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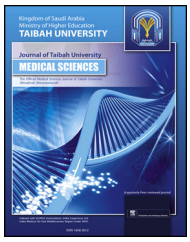

Original Article

Cold plasma enhances the generation of reactive oxygen species and the uptake of nanoparticles in cancer cells

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الملخص

أهداف البحث: البلازما الباردة، التي تعد مصدرا غنيا بأنواع الأكسجين التفاعلية وتولدها أيضا عندما تتلامس مع الخلايا، يتم التحقيق فيها لإمكاناتها في استهداف الخلايا السرطانية. تعد أنواع الأكسجين التفاعلية حاسمة في التسبب في تلف الورم وتنقسم إلى أنواع طويلة الأمد وقصيرة الأمد. من بينها، يعد تحليل الجذور قصيرة العمر أكثر أهمية لأنها مسؤولة في المقام الأول عن الضرر البيولوجي ويمكنها أيضا توليد جذور طويلة العمر. علاوة على ذلك، يعد اختيار الجسيمات النانوية لعلاج السرطان أمرا مهما، ولكن ما يهم أكثر هو مدى امتصاص الورم للجسيمات النانوية. من أجل تقليل الآثار الضارة، تكافح العديد من علاجات السرطان مع الانتقائية. يتم استخدام البلازما الباردة وجسيمات النانو الغليكوزيلية في الدراسة المقدمة لاستهداف خلايا الورم الميلانيني وزيادة إنتاج أنواع الأكسجين التفاعلية وامتصاص جسيمات النانو الغليكوزيلية.

طريقة البحث: تضمن البحث اختبار الكفاءة على خلايا الورم الميلانيني والانتقائية على الخلايا الليفية السليمة. قامت قياس التدفق الخلوي بقياس معدلات موت الخلايا المبرمج، بينما تم أيضا قياس كمية الجذور الحرة الناتجة في كل مجموعة علاجية باستخدام مقياس الجرعات الكيميائية. تم قياس متوسط محتوى الذهب الممتص في كل خلية باستخدام مطيافية الانبعاث الضوئي للبلازما المقترنة بالحث مع وبدون العلاج بالبلازما الباردة.

النتائج: أظهرت النتائج أنه في حين أن البلازما الباردة تسبب في خضوع الخلايا السرطانية لموت الخلايا المبرمج، فإن الخلايا السليمة تظل غير متأثرة. عند استخدام جزيئات الذهب النانوية، يكون هذا التأثير أعظم. أدى وجود البلازما الباردة إلى إنتاج كبير للجذور الهيدروكسيلية. بالإضافة إلى ذلك، لوحظ أن البلازما الباردة عززت امتصاص جزيئات الذهب النانوية في الخلايا السرطانية بينما لم يكن لها تأثير على الخلايا السليمة.

الاستنتاجات: تشير النتائج إلى أن الجمع بين الجسيمات النانوية الذهبية والبلازما الباردة يمكن أن يوفر خيارا علاجيا مستهدفا ومحسنا لمعالجة التحديات المرتبطة بإدارة الورم الميلانيني.

الكلمات المفتاحية: الجسيمات النانوية الذهبية؛ البلازما الباردة؛ الانتقائية؛ أنواع الأكسجين التفاعلية؛ موت الخلايا المبرمج

Abstract

Objectives: Cold plasma, which is a rich source of reactive oxygen species (ROS) and also generates them when it comes into contact with cells, is being investigated for its potential to target cancer cells. ROS are crucial in causing tumor damage and are divided into long- and short-term species. Among them, the analysis of short-lived radicals such as hydroxyl radicals ($HO^{\bullet}$) is much more important because they are primarily responsible for biological damage and can also generate long-lived radicals. Moreover, selecting nanoparticles (NPs) to treat cancer is important; however, what matters most is how well the NPs are absorbed by the tumor. To minimize adverse effects, the challenge of many cancer treatments is selectivity. Cold plasma and gold (GNPs) were used in this study to target melanoma cells, and increase ROS production and GNP absorption.

Methods: The research involved testing efficiency with an MTT assay on melanoma cells and selectivity on healthy fibroblast cells. Flow cytometry measured apoptosis rates, whereas a chemical dosimeter measured the amount of free radicals generated in each treatment group. The average gold content absorbed in each cell

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was measured with inductively coupled plasma optical emission spectroscopy (ICP-OES) with and without cold plasma therapy.

Results: The findings demonstrated that while cold plasma caused cancer cells to undergo apoptosis, healthy cells remained unaffected. This effect was greatest when GNPs were used. The presence of cold plasma led to the significant production of HO•. Additionally, it was observed that cold plasma enhanced the uptake of GNPs in cancer cells while having no effect on healthy cells.

Conclusion: The findings of this study suggest that the approach of combining GNPs and cold plasma could offer an optimized targeted therapeutic option for addressing the challenges associated with melanoma management.

Keywords: Apoptosis; Cold plasma; Gold nanoparticles; Reactive oxygen species; Selectivity

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Introduction

Melanoma is a serious form of skin cancer that originates from melanocytes in the skin.[1] Traditional cancer treatments face limitations such as toxicity and resistance, leading to a shift towards combination therapies to reduce side effects and target cancer cells.[2,3] Combination therapies have been widely used to reduce side effects and enable targeted therapy.[4,5] Nanoparticles (NPs), specifically gold NPs (GNPs), are being used for their stability and effectiveness in cancer treatment.[6,7]

Cold atmospheric plasma (CAP) is also being explored for its unique properties in cancer therapy, such as producing reactive oxygen species (ROS) that damage cancer cells.[8–10] Plasma is recognized as one of the four fundamental states of matter, possessing numerous properties attributed to the diverse compounds within its composition. It has been suggested that its electrical characteristics facilitate the opening of membrane channels, thereby enhancing the absorption of drugs. Furthermore, CAP causes anticancer effects due to ROS production.[11,12] ROS are divided into long- and short-term species.[13] In addition to the fact that CAP is a rich source of ROS, the interaction of CAP with the organic cellular component, which contains a large amount of specific amino acids, leads to more ROS production.[14] Plasma-induced ROS causes permanent DNA damage, apoptosis, and lipid peroxidation.[15–17] Most studies refer to long-term species measurement (H_2O_2, O_3, $H3O^+$, NO_2^-, and NO_3^-) due to their controllable reactivity and ease of measurement. However, the analysis of short-lived radicals ($HO^•$, $O_2^{-•}$, $HOO^•$, and $OONO^-$) is much more important because they are primarily responsible for biological damage and can also generate long-lived radicals.[18] Hydroxyl radicals ($HO^•$) are the most reactive species produced during treatment by CAP devices. The detection and measurement of $HO^•$ are often done indirectly and non-quantitatively due to experimental limitations.[19] One way to detect radicals is chemical dosimeter probes.[20] $HO^•$ react with chemical probes to produce stable compounds that can be detected by fluorescence analysis.

To realize the clinical potential applications of CAP, we investigated how helium-generated plasma affects the uptake of GNPs in metastatic melanoma and healthy cells. To this end, the study measured the absorption of GNPs in cancer and healthy cells before and after plasma exposure to develop a highly selective and effective cancer treatment. Synergistic effects were observed when plasma was combined with GNPs, showing promise for future therapies. Additionally, the study examined the levels of ROS, apoptosis/necrosis, and cell viability in various concentrations of GNPs and exposure times to plasma.

Materials and Methods

Materials

Gold (III) chloride hydrate (99.995 %) was obtained from Alfa Aesar (Haverhill, MA, USA). Fetal bovine serum (FBS) was obtained from Gibco (Grand Island, NY, USA). Perchloric acid, terephthalic acid (TA), dimethyl sulfoxide, sodium hydroxide (NaOH), and nitric acid were obtained from Merck (Darmstadt, Germany). The Apoptosis Detection Kit (Annexin V labeled with FITC/propidium iodide [PI]) was obtained from BD Biosciences (Franklin Lakes, NJ, USA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Dulbecco's Modified Eagle Medium (DMEM), Roswell Park Memorial Institute (RPMI-1640) cell culture medium, penicillin-streptomycin, trisodium citrate dihydrate, and trypsin-ethylene diamine tetraacetic acid (EDTA) were obtained from Sigma−Aldrich Company (St. Louis, MO, USA).

Methods

Cell experiments

Cell culture. RPMI and DMEM were used to culture the human melanoma cancer cells (DFW) and healthy fibroblast cells (human foreskin fibroblasts [HFFs]), both of which were obtained from the Pasture Institute (Tehran, Iran). Each medium contained 100 µg/mL streptomycin, 100 units/mL penicillin, and 10 % FBS. The cells were maintained at 37 °C in a 5 % carbon dioxide atmosphere.

Cytotoxicity assay

DFW cells (10,000/well) and HFF cells (7000/well) were inoculated into 96-well plates. Following a 24-h incubation period to facilitate cellular adhesion, GNPs (0−80 mg/L) were added to the cells and incubated for an additional 24 h to promote NP penetration. Then the cells were wasted three times with phosphate-buffered saline (PBS) to remove any free GNPs. Finally, the MTT assay was conducted.

Uptake assay

The *in vitro* intracellular uptake of GNPs was quantified by the estimation of gold, which was added to cells before and after CAP treatment. DFW cancer cells (5×10^5/well) and HFF cells (3×10^5/well) were seeded in 6-well plates. After a

full day of incubation, the cells were separated into treatment and control groups. In the control group, the cells were incubated with 20 and 40 mg/L GNPs alone. In the treatment group, cells were treated with CAP for 30 s after incubation with 20 and 40 mg/L GNPs. Then the free GNPs in the cell cultures were removed by washing the cells with PBS three times followed by trypsinization and digestion with perchloric and nitric acid solution after centrifugation. Inductively coupled plasma optical emission spectroscopy (ICP-OES) was used to calculate the amount of gold in cells. Finally, the average gold content per cell was calculated for all groups.

In vitro experiments

In the plasma jet device, a recurrent pulsed high voltage was used to create plasma between the earth electrode and the high-voltage electrode. The plasma inside the test tube was created using 99.99 % pure helium gas. The DFW and HFF cells were seeded in 96-well plates at a density of 10,000 and 7000 cells per well, respectively, and were cultured for a full day in a CO_2 incubator. Then GNPs were added to the cells at a concentration of 20 mg/L. Following a 24 h incubation, the plates were treated with CAP for 30, 60, and 90 s. The established distance between the plate and the nozzle was 20 mm. The treatment setup is shown in Figure 1. Cell survival was measured by the MTT assay for all groups. Samples were analyzed in triplicate for each experiment, and statistical analyses were performed on the resulting data.

Apoptosis assay

The apoptosis of DFW and HFF cells was detected by employing the mitochondrial staining method with FITC-Annexin V and PI (BD Biosciences) in accordance with the manufacturer's instructions. Briefly, cells were exposed to CAP for 30 and 60 s, with and without treatment with 20 mg/L GNPs. A 24-h treatment period was followed by the addition of trypsin−EDTA (0.01 mL) to each well. After being transferred to a 1.5 mL microtube, the contents of each well were centrifuged for 5 min at 3000 rpm. After the supernatant was slowly removed, the cells were transferred to a flow cytometry tube and dissolved in 0−5 mL of the kit's buffer. Then FITC-Annexin V and PI solutions (2 µL each) were added to each sample, followed by incubation for 10 min in the dark at room temperature. Finally, the samples were analyzed with the BD FACSCalibur flow cytometer.

ROS measurement assay

The efficiency of CAP therapy based on helium gas was evaluated by monitoring $HO^{\bullet}$ generation after CAP treatment using terephthalic acid (TA) dosimetry. TA is a non-fluorescent material that is easily hydroxylated by the presence of $HO^{\bullet}$ to form 2-hydroxy terephthalic acid (HTA), which can be detected by a spectrofluorometer. In brief, the dosimetric solution was prepared with 2 mM TA dissolved in 100 mL PBS and 700 mL deionized water while heated. Then 5 mL NaOH (1 M) was added to increase the pH of the TA solution. TA solution was incubated with CAP for 30 and 60 s with and without GNPs. As a control, 2 mL untreated TA solution with and without GNPs was used to identify the intensity of fluorescence induced by the treatments. The fluorescence signal was assessed at excitation and emission wavelengths of 310 and 425 nm, respectively, using a spectrofluorimeter (FP-6200; Jasco, Tokyo, Japan).

Statistical analyses

To collect all data, the experiments were repeated three times and the average was calculated. The results are presented as the mean ± standard deviation. Using the non-parametric Kolmogorov−Smirnov test, the normality of the data was determined. Then the mean differences were compared using one-way analysis of variance analysis run through SPSS software (version 21.0; IBM Corp., Armonk, NY, USA). $P < 0.05$ was considered statistically significant.

Results

Cytotoxicity of GNPs

Figure 2 shows the changes in mean cell viability induced by GNP toxicity at different concentrations in DFW and HFF cell lines. A gradual decline in the viability of both cell types was observed at GNP concentrations lower than 20 mg/L. This trend was consistent across both cell types as the concentration increased. Additionally, the findings indicated that the toxicity of GNPs was greater in cancer cells compared to healthy cells, although this difference was not statistically significant ($P > 0.05$).

Uptake assay

The effect of 30−90 s CAP treatment on GNP uptake in cancer and healthy cells, as assessed by ICP, is shown in Table 1. The results showed a concentration-dependent increase in NP uptake in both cell lines. Research has demonstrated that the presence of plasma significantly influences the uptake of nanoparticles, revealing a twofold increase in the absorption of GNPs at concentrations of 20 and 40 mg/L following the treatment of DFW cells with CAP. It is worth noting that there was no difference in GNP uptake in healthy fibroblasts before and after plasma treatment.

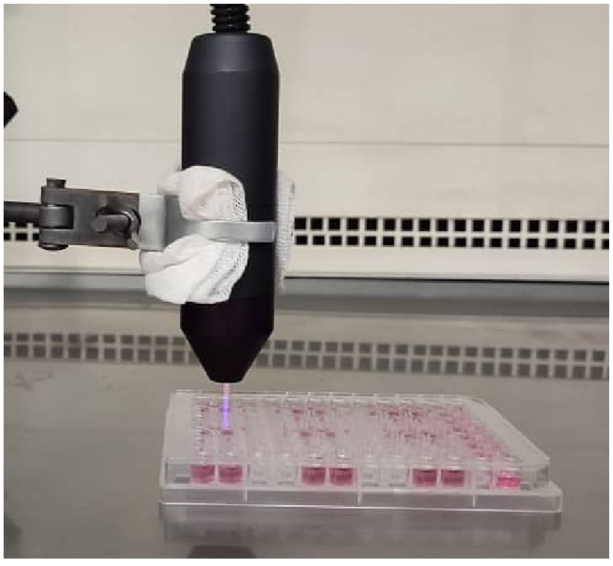

Figure 1: CAP treatment setup.

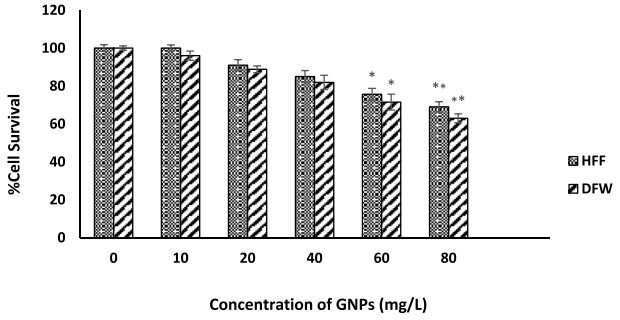

Figure 2: The average value along with its standard deviation is presented for the collected data. A detailed examination highlighting the distinctions between each treatment group and the control group is expressed as significantly different at levels of *P < 0.05 and **P < 0.01.

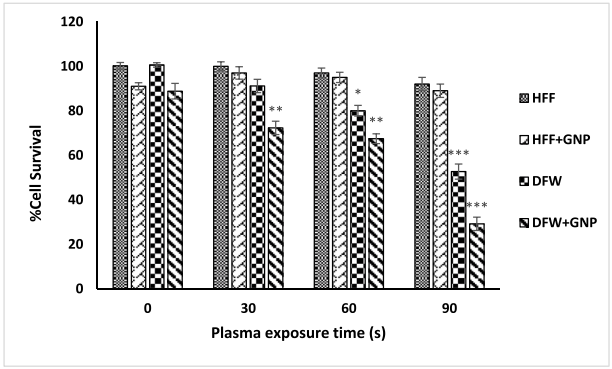

Figure 3: MTT assay results after 24 h on fibroblast (HFF) and melanoma (DFW) cell lines. The results are shown as the mean ± standard deviation. *P < 0.05, **P < 0.01, and ***P < 0.001 are the results of comparison analyses between each experimental group and the control group.

Evaluation of treatments by the MTT assay

Figure 3 shows the changes in survival of DFW and HFF cells with CAP and GNPs (20 mg/mL) and various irradiation times. The survival of melanoma cells in the absence of GNPs decreased as the duration of CAP treatment increased. The results were significantly different with CAP treatment for 90 s compared to the control group and other irradiation times, whereas treatment with 60 s CAP did not lead to a significant difference compared to the control group and the treatment group with 30 s CAP ($P = 0.009$ vs. the control group and $P = 0.035$ vs. 60 s).

GNPs incubated with CAP were much more effective than the corresponding drug-free group in eliminating cancer cells ($P < 0.05$). This effect was enhanced with treatment irradiation time. The lowest survival was observed with 20 mg/L GNPs and 90 s CAP irradiation (69 %), which was significantly different compared to the other treatment groups ($P < 0.01$).

HFF cells were treated similarly to determine whether plasma therapy is specific for cancer cell lines. Figure 3 illustrates that fibroblast cell survival was not significantly impacted by CAP, indicating that enhancing the plasma irradiation duration or dosage of GNPs will not have a major impact on CAP treatment outcomes.

Evaluation of cell apoptosis

To assess the suppression of cellular proliferation 24 h after therapeutic interventions, the quantitative levels of necrosis and apoptosis in the DFW and HFF cell lines subjected to CAP with or without GNPs were evaluated utilizing the FITC-Annexin V Apoptosis Detection Kit with PI staining. In Figure 4, the lower and upper right quadrants and the lower and upper left quadrants refer to the early and final stages of the apoptotic process, and viable and necrotic cells, respectively. The number of treated cells located in each of the defined squares (Figures 4a and 4c) was quantitatively extracted and shown graphically in Figures 4b and 4d. The results showed that CAP induced DFW cells to undergo apoptosis and inhibited their ability to proliferate in a dose-dependent manner. Consequently, the apoptosis rate increased from 16 % to 50 % when the irradiation time was extended from 30 to 90 s. In the presence of GNPs, the intensity of apoptosis reached 31 % and 76 % at 30 and 90 s plasma therapy, respectively. Moreover, the necrosis rate was nonsignificant in all treatment groups.

Additionally, it was shown that CAP and GNPs had no effect on the rate at which fibroblast cells underwent necrosis and apoptosis.

ROS measurement assay

A terephthalic acid chemical dosimeter was used to confirm the presence of $HO^{\bullet}$ in the plasma treatment and evaluate the effect of GNPs on the amount of radical production in the combined plasma treatment. Figure 5 displays the results of the fluorescence intensity of the TA solutions at plasma treatment times of 30 and 60 s both with and without GNPs. The fluorescent intensity significantly increased with increasing treatment time. It is also worth noting that GNPs alone

Table 1: Uptake of GNPs with and without plasma therapy.

	GNP-20	GNP-40	CAP30+ GNP-20	CAP30+ GNP-40	CAP60+ GNP-20	CAP60+ GNP-40	CAP90+ GNP-20	CAP90+ GNP-40
GNP/DFW cells (pg)	10.25 ± 0.05	17.87 ± 0.04	22.37 ± 0.03	32.05 ± 0.07	36.55 ± 0.02	51.88 ± 0.05	55.96 ± 0.02	66.23 ± 0.04
GNP/HFF cells (pg)	13.24 ± 0.06	20.04 ± 0.06	12.91 ± 0.05	20.53 ± 0.08	15.4 ± 0.05	23.02 ± 0.02	17.78 ± 0.09	25.14 ± 0.09

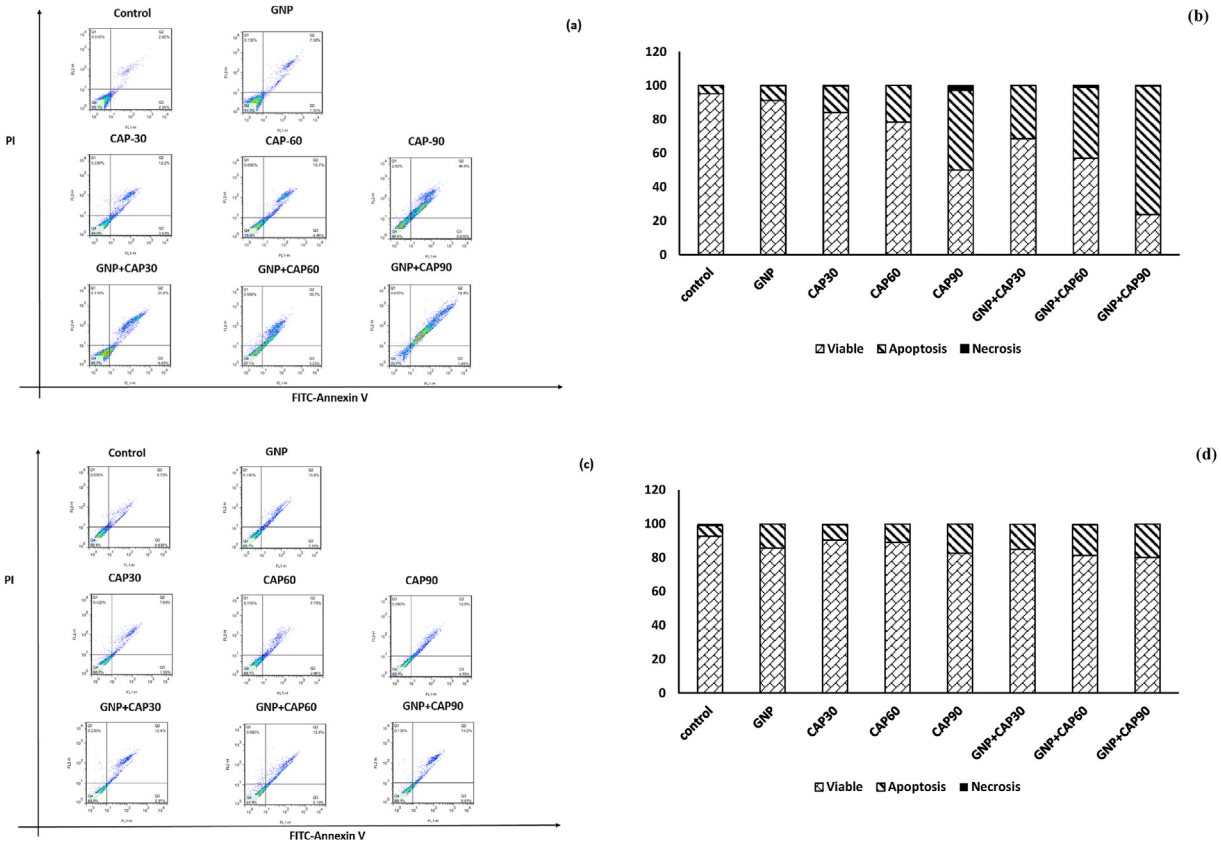

Figure 4: Findings from flow cytometry on DFW and HFF cell lines include: (a) Annexin-V-FITC assay for detecting DFW cell apoptosis and necrosis; (b) Chart displaying the percentage of necrotic and apoptotic DFW cells after treatment in different groups; (c) Annexin-V-FITC assay for detecting HFF cell apoptosis and necrosis; and (d) Chart displaying the percentage of necrotic and apoptotic HFF cells in various groups. Abbreviations: GNPs: Gold nanoparticles; CAP30: Treatment with CAP for 30 s; CAP60: Treatment with CAP for 60 s; CAP90: Treatment with CAP for 90 s; GNP + CAP30: Combination of gold nanoparticles and plasma therapy for 30 s; GNP + CAP60: Combination of gold nanoparticles and plasma therapy for 60 s; GNP + CAP90: Combination of gold nanoparticles and plasma therapy for 90 s.

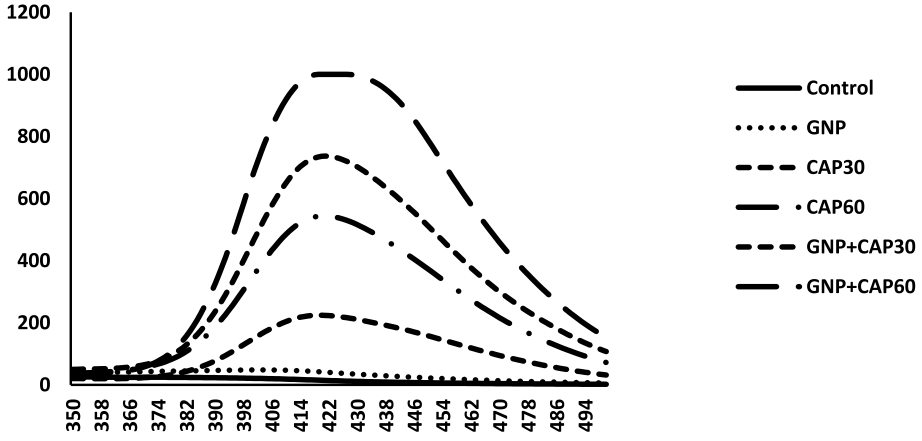

Figure 5: ROS measurement. The fluorescence signals measured by terephthalic acid dosimeter after plasma treatment at different times with and without GNPs. Abbreviations: see legend to Fig. 4.

caused a small amount of $HO^{\bullet}$, which significantly increased in combination with CAP therapy. Plasma treatment in combination with GNPs produced approximately 2-fold more $HO^{\bullet}$ than CAP treatment without GNPs.

Discussion

Since malignant melanoma is aggressive, resistant, and responds poorly to treatment, new antitumor strategies are

needed. It is appealing to make cancer treatments more selective to reduce treatment side effects.[21] CAP is a novel cancer therapy that is still being studied as its mechanisms are not fully understood. We proposed an effective strategy to improve the therapeutic efficacy of melanoma cancer, and GNPs were used in combination with CAP to enhance efficiency.[22] The mechanism of CAP is linked to the production of ROS, and GNPs enhance the ROS generated during CAP treatment.[10] CAP enhances NP uptake and distinguishes between cancer and healthy cells based on biological characteristics.[21,23]

It was observed that the toxicity of GNPs was higher in cancer cell lines than healthy cells. In line with the findings of the current investigation, Bhamidipati found that spherical GNPs are more toxic to cancer cells than fibroblast cells.[24] When CAP is applied prior to GNP incubation, it significantly enhances the uptake of GNPs in cancer cells, doubling their accumulation, while healthy cells remain unaffected. This indicates that CAP effectively increases NP uptake specifically in cancer cells.

Several mechanisms contribute to the enhanced uptake of GNPs. CAP treatment can induce temporary changes in cell membrane permeability, facilitating GNP uptake through endocytosis. Additionally, CAP may alter the surface charge or structure of DFW cells, potentially increasing their affinity for GNPs, which can also be functionalized for improved binding. Furthermore, the oxidative stress generated by ROS may activate specific signaling pathways that promote endocytosis or phagocytosis, further enhancing NP uptake.[25,26] Cheng et al. employed an atomic force microscope to demonstrate the impact of plasma on the cell membrane by recording the detailed membrane morphology.[27] They observed that cancer cell membranes changed from smooth, non-granular ridges to uneven surfaces with small pores. However, these changes were not observed in healthy cells. The absence of a confocal laser scanning microscope to identify the location of GNPs in the cells was one of the study's limitations.

The results of the MTT assay showed that CAP treatment led to a time-dependent increase in the death of melanoma cells, such that after 90 s of exposure, 50 % of the cancer cells died. A significant observation regarding plasma was the increased efficacy of plasma treatment when GNPs were incorporated. Following a duration of 90 s of plasma exposure in the presence of GNPs, a mere 30 % of the cancer cells were viable, indicating that GNPs facilitated the enhancement of the plasma's functionality. There are several possible reasons for this enhancement. The high surface area-to-volume ratio of GNPs may improve interactions with nearby molecules and increase the generation of ROS. In addition, GNPs can serve as catalysts in a variety of chemical reactions. It is also possible that the presence of GNPs speed up reactions that produce more ROS. Additionally, the interaction of GNPs with the ionized species in CAP may result in improved energy transfer, which promotes the production of ROS. Furthermore, GNPs might change the balance of ROS production and scavenging by influencing how cells react to oxidative stress.[28] Lastly, CAP and GNPs together might have synergistic effects that increase ROS levels more than either treatment alone.[10,11,16] However this hypothesis has only been tested on *in vitro* models and needs to be further investigated on *in vivo* models. Cheng's study showed a 30 % increase in cell death with combined treatment of GNPs and CAP in glioblastoma cells, as they increased intracellular ROS levels and oxidative stress.[29]

Increasing the selectivity of cancer treatments is a desirable goal as it may reduce adverse effects. CAP is a novel cancer treatment that works by disrupting the intracellular oxidative balance, which can improve selectivity. Retrospective analyses of studies on CAP revealed differences in several biological factors, despite reports claiming that CAP treatment is selective. In this study, cells with similar biological factors were used to investigate the selective effects of CAP therapy. As shown in Figure 3, CAP had a selectivity effect. In agreement with the present study, Shahmirani and colleagues exposed fibroblast cells (L929 cell line) to 120 s of irradiation with CAP activated by helium gas in the presence of GNPs at a concentration of 375 ppm, and ultimately did not observe a decrease in cell survival.[30]

The flow cytometry results showed that the apoptotic effect of CAP was much stronger with 24 h incubation of GNPs and plasma irradiation than with GNPs alone. The most important mechanism underlying the effect of plasma on living cells and induction of apoptosis is the generation of ROS.[15,31] CAP has energetic electrons, which collide with molecules of helium gas and air that generate ROS. Lipid peroxidation of the cell membrane or DNA damage is one of the processes that triggers apoptosis. When ROS produced by plasma attach to DNA or cause cell membrane lipid oxidation, the apoptotic pathway is activated, which was confirmed by the ROS measurement results in this study.

To compare the results of flow cytometry with those of the MTT assay, cells were treated under the same conditions as the MTT test. The first point to note is that the highest death rate in all groups was of the apoptotic type, and there was no difference in necrotic death rate before and after treatment. Quantitative assessment of apoptosis by flow cytometry in healthy HFF fibroblasts revealed no significant difference between CAP treatment alone or in combination with GNPs. In another study, Nitsch et al. used the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay and caspase 3/7 evaluation to investigate the apoptosis/necrosis rate of chondrosarcoma cancer cells after CAP treatment.[32] They found that 10 s plasma irradiation caused a significant increase in cell apoptosis versus the control group. A study by Saadati et al. also demonstrated that plasma activated p53 and B-cell lymphoma 2 (Bcl-2)/Bcl-2-associated X protein, which resulted in the apoptosis of melanoma cells.[33]

As mentioned in the Introduction section, the ROS generated by the plasma include long- and short-lived radicals; most studies have measured long-lived radicals.[11,18] However, the analysis of short-lived radicals is much more important because they are primarily responsible for biological damage and the origin of generation of long-lived radicals. $HO^{\bullet}$ are the most reactive species produced during water treatment by CAP devices. In this study, the rate of increase of $HO^{\bullet}$ under plasma irradiation was measured at different irradiation times and in the presence of GNPs with a chemical dosimeter. It has been confirmed that CAP produces many $HO^{\bullet}$ radicals, which are increase in the presence of GNPs. Brun et al. found that 5 min after CAP treatment, flow cytometry analysis with 2′,7′-dichlorofluorescein

showed the formation of ROS in cultured cells. Compared to intracellular ROS induced by plasma, it has been shown that hydrogen peroxide has lower concentrations and shorter half-lives.[34]

Conclusion

Clinically, melanoma patients face several challenges, and conventional therapies do not provide definitively effective treatments. Treating these patients has continued to be a major challenge for physicians. In this study, we treated metastatic melanoma cells with GNPs in combination with CAP therapy. Our findings indicate that this synergy has a great deal of potential to lessen damage to healthy cells and increase the effectiveness of cancer therapy. GNPs increased the production of $HO^{\bullet}$, in combination with CAP, indicating that $HO^{\bullet}$ are the reason for the synergistic effects of combination treatment. Moreover, CAP was found to enhance GNP uptake in cancer cells, while healthy cells were unaffected, showing that CAP is a good drug delivery method with minimal damage to healthy cells. The results of this study reveal a novel and potentially effective treatment, providing hope for a positive impact on patients' lives.

Conflict of interest

The authors have no conflict of interest to declare.

Ethical approval

The current experimental study received ethical approval from the Research Ethics Committee of the Alzahra Research Centers (Approval No. IR. ARI. MUI. REC. in 2023.10, 1402.171.11).

Authors contributions

FS was involved in the statistical analyses, data collection, and study design. SM was in charge of general supervision, analysis, and data evaluation, and participated in the study conception and design. AS greatly aided with the interpretation of the findings and the conclusions. Each author contributed to the final version of the manuscript, edited the final draft, and agreed to the submission of the manuscript. Each author is eligible for authorship and has verified that the work is free of plagiarism. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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