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DOSE ENHANCEMENT EFFECTS BY DIFFERENT SIZE OF GOLD NANOPARTICLES UNDER IRRADIATION OF MEGAVOLTAGE PHOTON BEAM

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ABSTRACT

Gold nanoparticles (AuNPs) have been extensively investigated as dose enhancement agent to increase the lethal dose to the tumours while minimizing dose to the normal tissue. Their intriguing properties and characteristics such as small size and shape provide favorable option in increasing radiotherapy therapeutic efficiency. In this study, the effects of AuNPs size on the dose enhancement effects irradiated under megavoltage photon beams were investigated. The study was conducted in-vitro on HeLa cells using AuNPs of 5 nm and 15 nm sizes. The cells samples were incubated with AuNPs and irradiated with photon beam of energy 6 MV and 10 MV at 100 cm SSD and 10 cm x 10 cm field size. Clonogenic assay were performed to observe the dose enhancement effects on cell survival. Dose enhancement factor (DEF) were extrapolated and evaluated from the cell survival curves. The results show that both sizes of AuNPs produce dose enhancement with the larger size AuNPs of 15 nm produce more dose enhancement compare to 5 nm AuNPs for 6 MV photon beam. Dose enhancements were observed for 10 MV photon beams but DEF for both sizes AuNPs shows no differences. In conclusion, larger size AuNPs produce higher dose enhancement compare to small size of AuNPs which conclude that nanoparticles size is important factor that need to be taken into account for AuNPs to be applied in radiotherapy.

Keywords: Dose enhancement, gold nanoparticles, megavoltage photon beam, radiotherapy

INTRODUCTION

The goal of radiation therapy is to maximize the dose to malignant cells while minimizing exposure to normal tissues. The effects of radiation are not immediate and benefit of the treatment occurs over time. Tumour controls are normally achieved by delivering highly conformal radiation dose to the tumour but in many cases complication to normal tissue is inevitable. Modernistic advances in nanotechnology have provided new opportunities for flourishing targeted radiotherapy modalities using gold nanoparticles (AuNPs) to further increase radiotherapy therapeutic efficacy. Hence better sparing of normal tissue could be accomplished.

In the recent years, application of nanoparticles in cancer diagnosis and treatment has been the issue of extensive research. Dose enhancement effects by gold nanoparticles in radiotherapy particularly have been explored for their potential clinical application and yet the results are still inconclusive.

The central rationale behind application of AuNPs in radiotherapy is mainly due to high atomic number (Z) properties that naturally have high radiation interaction cross section that leads to increase absorbed dose (Mesbahi, 2010). In addition, gold (Au) is inert and stable metal. Gold atoms present in nanometer sized particles have their basic properties of metal type materials such as melting point, crystal structure, conductivity, magnetic properties and optical properties completely altered from bulk materials (Daniel and Didier, 2004). Utilisation of AuNPs for radiotherapy is highly desirable because of its ability to absorb radiation and therefore increase the dose to the tumours if they were included in the tumour prior irradiation (Mesbahi, 2010). Targeting AuNPs as dose enhancement agents will facilitate escalation of radiation dose to the tumours and hence increase cancer cell damage. Compared to the other common metallic contrast agents employed in radiotherapy for dose enhancement such as iodine and gadolinium, gold has a higher Z (Hainfeld et al., 2008). Moreover, gold nanoparticles have the potential to treat cancer without any of the harmful side effects associated with current treatment methods (Hainfeld et al., 2008).

Preclinical investigations have reported the effectiveness of small AuNPs to enhance radiotherapy (Hainfeld et al., 2004; Rahman et al., 2009). The size dependency of AuNPs dose enhancement effects have been reported by Zhang et al. (2011) found optimal size be around 12 to 27 nm. Other study reported dose enhancement by AuNPs of 4.6 and 6.1 nm (Liu et al., 2010). It is suggested that 50 nm induced more radiosensitivity compare to14 and 74 nm gold particles due to their higher uptake in cells (Chithrani et al., 2010). Significant dose enhancements were also observed for 10 nm AuNPs coated with cysteamine and glucose when irradiated with x-rays of different energy (Kong et al., 2008). Similar to previous studies, kilovoltage beam energy is being preferred to produce higher dose enhancement. However, kilovoltage beam have limited application in clinical radiotherapy that mainly used megavoltage beam from linear accelerator to treat various types of cancer. Therefore, in this study the size dependency of AuNPs dose enhancement effects were investigated using clinical megavoltage photon beam. Different photon beam energy is used to elucidate the energy dependency effects of AuNPs dose enhancement.

MATERIALS AND METHODS

Gold Nanoparticles Preparation

The experiment was carried out using two different sizes of Gold nanoparticles; 5 nm and 15 nm. The first batch of gold nanoparticles (AuNPs) was purchased from Nanoprobes (Aurovist^{TM}) whereas the second batch of were purchased from NanoHybrids Advanced Imaging Solutions. The AuNPs were prepared for in-vitro studies by diluting in Dulbecco's Phosphate Buffered Saline (D-FBS) (Gibco, Life Technologies, CAL, USA).

Cell Culture Protocol

HeLa cell line was purchased from ATCC-HTB-4^{TM}. The cells were maintained in Dulbecco's Modified Eagles medium (DMEM), 10% fetal bovine serum (FBS) and 1% antibiotics (10,000 units/mL penicillin and 10,000 µg/mL streptomycin) (Gibco, Life Technologies). In a humidified environment of 95% air and 5% CO_2 at 37°C, the cells were incubated and subcultivated twice a week.

Cells Irradiation

The cell samples were prepared to have approximately 1000 cells per samples with and without AuNPs. Concentration of AuNPs used for both sizes are 1 mMol/L. The cells irradiations were performed with 6 MV and 10 MV photon beams from Primus linear accelerator (Siemen Healthcare, USA) at Nuclear Medicine, Radiotherapy and Oncology Department, Hospital Universiti Sains Malaysia. The cells samples were irradiated at source to surface distance (SSD) of 100 cm and field size 10 x 10 cm^2 according to the setup in Figure 1. Bolus was placed on top of the cell samples as a buildup so that the maximum dose was delivered to the cells (bolus thickness for 6 MV: 1.5 cm, 10 MV: 2.5 cm). Irradiations were done with constant dose rate of 100 MU/min of radiation doses ranging from 0 Gy to 10 Gy.

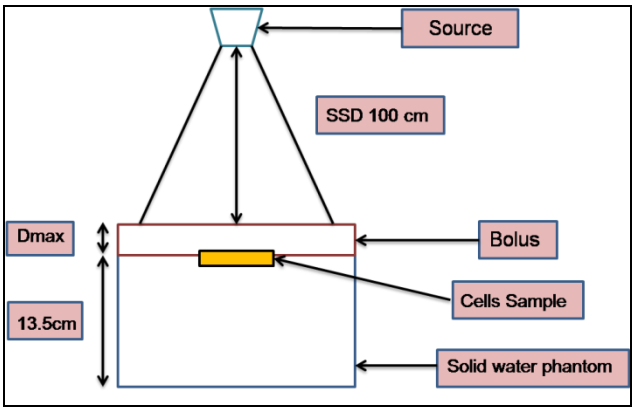

Figure 1: The experimental setup for cells irradiation

Clonogenic Assay

Subsequently after irradiation, the cell samples were seeded in 6-well plates (SPL Life Sciences, Korea) and were incubated at 37°C in a humidified environment of 95% air and 5% CO_2 for 7 days. After 7 days the cells were fixed with cold methanol for 30 minutes. Then the fixed cells were stained with crystal violet for 1 hour. The crystal violets were rinsed with tap water and the stained cell samples were left to dry. The visible colonies were then counted with naked eyes.

Cell Survival Analysis

The cell survivals were analyzed by calculating the survival fraction from the cell colonies according to the equation 1. The survival fraction for samples with and without different sizes of AuNPs were then plotted and fitted according to the linear quadratic (LQ) model using OriginPro 8.5 software. The dose enhancement factors (DEF) were extrapolated and calculated by taking the ratio of dose that produces 50% of cell survival fraction for control cells to treated cells with AuNPs as depicted in equation 2.

$$Survival\ fraction = \frac{Number\ of irradiated\ cell\ colonies}{Number\ of\ control\ cell\ colonies} \quad \text{.....(1)}$$

$$DEF_{50} = \frac{D_{50,control}}{D_{50,AuNPs}} \quad \text{.....(2)}$$

Statistical Analysis

The data are expressed as the mean ± standard deviation of three samples. Statistical analysis was performed using one way analysis of variance (ANOVA) using OriginPro 9.2 software.

RESULTS

The cell survival curves for cells irradiated with 6 MV photon beam is depicted in Figure 2. The cell survival curves show the reduction of cell survival and potential cellular damage cause by inclusion of 5 nm and 15 nm AuNPs in the cells irradiated under 6 MV photon beam. The value of DEF obtained is 1.78 and 2.29 for 5 nm and 15 nm AuNPs, respectively. Irradiation of cells with 10 MV photon beam garnered similar DEF for both 5 nm and 15 nm which is around 1.31. Figure 3 show the cell survival curves irradiated with 10 MV photon beams. Similar to irradiation with 6 MV photon beam, enhancement of dose was also observed in cells irradiated with AuNPs compare to control. DEF values indicate that 15 nm AuNPs show better dose enhancement effects compare to 5 nm AuNPs. The DEF values for all beam energies and AuNPs sizes investigated are tabulated in Table 1.

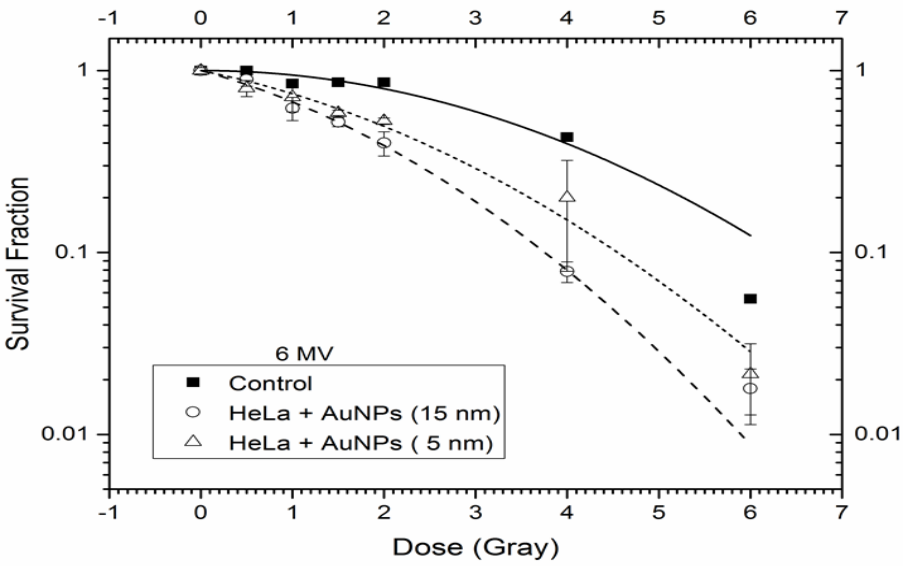

Figure 2: Survival curves of HeLa cells with 5 nm and 15 nm AuNPs irradiated with 6 MV photon beam

Radiobiological analysis from linear quadratic formalism shows the increment of α and β values for AuNPs of 5 nm and 15 nm compare to control. The α value representing direct killing of cells by single hits (linear parameters) meanwhile the β values indicate the impacts of cells killing from double hits (quadratic parameters). The α/β ratio is the point at which linear cell kill is equivalent to quadratic cell kill. The value α/β also increases for AuNPs samples which are 0.389 for 5 nm of AuNPs and 4.039 for 15 nm of AuNPs with irradiation 6 MV and 0.904 for 5 nm and 1.496 for 15 nm with irradiation 10 MV, respectively compare to 0.025 for control.

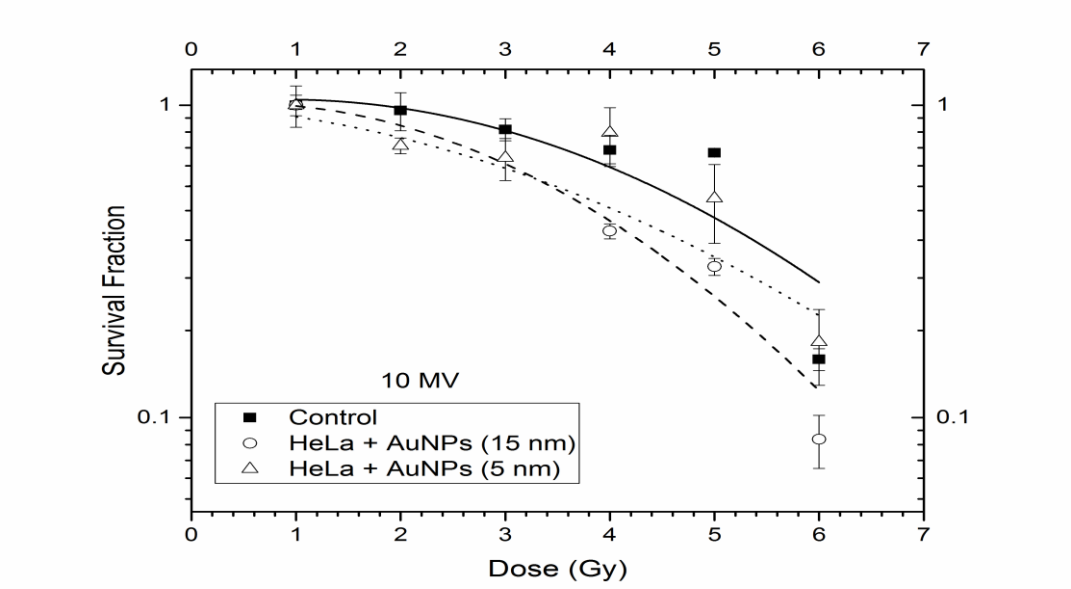

Figure 3: Survival curves of HeLa cells with 5 nm and 15 nm AuNPs irradiated with 10 MV photon beam

Table 1: The DEF value and radiobiological Parameters of Linear Quadratic Model

Photon Beam Energy	Size	Alpha (α)	Beta (β)	α/β Ratio	DEF
	Control	-0.00148 ± 0.04525	0.05823 ± 0.01524	0.025	1
6 MV	5 nm AuNPs	0.23329 ± 0.03664	0.05992 ± 0.01616	0.389	1.78
	15 nm AuNPs	0.31755 ± 0.05808	0.07863 ± 0.03305	4.039	2.29
10 MV	5 nm AuNPs	-0.06221 ± 0.06487	0.06882 ± 0.01552	0.904	1.31
	15 nm AuNPs	0.05155 ± 0.0604	0.03446 ± 0.01608	1.496	1.31

DISCUSSIONS

The dose enhancement effects are seemed to be prominent for larger sizes AuNPs. The cell survival curves for 15 nm AuNPs show highest reduction in cell survival compare to cell survival for 5 nm AuNPs. This effect appears to be related to the higher number of AuNPs present in the cells since the amount of intercellular gold increased with the size of the nanoparticles (Chithrani et al., 2010). Thus, more radiation interaction occurs corresponding to the amount of AuNPs in the cells. Larger sizes nanoparticles also may contain more gold atom which increase the radiation interaction cross section and production of secondary electron.

The most probable interaction that happened at megavoltage energy is Compton interaction (Mesbahi, 2010). Compton interaction occurs when the photon interacts with the free electron of the atoms or loosely bound electron. The electron will receive some of the energy from the incident photon and the rest of the energy will be emitted as a scattered photon. In this process, the incident energy must be large compared to the binding energy of the electron. The presence of gold atoms with high Z inside the cell might generate a larger number of secondary electrons from the radiation interactions in comparison to those generated in the absence of AuNPs. The number of secondary electrons is increase in conjunction with the interactions and the resulting "free radicals" leads to an increase in cell death, since these free radicals can damage the DNA molecules inside the cells (Mesbahi et al., 2013).

In this study, energy dependency is difficult to clarify considering clinical beam are generated in spectrum. Previous study shows there is no significant dose enhancement with high energy photons. As discussed by Mesbahi et al. (2013), the dose enhancement values for the low energy beams were meaningfully higher than megavoltage beams, because the photoelectric absorption coefficients of gold at K- (80.7 keV) and L- (11.9 - 14.4 keV) were high. Our results show promising outcome for application of AuNPs for megavoltage beam radiotherapy. In term of AuNPs sizes, the results in this study were close agreement with previous study conducted by Chithrani et al. (2010) who used nanoparticles in the size range from 14 – 74 nm. In agreement with results from this study, they also found evidence of dose enhancement effects at clinically relevant photon energies for cells with internalized AuNPs (Chithrani et al., 2010).

CONCLUSIONS

This study has found that the dose enhancement effects occur at megavoltage radiotherapy beams with 5 nm and 15 nm sizes of AuNPs. HeLa cells irradiated with 15 nm AuNPs showed higher DEF value compare to 5 nm AuNPs when irradiated with 6 MV photon beams meanwhile no difference were observed for 10 MV photon beams. The curves also demonstrate steepest slope and small shoulder for larger size AuNPs which indicate increase in biological effects. The LQ parameters such as alpha, beta and alpha/beta ratio are in agreement with the DEF results. The results strengthen the idea that the dose enhancement effects could be achieved for megavoltage photon beams and are dependent on the size of AuNPs. Considerably more work need to be done on different sizes, concentration and other properties of AuNPs in order to ensure clinical translation of AuNPs in radiotherapy.

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