparticles in drug delivery, targeting and imaging applications. Molecules. 2017;22(9):1445. doi:10.3390/molecules22091445
12. Mendes R, Pedrosa P, Lima JC, Fernandes AR, Baptista PV. Photothermal enhancement of chemotherapy in

breast cancer by visible irradiation of Gold Nanoparticles. Sci Rep. 2017;7(1):10872. doi:10.1038/s41598-017-11491-8
13. Kefayat A, Ghahremani F, Motaghi H, Mehrgardi MA. Investigation of different targeting decorations effect on the radiosensitizing efficacy of albumin-stabilized gold nanoparticles for breast cancer radiation therapy. Eur J Pharm Sci. 2019;130:225-33. doi:10.1016/j.ejps.2019.01.037
14. Norouzi H, Khoshgard K, Akbarzadeh F. In vitro outlook of gold nanoparticles in photo-thermal therapy: a literature review. Lasers Med Sci. 2018;33(4):917-26. doi:10.1007/s10103-018-2467-z
15. Spirou SV, Basini M, Lascialfari A, Sangregorio C, Innocenti C. Magnetic hyperthermia and radiation therapy: radiobiological principles and current practice. Nanomaterials. 2018;8(6):401. doi:10.3390/nano8060401
16. Mahmood J, Shukla HD, Soman S, Samanta S, Singh P, Kamlapurkar S, et al. Immunotherapy, radiotherapy, and hyperthermia: a combined therapeutic approach in pancreatic cancer treatment. Cancers. 2018;10(12):469. doi:10.3390/cancers10120469
17. Brero F, Albino M, Antoccia A, Arosio P, Avolio M, Berardinelli F, et al. Hadron therapy, magnetic nanoparticles and hyperthermia: A promising combined tool for pancreatic cancer treatment. Nanomaterials. 2020;10(10):1919. doi:10.3390/nano10101919
18. Dziawer Ł, Majkowska-Pilip A, Gaweł D, Godlewska M, Pruszyński M, Jastrzębski J, et al. Trastuzumab-modified gold nanoparticles labeled with 211At as a prospective tool for local treatment of HER2-positive breast cancer. Nanomaterials. 2019;9(4):632. doi:10.3390/nano9040632
19. Esmaeili Govarchin Ghaleh H, Zarei L, Mansori Motlagh B, Jabbari N. Using CuO nanoparticles and hyperthermia in radiotherapy of MCF-7 cell line: synergistic effect in cancer therapy. Artif Cells Nanomed Biotechnol. 2019;47(1):1396-403. doi:10.1080/21691401.2019.1600529
20. Priya MRK, Iyer PR. Antiproliferative effects on tumor cells of the synthesized gold nanoparticles against Hep2 liver cancer cell line. Egyptian Liver Journal. 2020 Dec;10(1):15. doi:10.1186/s43066-020-0017-4
21. Jabbari N, Zarei L, Esmaeili Govarchin Galeh H, Mansori Motlagh B. Assessment of synergistic effect of combining hyperthermia with irradiation and calcium carbonate nanoparticles on proliferation of human breast adenocarcinoma cell line (MCF-7 cells). Artif Cells Nanomed Biotechnol. 2018;46(sup2):364-72. doi:10.1080/21691401.2018.1457537
22. Azemati F, Kondori BJ, Ghaleh HE. Therapeutic potential of nanoparticle-loaded hydroxyurea on proliferation of human breast adenocarcinoma cell line. Iran J Pharm Res. 2020;19(1):271-81. doi:10.22037/ijpr.2020.1100921
23. Hainfeld JF, Lin L, Slatkin DN, Dilmanian FA, Vadas TM, Smilowitz HM. Gold nanoparticle hyperthermia reduces radiotherapy dose. Nanomedicine. 2014;10(8):1609-17. doi:10.1016/j.nano.2014.05.006
24. Moradi S, Mokhtari-Dizaji M, Ghassemi F, Sheibani S, Amoli FA. The effect of ultrasound hyperthermia with gold nanoparticles on retinoblastoma Y79 cells. Gold Bull. 2020;53(2):111-20. doi:10.1007/s13404-020-00279-w