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Original paper

Enhancing the effect of 4 MeV electron beam using gold nanoparticles in breast cancer cells

Somayeh Sadat Mehrnia [a], Bijan Hashemi [a,*], Seyed Javad Mowla [b], Azim Arbabi [c]

[a] Department of Medical Physics, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
[b] Department of Molecular Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran
[c] Department of Radiotherapy, Imam Hossein (A.S.) Hospital, Shahid Beheshti University of Medical Science, Tehran, Iran

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ABSTRACT

Gold nanoparticles (GNPs) have been applied as radiosensitizer in radiotherapy. Limited reports have shown that GNPs may be effective as a dose enhancer agent for electron radiation therapy. Some Monte Carlo Simulation studies have shown that selecting suitable size of GNPs and electron energies are critical for effective dose enhancement. The aim of this study was to assess possible radiosensitization effect of GNPs on cancer cell treated with 4 MeV electron beams. Approximately 10 nm GNPs were synthesized and characterized by electron microscope and dynamic light scattering. MCF-7 and MDA-MB-231 breast cancer cells were used and their viability was measured by MTT assay. Radiosensitization effect of GNPs under 4 MeV electron beams was measured by clonogenic assay. The result showed a concentration dependent uptake of GNPs without reducing cell viability at concentrations $\leq$50 mg/L. Incubation of cancer cells with GNPs caused a significant decrease in their viability following exposure to electron beams as well as a decrease in their survival fraction when compared to control. The sensitizer enhancement ratio (SER) by electron beams in MCF-7 cells was 1.43 and 1.40 in presence of 25 and 50 mg/L GNPs, respectively. For MDA-MB-231 cells, it was 1.62 in presence of 25 mg/L GNPs. Our data demonstrated the significant dose enhancement of the GNPs in combination with 4 MeV electron beams that could be applicable for the treatment of superficial tumors and intra operative radiation therapy.

1. Introduction

In radiotherapy ionizing radiation is used to kill cancer cells and provide tumor control while simultaneously spars the surrounding tissues. Numerous studies have shown that the dose to tissue volumes can be significantly increased by the addition of gold nanoparticles (GNPs). This effect mainly due to greater photon absorption suggesting considerable potential for increasing cell killing through their selective delivery to cell [1–6]. Reports from different laboratories have indicated the potential of the nanoparticles as radiosensitizers [7–10]. GNPs have shown the potential to act as a radio sensitizer in ortho- and Mega-voltage photon energies [3,11–16]. In addition, GNPs in suitable concentrations are non-toxic and bio-compatible [17] therefore they can enhance the biological effect of ionizing radiations and the treatment outcomes.

Electron beams are usually selected to irradiate superficial tumors. The main reason is the relatively limited range of penetration of an electron beam compared to photons. In clinic, electron beams generated by the linear accelerators are increasingly used instead of ortho-voltage beams for skin, subcutaneous tumors and Intra Operative Radiation Therapy (IORT). This is mainly due to its distinct advantages in terms of dose uniformity in target volume and minimizing the dosage to deeper healthy tissues [18]. As an example of IORT application in breast cancer, recently Irish researchers reported the application of X-ray radiation and its protection issues following the surgical lumpectomy procedure. They irradiated the tumor bed in a group of patients with 50 kVp X-rays [19].

Although GNPs could maximize tumor dose enhancement with ortho-voltage beams, it has technical restrictions. The theoretically predicted enhancement in cell killing and the experimentally observed results are incoherent. Concerning the electron beams with growing clinical use, a theoretical study suggest the possibility of dose enhancement by gold atoms [20]. Both *in vitro* and *in vivo* studies showed that the combination of GNPs with electron beams could result in notable decrease in survival fraction of cancer cells and prolonged survival of treated mice [11,21,22].

* Corresponding author at: Department of Medical Physics, Faculty of Medical Sciences, Tarbiat Modares University, P.O. Box: 14115-331, Tehran, Iran.
E-mail address: bhashemi@modars.ac.ir (B. Hashemi).

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Chow et al. [23] showed that GNPs in suitable size increase secondary electron energy deposition ratio when applied with keV or "low" Mega-voltage energy of electron beams. Their simulation showed that the mean effective range of the secondary electrons produced by GNPs was dependent on the diameter of GNPs and the energy of electron beam. The calculated mean effective ranges of secondary electrons for electron beam energies ranging from 50 keV to 4 MeV and GNPs diameter ranging from 2 to 100 nm were 0.5–15 μm outside the nanoparticles, which is approximately within the dimension of a living cell. This is important for the transport of energy deposition. A longer effective range can enhance the cell killing probability [17,24]. However, related experimental works on GNPs irradiated by electron beams is not available. This study evaluates the possible dose enhancing effect of 10 nm-GNPs on 4 MeV electron beams.

2. Materials and methods

2.1. Preparation of GNPs

GNPs were synthesized based on previous methods [25] with some modifications. Briefly, all experimental dishes were thoroughly washed in Aqua Regia (3 parts HCl and 1 part HNO3), and all solutions were prepared using 18-MΩ-deionized water. 50 mL of HAuCl4 (0.25 mM) was reduced with sodium citrate (1%w/v, 2 mL) by boiling and vigorous stirring for 10 min. The resultant burgundy suspension was cooled, sterile-filtered and stored in glass bottles at 4 °C. The quality of GNPs was checked using dynamic light scattering (DLS) and transmission electron microscopy. In the method used, GNPs are typically synthesized using citrate-reduction of Au ions in an aqueous solution, which results in the coverage of GNP surface with citrate anions. Some reports have shown that citrate covered GNPs may be toxic at doses higher than 500 μM when exposed to the cells for 48 h [26]. However, as mentioned before we used significantly lower doses and 24 h exposure to avoid possible direct toxicity by synthesized GNPs.

2.2. Cell culture

Breast cancer cell lines MCF-7 and MDA-MB-231 were obtained from Iranian Biological Resource Center. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 1% penicillin -streptomycin, 2 mM L-glutamine, and 10% fetal bovine serum (FBS) at 37 °C in an atmosphere of 5% CO2 incubator.

2.3. MTT assay

the cells' viability was assessed using MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma-Aldrich) assay to determine their toxicity following exposure to various GNPs concentrations and/or electron beam. The cells were seeded in the 96-well plates and 24 h later were treated with different concentrations of GNPs (0, 12.5, 25 and 50 mg/L) for additional 24 h. The cytotoxicity of the GNPs was assessed by adding MTT solution in fresh medium for 4 h. The cells were collected in DMSO (Dimethyl sulfoxide) and placed on shaker for 5 min. The absorbance of final solution was measured at 540 nm using a 96-well plate reader (ELISA-Reader, Hyperion, Canada). Results were normalized to control and presented as percentages of absorbance for untreated control cells. Three independent experiments were done for each data point. For determining the effect of radiation on the cell survival, the irradiated cells were seeded in 96-well plates for 3 days. Afterward, MTT assays were performed as mentioned above.

2.4. GNPs uptake assay

The uptake of GNPs by cells was quantified by atomic absorption spectroscopy (AAS) (SHIMADZU AA-670G, Japan). For ASS measurements, 70,000 cells were seeded per well in 24-well plates in 0.5 mL of complete culture medium. 24 h post seeding, cells were treated for another 24 h with 6, 12.5, 25 and 50 mg/L of GNPs solutions. At the end of the exposure time, the medium was removed, wells were washed for 3 times with phosphate-buffered saline (PBS), and exposed to 0.05% Trypsin-EDTA for 2–3 min. Fresh complete medium was added and cells were collected for counting. Then each sample was collected in a separate tubes. The amount of Au was analyzed by AAS after mineralization with Aqua Regia and sonication and normalized to cell count. Three independent experiments were carried out and results were expressed as Au concentration ng/cell.

2.5. Irradiation procedure

4 MeV electron irradiations were performed using a Varian linear accelerator (LINAC) (Varian, Clinac 2300C/D, USA) following a dosimetric calibration. Cells were seeded in 12-well plates 48 h prior to irradiation. 24 h post seeding, cells were exposed to GNPs in different concentrations (0, 12.5, 25, 50 mg/L) for another 24 h. Before irradiation, GNP containing medium was replaced with the fresh complete medium. Medium level was adjusted to 7 mm over the cell monolayer to place the cells at d_{max} of radiation dose. Irradiations were performed using a 25 × 25 cm applicator positioned at the top of dish. The cells were set at 100 cm distance from the electron source. Irradiations were done in single fractions with constant dose rate of around 1 Gy per monitor unit. The cell culture plate was placed at the center of the electron beam to ensure that all the cells receive a uniform radiation dose. The radiation dose was also confirmed using a parallel-plate ion chamber.

2.6. Clonogenic survival assays

The effectiveness of GNPs, radiation and their combination on cell survival and renewal was assessed by clonogenic assay. Clonogenic assay is a gold standard assay for measuring the distractive effect of radiation on cell genome. Following exposure to 0–6 Gy electron beams, cells were washed 3 times by PBS, trypsinized for 2–3 min, and re-suspended in complete medium, counted, and replated in six-well plates. The cultures were maintained incubated for 14 days without medium change. Cellular colonies were fixed using 10% formalin and then stained with 0.1% crystal violet for colony count. Surviving fractions (SF) were calculated relative to starting plated cells and normalized to the non-irradiated control cells. Data was plotted and fitted to the linear-quadratic equation, $SF = \exp(-\alpha D - \beta D^2)$.

2.7. Statistical analysis

All experiments were carried out in triplicate and repeated for 3 times. Results are expressed as Mean ± SEM. Statistically significant differences were calculated using one-way analysis of variance for MTT assays and Two-way analysis of variance for clonogenic assays followed by Tukey post hoc. P values less than 0.05 were considered as statistically significant.

3. Result

3.1. Characteristics of GNPs

Fig. 1A shows the UV–Visible spectrum GNPs solution. Maximum absorption was observed at 516 nm. Fig. 1B shows the GNPs