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Research Article

The Impact of Different Nanoparticle Concentrations Combined with Gamma Rays on Breast Cancer Cells

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Abstract

Background: Cancer is the second leading cause of death worldwide. To treat cancer effectively, radiation must maximize cytotoxicity against tumor cells while limiting injury to healthy cells. Nanotechnology and biomedical technologies can help improve cancer detection and treatment, but they also create challenges.

Objective: To evaluate the effect of different concentrations of gold nanoparticles (25%, 50%, or 100%) exposed to varying doses of gamma radiation (200Gy, 400Gy, 600Gy) on breast cancer cells in women in comparison with normal cells.

Method: For 24 and 48 hours, breast cancer cells and normal breast cells were treated with 25%, 50%, or 100% gold nanoparticles (AuNPs). Another comparable method was done after exposing concertation AuNPs to 200Gy, 400Gy, and 600Gy gamma radiation. After 24 and 48 hours, the results were compared to Retinoblatoma-Endothelial Factor (REF) and Malondialdehyde (MDA) values. The study was conducted in the Biotechnology Research Center, Al-Nahrain University, and the Laboratory of Biophysical Techniques, Sciences College of Sciences for Women, University of Baghdad, from October 2023 to February 2024.

Results: Significant changes in cell growth suppression between MDA and REF after 24 and 48 hours were seen with AuNPs alone (25%, 50%, 100% concentration) and with gamma-ray dosages 200Gy, 400Gy, 600Gy. The mean growth inhibition of cells treated with AuNPs irradiated by gamma-rays (200Gy, 400Gy, 600Gy) compared to AuNPs alone was highly significant ($P < 0.001$) in instances REF (24 and 48 hours) and MDA (24 and 48 hours).

Conclusion: A high dose of gamma rays combined with AuNps can keep its destructive effect on cancerous cells for 24 - 48 hours. Gamma radiation has the potential to perform a valuable function in enhancing the effectiveness of AuNPs. Gold nanoparticles carrying this dose of radiation will cause minimal damage to normal tissue.

Keywords: AuNPs; Breast cancer; Cell line; Gamma Ray; Plasma.

Introduction:

Malignant tumors are one of the leading causes of human mortality globally (1, 2). To achieve effective cancer treatment, it is essential to enhance the cytotoxicity of radiotherapy targeted specifically at tumor cells while minimizing the adverse effects on healthy cells. The integration of nanotechnology and biomedical techniques offers promising opportunities but also presents significant challenges in developing more precise cancer diagnostics and therapeutic strategies (3, 4). One optimal approach is the development of effective nanoscale radiosensitizers that selectively target tumor cells. For instance, gold nanoparticles (AuNPs) have been shown to accumulate in tumor tissues through passive targeting mechanisms, and experiments using AuNPs as radiosensitizers have yielded promising results (5).

A nanoparticle is a small particle ranging in size from 1 to 100 nanometers. Due to their small size, nanoparticles can easily penetrate cellular membranes and the blood-brain barrier, making them. Ideal for delivering drugs and other therapeutic agents to cancer cells (8). Additionally, nanoparticles can selectively bind to malignant cells, allowing for precise detection of the location and characteristics of the disease. Gold nanoparticles (AuNPs) are particularly significant, having found extensive use in both medical and non-medical fields, owing to their unique properties, including stability, biocompatibility, and low toxicity (9).

In the context of breast cancer treatment, conjugating gold nanoparticles with pharmaceuticals offers two potential benefits: First, enhancing drug uptake into cancer cells, and second, providing a potential solution to drug resistance (10, 11, 12). Firstly, it can augment the uptake of pharmaceuticals. Secondly, it offers a potential solution to address the issue of pharmaceutical resistance (12).

Gamma rays, a form of ionizing electromagnetic radiation, can induce various cellular changes that may lead to cancer in the affected area. While the primary objective of radiotherapy is to eliminate

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cancer cells by inducing DNA damage, the presence of radioresistant cancer cells poses a significant challenge to its success (15). Gamma radiation can cause double-strand breaks in cellular DNA, leading to irreversible damage (16). Radiation therapy is highly effective at destroying cancer cells while having a relatively limited effect on healthy cells, which are more capable of withstanding and regenerating from damage (17).

Researchers have conducted a proof-of-principle study of the ability of gold nanoparticles to ameliorate the effects of radio-sensitization to DNA damage caused by high-energy electrons (18). The researchers used plasmid DNA and exposed it to 60 keV, either separately or in combination with gold nanoparticles, at a DNA-to-gold nanoparticle ratio of 1:1 or 1:2. This resulted in a 2.5-fold increase in the number of double-strand breaks. Another study yielded intriguing results (19), in that its authors concluded that the auger electron cascade is the prevailing phenomenon when employing photon energies below the K-edge. Consequently, it is necessary to position nanoparticles of small size near the intended target areas within cellular compartments (20). Nevertheless, the utilization of photon sources positioned above the K-edge necessitates a greater concentration of gold within the tumor region. However, in such instances, the dimensions and placement of the gold nanoparticles are not of any particular importance (21).

Therefore, the main objective of the current study was to evaluate the effects of different concentrations of gold nanoparticles (25%, 50%, and 100%) when exposing breast cancer cells to different doses of gamma rays (200 Gy, 400 Gy, and 600 Gy) in comparison to normal cells.

Materials and Methods:

The investigation was carried out at the Laboratory of Biophysical Techniques, College of Sciences for Women, University of Baghdad, and Al-Nahrain University's Biotechnology Research Center in Cooperation with the College of Medicine, University of Baghdad, in the period from October 2023 to February 2024.

Preparation of the gold solution: The gold solution was prepared in the same lab as above, using aqueous tetrachloride salts ($HAuCl_4.3H_2O$) at a concentration of 0.5 mM in 100 mL of distilled water. This was added to the beaker to function as a catalyst to help dissolve the aqueous tetrachloride salts (99% purity, 411.8476 g/mol mol mass). Equation (1) can be used to calculate the required concentration (mol L^{-1}) (22).

Concentration= [mass / (molecular weight* volume)] …….. (1)

Where mass is the mass solution of AuNPs, volume is the volume solution of AuNPs, and molecular mass represents of molecular mass of gold

Preparation of AuNPs: Gold nanoparticles (AuNPs) were prepared using jet plasma formation via the techniques described below (23)

The compressor bottle was pressurized with Argon gas, and the metal tube had a fixed diameter of 1 mm. Once a gold solution of the specified concentration and size had been produced, it was deposited on the holder beneath the metal tube as previously described. The beaker had a rounded shape transitioning from the metal tube to the liquid surface, with the tube's tip measuring 1 mm. The gas flow in the metal tube was controlled via a flow meter to manage the gas exiting from the tank. The voltage provided to the system was incrementally raised until plasma formed between the tube and the liquid surface containing a gold solution which was then maintained for four minutes, resulting in the transformation of the solution into nanoparticles.

Sample irradiation method: Following the preparation of the AuNPs solution, three containers were used, each containing 5 ml of 100% AuNPs concentration (no normal or cancer cells were present). Each container was directly exposed to a gamma ray source (Co^{60} with 14Gy/hr) at a distance of 50 cm from the window, ensuring an exposure depth of 10 mm. The first samples were exposed to a dose of 200 Gy, the second to 400 Gy, and the third to 600 Gy, as shown in Figure 1. Each sample was then diluted to 25%, 50%, and 100%. The irradiated gold particles were then administered to the cells.

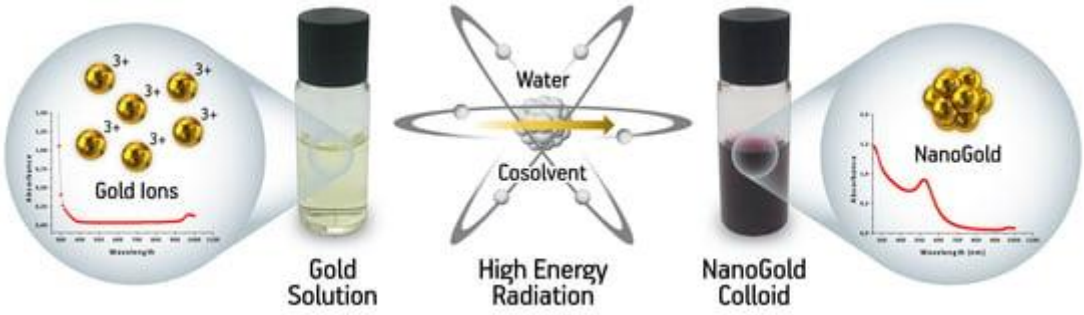

Figure 1: Interaction of gamma-ray with gold solution [24]

Cell lines in the current study: The current study used two cell lines: the first was breast cancer (MDA) cells, whilst the second was normal cells (REF). These cells were provided by the Al-Nahrain University's Biotechnology Research Center. Cells were added to the free serum medium (FSM) and Trypsin solution, and distributed within a microplate of 96 wells at a concentration of 10,000 cells per well.

All of them incubated at 37°C for 24 hours and 48 hours to generate a monolayer. Excess dye was removed by washing the plates with distilled water several times. The microplate was divided into groups: the first group consisted of five wells exposed to AuNPs at concentrations of 25%, 50%, and 100%; the second group was exposed to the combination of AuNPs at different concentrations (25%, 50%, and 100%) which had been irradiated with gamma rays at doses of 200, 400, and 600 Gy. Crystal violet assay was added, and the cells were incubated for 20 min at 37°C. The inhibitory rate was calculated according to GI% [25] by converting the reading of the absorbance for the optical density in the ELISA device into percentages according to the following equation (2):

$$GI\% = \frac{v\ control - v\ treated}{v\ control} \times 100\% \quad \text{..... (2)}$$

Statistical analysis: Statistical analyses were performed using SPSS for Windows (IBM, Inc.) version 22. Statistical analysis was carried out on absorbance readings to calculate the IC50. The differences between treated MDA and REF cells, separately, the means and standard deviations (SD) were calculated, and the differences between group means were tested using the T-test, with the p-value of < 0.05 considered to be significant.

Results

Cell lines treated with nanoparticles and gamma rays: The outcomes of examining the effects of the three concentrations of gold nanoparticles (25%, 50%, and 100%) on normal cells (REF) and cancer cells (MDA) both without and after radiation treatment with three gamma ray doses (200 Gy, 400 Gy, and 600 Gy) are presented in Tables 1, and Figures 2.

Table 1: The impact of (A) 25%, (B) 50%, and (C) 100% AuNP concentration, both with and without radiation treatment, on mean REF and MDA values (for 24 and 48 hours)

(A) 25% AuNP concentration

	REF 24(%)	REF 48 (%)	MDA 24(%)	MDA 48(%)
AuNPs	7.0±1.25	8.3±1.33	27.0±1.58	34.0±2.21
AuNPs + 200Gy	11.1±1.33	16.1±1.35	39.0±1.86	40.3±2.92
AuNPs + 400Gy	14.3±1.29	19.3±1.52	45.0±2.45	46.0±2.84
AuNPs + 600Gy	17.7±1.34	22.3±1.62	50.3±3.25	54.0±3.5

(B) 50% AuNP concentration

AuNPs	REF 24(%)	REF 48 (%)	MDA 24(%)	MDA 48(%)
AuNPs + 200Gy	9.5±1.25	11.1±1.28	31.3±2.4	40.3±3.25
AuNPs + 400Gy	13.3±1.32	17.0±1.29	43.3±2.53	52.3±3.45
AuNPs + 600Gy	16.3±1.38	20.0±1.37	47.0±3.1	56.7±3.56
AuNPs	20.0±1.29	23.3±2.25	52.7±3.75	61.0±3.95

(C) 100% AuNP concentration

	REF 24(%)	REF 48(%)	MDA 24(%)	MDA 48(%)
AuNPs	11.3±1.35	14.0±1.45	37.3±2.15	44.0±2.95
AuNPs + 200Gy	14.9±1.39	18.7±1.5	47.7±2.25	57.0±3.15
AuNPs + 400Gy	17.9±1.48	23.0±1.55	50.3±3.1	61.0±3.25
AuNPs + 600Gy	21.6±1.58	25.3±1.48	57.3±3.5	67.0±4.15

In Table 1, the differences in the mean growth inhibitions between MDA and REF cells after 24 hours, and after 48 hours were found to be statistically highly significant ($P < 0.001$) compared to those treated using AuNPs alone [(A) 25%, (B) 50% and (C) 100% concentration) and those treated using AuNPs irradiated with gamma-ray doses of 200 Gy, 400 Gy, and 600 Gy. The differences between the mean growth inhibition of cells treated using AuNPs irradiated with gamma-ray doses of 200 Gy, 400 Gy, and 600 Gy and those treated by AuNPs alone were also highly statistically significant ($P < 0.001$) in the instances of REF after 24 hours and 48 hours, and MDA after 24 hours and 48 hours, as illustrated in Figure 1..

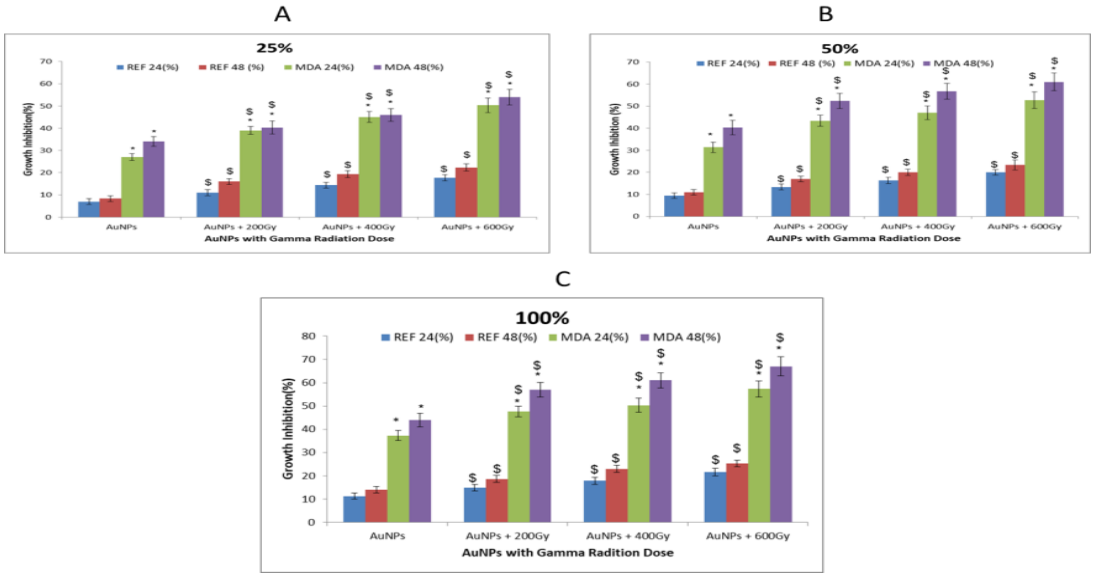

Figure 1: The relationship between the effect of (A) 25% (B) 50% and (C) 100% nanoparticle concentration, both with and without radiation treatment, on REF and MDA (24 and 48 hours).

**Significant differences compared to the mean for growth inhibition cells for MDA 24 (%) and REF 24 (%), and MDA 48 (%) and REF 48 (%) using the same treatment ($P < 0.001$).*

$^{\$}$significant differences compared to the mean for growth inhibition cells treated using AuNPs irradiated with gamma radiation doses of 200 Gy, 400 Gy, and 600 Gy) and cells treated using AuNPs alone.

Discussion:

Discovering novel and inventive therapeutic strategies to treat cancer constitutes a significant global challenge. Over the past decade, the therapeutic efficacy of certain malignant tumors has markedly improved due to advancements in cancer treatment technologies and the implementation of tailored therapy strategies. Chemotherapy is a prevalent and sanctioned method for cancer treatment (26, 27, 28). Currently, radiation is employed to treat approximately 50% of cancer patients (29). In radiotherapy technologies, it is essential to effectively direct radiation to the tumor while minimizing radiation exposure to the surrounding area to protect the integrity of nearby healthy tissue (30, 31). Therefore, our research focuses on a method to deliver radiation directly to the tumor using radioactive gold nanoparticles without exposing healthy tissue.

Various research efforts have succeeded in generating enhanced radio-sensitivity through the use of gold nanoparticles (32, 33); HeLa cells were found to be sensitive to radiation that emanated from gold nanoparticles (50 nm) when exposed to 220 keV peak X-rays (36). Previous studies show that gold nanoparticles can absorb radiation much better than tissues because they have a high atomic number (Z=79), being about 100 times more effective at keV energies. Gold nanoparticles emit X-rays when interacting with high-energy external photons that overcome the binding energy of electrons to the atom (due to the internal conversions) (34, 35). Therefore, in the current study, gold nanoparticles were exposed to high doses of radiation to convert them into unstable (radioactive) particles.

In the current study, the results showed an increase in the rate of cell death with an increasing concentration of radioactive gold nanoparticles, see table 1 and figure 1. This is because increasing the concentration of AuNPs leads to a rise in temperature due to the interaction of gold nanoparticles with cells, destroying the cell membrane, breaking DNA chains, and ultimately killing the cells. This suggests that the photo-thermal properties of AuNPs play a significant role in the biomedical application during destroying the membrane of the cancer cell (36). The results also showed an increase in the rate of cell death with increasing doses of radiation of gold nanoparticles at a given concentration. This is because increasing the dose leads to increased electron production due to the interaction of gold nanoparticles with radiation, which leads to an increase in free radicals inside the cells. This leads to the destruction of the cell, the breaking of DNA chains, and ultimately cell death. As mentioned by Dizdaroglu, there is mounting evidence for an important role by free radical-induced DNA damage resulted from interaction of radiation with water into cells. Gold nanoparticles interact with the cancer cells when injected into the body; their shape, size, and surfaces are essential in regulating their chemical and physiological reactions within the human body. Therefore, nanoparticles should be administered to patients in proportion to their body mass index (37).

Limitations:

To achieve more accurate and reliable results, the number of doses of gamma rays and concentration of AuNPs in this study should be increased. Additionally, it is preferable to thermometer to measure the increase in temperatures of the cell line when treated by AuNPs.

Recommendation:

The main recommendations of our study are as follows:

1. Employ nanotechnology as a technique for cancer treatment, exposing gold nanoparticles to a high radiation dose in experimental animals rather than cell cultures, to investigate the impact of gold nanoparticles on cancer treatment and their potential side effects on normal body tissues.
2. Using nanotechnologies to deliver chemotherapy and radiation together, study their effect on cancerous cells and normal cells, and compare the results with those of the traditional method of cancer treatment.
3. Collaborate with a radiologist and oncologist to determine the most effective method for delivering these nanoparticles to the specific cancer area, ensuring they do not circulate in the bloodstream or throughout the entire body, a process that could be accomplished through catheterization.

Conclusion:

A high dose of gamma rays combined with AuNps can keep its destruction effect on cancerous cells for 24 - 48 hours. Gamma radiation has the potential to perform a valuable function in enhancing the effectiveness of AuNPs. Gold nanoparticles carrying this dose of radiation will cause minimal damage to normal tissue.

Authors' declaration:

We confirm that all the Figures and Tables in the manuscript belong to the current study. Besides, the Figures and images, which do not belong to the current study, have been given permission for re-publication attached to the manuscript. Authors sign on ethical consideration's Approval-Ethical Clearance: The project was approved by the local ethical committee in College of Medicine, University of Baghdad and with a cooperation was made with the Biotechnology Research Center, Al-Nahrain University' and the Laboratory of Biophysical Techniques, Sciences College of Sciences for Women, University of Baghdad, according to the letter issued by the Deanship of the College of Medicine, University of Baghdad No. (4937) at (11/12/2023).

Conflict of Interest: None

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Authors' contributions:

Study conception & design: (Numan S. Dawood and Noor K. Fadhil). Literature search: (Numan S. Dawood and Noor K. Fadhil). Data acquisition: (Noor K. Fadhil). Data analysis & interpretation: (Noor K. Fadhil, Numan S. Dawood and Maan H. Al-khalisy). Manuscript preparation: (Noor K. Fadhil, Numan S. Dawood and Maan H. Al-khalisy). Manuscript editing & review: (Numan S. Dawood and Maan H. Al-khalisy).

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تأثير تركيزات مختلفة من الجسيمات النانوية الممزوجة بأشعة جاما على خلايا سرطان الثدي

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[2] فرع التشريح، كلية الطب، جامعة بغداد، بغداد، العراق.

الخلاصة
الخلفية: يُعد السرطان ثاني سبب للوفاة في العالم. ولمعالجة السرطان بفعالية، يجب أن يُعزز الإشعاع السمية الخلوية ضد الخلايا السرطانية مع الحد من إصابة الخلايا السليمة. تُساعد تقنية النانو والتقنيات الطبية الحيوية على تحسين الكشف عن السرطان وعلاجه، إلا أنها تُشكل تحديات أيضًا.
الهدف: تقييم تأثير تراكيز مختلفة من جسيمات الذهب النانوية (25%، 50%، أو 100%) المُعرَّضة لجرعات متفاوتة من أشعة غاما (200 غراي، 400 غراي، 600 غراي) على خلايا سرطان الثدي لدى النساء ومقارنتها بالخلايا الطبيعية.
المنهجية: عولجت خلايا سرطان الثدي وخلايا الثدي الطبيعية بجسيمات الذهب النانوية (AuNPs) بتركيز 25%، 50%، أو 100% لمدة 24 و48 ساعة. كما طُبِّقت طريقة أخرى مماثلة بعد تعريض جسيمات الذهب النانوية المُركَّبة لجرعات 200 غراي، 400 غراي، و600 غراي من أشعة غاما. بعد ٢٤ و٤٨ ساعة، قورنت النتائج بقيم REF وMDA. أُجريت الدراسة في مركز أبحاث التكنولوجيا الحيوية بجامعة النهرين، وامتدت من أكتوبر ٢٠٢٣ إلى فبراير ٢٠٢٤.
النتائج: لوحظت تغيرات ملحوظة في تثبيط نمو الخلايا بين MDA وREF بعد 24 و48 ساعة، وذلك باستخدام جسيمات النانو الذهبية وحدها (بتركيز 25%، 50%، 100%) وبجرعات أشعة غاما 200 غراي، 400 غراي، 600 غراي. كان متوسط تثبيط نمو الخلايا المعالجة بجسيمات النانو الذهبية المُشعة بأشعة غاما (200 غراي، 400 غراي، 600 غراي) مقارنةً بجسيمات النانو الذهبية وحدها ذا دلالة إحصائية عالية ($P< 0.001$) في حالتي REF (24 و48 ساعة) وMDA (24 و48 ساعة).
الاستنتاج: يمكن للجرعة العالية من أشعة غاما، إلى جانب جسيمات النانو الذهبية، أن تحافظ على تأثيرها التدميري على الخلايا السرطانية لمدة 24-48 ساعة. لأشعة غاما دورٌ قيّم في تعزيز فعالية جسيمات النانو الذهبية. ستُسبب جسيمات النانو الذهبية التي تحمل هذه الجرعة من الإشعاع ضررًا طفيفًا للأنسجة الطبيعية.
الكلمات المفتاحية: سرطان الثدي، جسيمات الذهب النانوية، البلازما، أشعة غاما، خط الخلية.