# THE POTENTIAL COMBINATION OF AUNPS WITH KILOVOLTAGE SYNCHROTRON RADIATION: RADIOBIOLOGICAL EVALUATION

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## ABSTRACT

*Optimum and selective approaches to treat cancer become crucial aspects in radiotherapy. The promising application radiosensitizer such as gold nanoparticles (AuNPs) that capable to be internalize and moving in the vicinity of the nucleolus environment could potentially be a suitable technique to achieve conformality in radiotherapy. AuNPs in combination with kilovoltage synchrotron beams is predicted to give an impeccable damage to tumor through the photoelectric effects and hence contribute the radiosensitization mechanisms. However minimal understanding in the radiobiological and physical impacts might hinder this application from clinical trials. In this study, kilovoltage synchrotron beam of energy 70 and 81.5 keV combined with AuNPs were tested on P815 with and without AuNPs. Cell viability assay were conducted and cell survival were calculated. Cell survival curves generated from radiobiological models were fitted to the experimental cell survival data. Radiobiological model of linear quadratic (LQ) and Pade' linear quadratic (PLQ) were implemented in this study. Sensitization enhancement ratio (SER) was used to measure the radiosensitization effects. The effects of AuNPs are obvious especially for 70 keV beam energy with highest SER of 2.32 extrapolated from PLQ's cell survival curves. The 81.5 keV produced less SER of 1.32 from PLQ's model cell survival curves. Meanwhile, LQ model depicted lower SER of 1.25 and 1.24 for 70 and 81.5 keV respectively. It is concluded, the combination of AuNPs and synchrotron beam irradiation may give optimum treatment towards targeted tumor with suitable radiobiological model to fully evaluate and understand the potential towards clinical breakthrough.*

## ABSTRAK

*Pendekatan optimum dan selektif untuk merawat barah menjadi aspek penting dalam radioterapi. Aplikasi radiosensitizer yang menjanjikan seperti nanopartikel emas (AuNP) yang mampu menginternalisasi dan bergerak di sekitar persekitaran nukleolus berpotensi menjadi teknik yang sesuai untuk mencapai kesesuaian dalam radioterapi. AuNP dalam kombinasi dengan pancaran kilovoltage synchrotron diprediksi akan memberikan kerosakan yang sangat baik pada tumor melalui kesan fotolistrik dan dengan itu menyumbang mekanisme radiosensitisasi. Walau bagaimanapun, pemahaman yang minimum mengenai kesan radiobiologi dan fizikal dapat menghalang aplikasi ini dari ujian klinikal. Dalam kajian ini, pancaran kilovoltage synchrotron tenaga 70 dan 81.5 keV digabungkan dengan AuNP diuji pada P815 dengan dan tanpa AuNP. Ujian daya maju* *sel dilakukan dan kelangsungan hidup sel dikira. Keluk kelangsungan hidup sel yang dihasilkan dari model radiobiologi dipasang pada data survival sel eksperimen. Model radiobiologi kuadratik linier (LQ) dan kuadratik linier Pade (PLQ) dilaksanakan dalam kajian ini. Nisbah peningkatan kepekaan (SER) digunakan untuk mengukur kesan radiosensitisasi. Kesan AuNP jelas terutama untuk tenaga pancaran 70 keV dengan SER tertinggi 2.32 yang diekstrapolasi dari keluk kelangsungan hidup sel PLQ. 81.5 keV menghasilkan kurang SER sebanyak 1.32 dari keluk survival sel model PLQ. Sementara itu, model LQ menggambarkan SER yang lebih rendah 1.25 dan 1.24 masing-masing untuk 70 dan 81.5 keV. Kesimpulannya, kombinasi AuNP dan penyinaran sinar synchrotron dapat memberikan rawatan yang optimum terhadap tumor yang disasarkan dengan model radiobiologi yang sesuai untuk menilai dan memahami sepenuhnya potensi penembusan klinikal.*

Keywords*:* gold nanoparticles; radiotherapy; synchrotron; radiobiological; in-vitro

## INTRODUCTION

The restriction of higher doses at tumour site became the main factor to avoid the side effects of ionizing radiation in surrounding normal tissues. Alternative way to overcome the problem, by implementing the concept of radiosensitization which aims to increase the absorbed dose at the accumulated high atomic number of materials in cancerous cells only, while sparing the normal tissues surround it.

The first attempt to apply radiosensitizer by Matsudaira et al (1980) was by using iodine as radioenhancer element. Nevertheless, the achievement in nanotechnology enable to produce nano-sized metal that have special characteristics such as biocompatible, easy to synthesis, high surface ratio and tuneable. The most favourable research was focused on the application of AuNPs as one of the potential candidate of radiosensitizer in radiation therapy.

Briefly, AuNPs feature particles that embody a several thousands of high atomic number atoms within each single nanoparticles unit. As a result, nano-sized gold capable to be internalize into the cells and moving in the vicinity of the nucleolus environment. Therefore significantly may elevate the radiotherapeutic doses toward nucleus molecules, mitochondria or other important organelles via resulting DNA damage and increasing reactive oxygen species (ROS) (Pelletier et al., 2018).

According to studies which are widely investigated on AuNPs, the most efficient or optimum energy in generating effective dose physical enhancement was by using kilovoltage energies which also considering the incident photon energy must be higher than the binding energy of the gold K-shell atoms, thus able to result photoelectric interactions for the benefit of dose enhancement factors (Hainfeld et al., 2004; Rahman et al., 2009; Liu et al., 2010).

The revealing impact on the predicted physical and biological mechanism in the implication of AuNPs as radiosensitizer combined with kilovoltage irradiation energy. Further investigation had been done on the concept in vitro or in vivo approaches. Khoshgard et al,. (2014) had investigated the effects of dose enhancement under orthovoltage superficial radiotherapy techniques, it was shown that the DEF quantification was reaching up to 1.64 and 1.35 for specialized GNPs obtained with the 180 kVp x-ray beam. Evidence on dose enhancement also found to be comparable by the finding of Rahman et al., (2009), proven that AuNPs effectively enhance the radiation effects with kilovoltage-energy-range x-ray irradiation. The research showed a significantly elevation of DEF with maximum up to 24 by 80 kVp x-ray at 1 mmol of AuNPs (Rahman et al., 2009). Somehow in order to quantify the DEF values which dependence on the beam energy, monoenergetic beam will translate into better dose enhancer factor cause by higher atomic number materials. It was further verified by experimental study using synchrotron-generated monoenergetic X-ray towards AuNPs as radioenhancer, using irradiations range from 30-100 keV. The outcome is somewhat expected that the radiosensitivity enhancement was obtained with DEF maximum at 40 keV with a value of 3.47.

Based on previous studies, utterly monoenergetic synchrotron light that can produced highly collimated beam in a thin laminar beam, emitted hard x-rays beam in very short pulses with a high degree of polarization were investigated to cause an effects to cells with AuNPS. In this study, the effects of AuNPs on cells were analysed by using different radiobiological models and radiosensization effects quantification approaches with different monochromatic synchrotron energy. In revealing the essential factor influence the cellular response to the interaction of AuNPs with synchrotron beam irradiation, hence able to quantify the radiosensitizing effect caused total damage of biological system.

## METHOD AND MATERIALS

The irradiations were performed using P815 cell lines with 70 and 81.5 keV of monoenergetic synchrotron x-ray beam. The cells samples were prepared in suspension into 0.5 ml microcentrifuge tube, with 1000 cells count/tube, then 1 mMol/L concentration of AuNPs were mixed directly into the cells sample. Each of the treatment samples were prepared triplicate and coupled with control samples (without AuNPs). All those preparations were performed at specialised cell culture lab in the Imaging and Medical beamline, Australian Synchrotron.

After irradiation the viability of the cells with and without AuNPs were analysed and cell survival were calculated. The cell survival curves were plotted and further characterized using two radiobiological models: Linear Quadratic (LQ) and Pade' Linear Quadratic (PLQ). The sensitization enhancement ratio (SER) was then extrapolated from the cell survival curves generated from both models.

### *Linear quadratic model*

The LQ model is the most commonly used radiobiological model to investigate the radiation response of both *in- vitro* and *in- vivo* radiation treatments. The LQ model is expressed in equation Error! Reference source not found.:

$$ln\ (S_F) = (\boldsymbol{\alpha} D + \boldsymbol{\beta} D^2) \qquad (1)$$

*D is the dose*

*$\alpha$ represents the direct killing of cell by "single hit",*

*$\beta$ represent the impact of cell killing from "double hits"*

This model stems from the curvilinear nature of dose-response curves of the log of cell survival. The curvature is assumed to be related to the production of DNA double-strand breaks (DSBs) by two different radiation tracts. Two of such DSBs or subset of DSB is required to produce a lethal lesion such as a dicentric chromosome aberration (Brenner, et.al, 1998).

### *Pade' linear quadratic model*

Pade' Linear Quadratic is known as a novel biophysical model. This model shows a smooth transition, which explained rational function fit automatically linear at both low and high doses.

The model is shown as in equation 2,

$$ln\ (S_F) = (\boldsymbol{\alpha} D + \boldsymbol{\beta} D^2)/(1 + \boldsymbol{\gamma}\ D) \qquad (2)$$

Consist of three parameters:

1. *Radiosensitivity $\alpha$ is a single event inactivation constant in units of $Gy^{-1}$.*
2. *Parameter $\beta$, which is in units of $Gy^{-2}$, represents the delay time response to irradiation imposed and associate with recovery time $\tau$.*
3. *Parameter $\gamma$, the reciprocal of the Michaelis-Menten constant, $K_M$, theoretically about the chemical kinetics for enzyme catalysis. Accumulate the quantity of the lesions concentration. (D. Belkic and K. Belkic, 2013)*

## RESULTS

Figure 1 show the cell survival curves at 70 keV and 81.5 keV fitted by using LQ models. The fitted survival curves indicated lack of agreement with the experimental data. On the other hand, the LQ parameters presented in Table 1 and 2 demonstrate increase in $\alpha$ value along with variable $\beta$ and $\alpha/\beta$ values . The LQ parameters significantly portray the interaction of double strand break at the early initial event which caused cell inactivation or death.

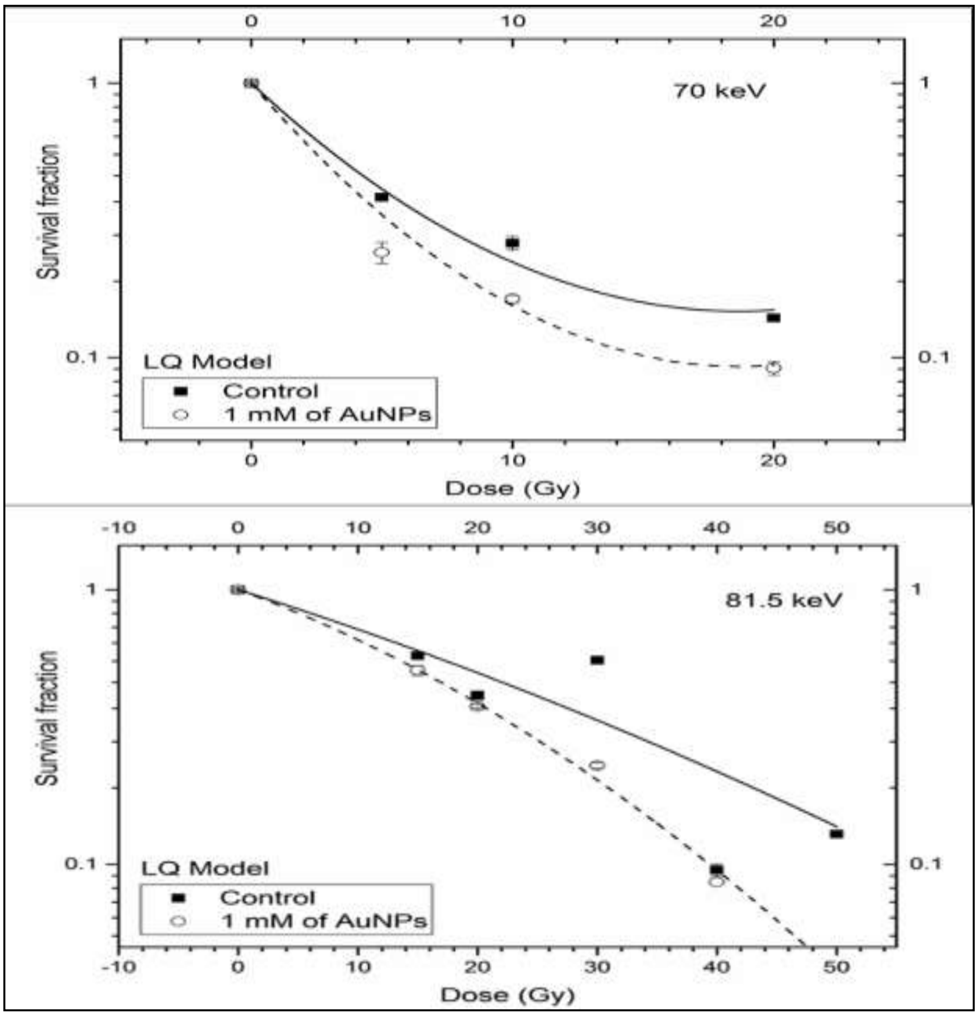


Fig. 1: Linear quadratic model curves for 70 keV and 81.5 keV.

Table 1: Parameters of linear quadratic model for 70 keV

| Cell Samples | LQ Parameters | | | |
|---|---|---|---|---|
| | α | β | α/β | $X^2$ |
| Control | 20.499 ±1.917 | -54.938 ±126 | 0.373 | 0.995 |
| AuNPs | 25.490 ±2.235 | -64.384 ±13.506 | 0.396 | 0.999 |

Table 2: Parameters of linear quadratic model for 81.5 keV

| Cell Samples | LQ Parameters | | | |
|---|---|---|---|---|
| | α | β | α/β | $X^2$ |
| Control | 3.162 ±1.774 | 1.617 ±5.716 | 1.954 | 0.873 |
| AuNPs | 3.642 ±0.363 | 5.588 ±1.485 | 0.652 | 0.998 |

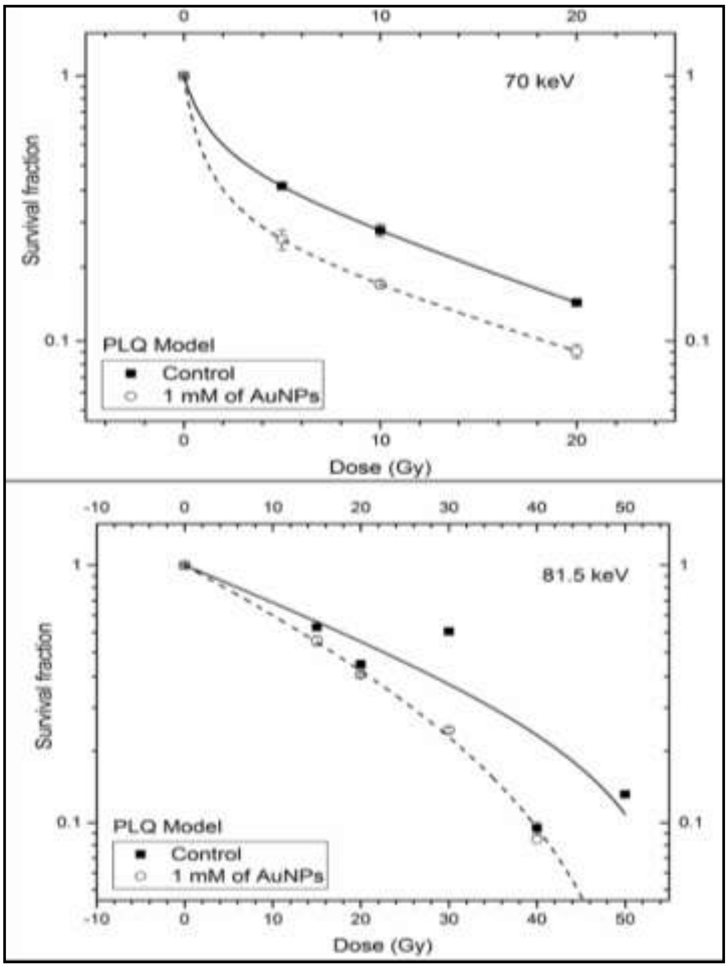


Fig. 2: Pade' linear quadratic model survival curves for 70 keV and 81.5 keV.

Table 3: Parameters of Pade' linear quadratic model for 70 keV

| Cell Samples | PLQ Parameters | | | |
|---|---|---|---|---|
| | α | β | γ | $X^2$ |
| Control | 57.81 ±2.51 | 324.46 ±324.46 | 57.32 ±4.02 | 1 |
| AuNPs | 114.91 ±3.04 | 367.96 ±1.56 | 73.89 ±2.55 | 1 |

Table 4: Parameters of Pade' linear quadratic model for 81.5 keV

| Cell Samples | PLQ Parameters | | | |
|---|---|---|---|---|
| | **α** | **β** | **γ** | $X^2$ |
| Control | 3.276 ±1.812 | -4.771 ±17.175 | -1.60 ±3.247 | 0.873 |
| AuNPs | 4.3013 ±0.238 | -5.421 ±2.192 | -1.592 ±0.273 | 0.998 |

A good agreement obtained by the curve generated using PLQ model for 70 keV, while for 81.5 keV shows less agreement at some point of the experimental data. The survival curve from PLQ models are depicted in Fig.2. and parameters from the models are presented in Table 3 and 4. The PLQ parameters (increment of $\alpha$, $\beta$ and $\gamma$ values) for AuNPs are found to increase for 70 keV beam which might correlate with the radiosensitization effects.

The radiosensitization effects were quantified by calculating the sensitization enhancement ratio (SER), which was assessed according to percentage of cell survival of control over cell curvival treated with AuNPs. The SER value obtained for 70 keV from LQ and PLQ models are 1.25 and 2.32, respectively and for 81.5 keV from both model are 1.24 and 1.32 respectively.

## DICUSSION

First analysis on cell survival generated by LQ and PLQ models indicate radiosensitization effects from the parameter prediction at 40 keV and 70 keV (refer to Table 5). The LQ model determined higher alpha value correlation of more radiosensitization occurred on cell AuNPs-treated with radiation. Rahman et al 2014, explained low energy (around 40 – 50 keV) produced higher of alpha value where the same pattern of DEF increment, also may act as the dose enhancement indicator. While for the $\beta$ values are considered to be insignificant as the linear part dominant the survival curve. Moreover, the uncertain values of $\alpha/\beta$ may influence by the variance in $\beta$ values (Rashid et al, 2018).

Table 5: Parameters of Radiobiological model and SER

| Radiobiological model | SER | |
|---|---|---|
| | 70 keV | 81.5 keV |
| LQ | 1.25 | 1.24 |
| PLQ | 2.32 | 1.32 |

Further discussion continued on PLQ model which had improved the fitting process by alterably shaping the curve formation with less bending at high doses region (Shuryak et al., 2015). It parameters shown the same trend with the increment values for '$\alpha$' '$\beta$' and '$\gamma$'. For the early parameters represent an event of inactivation that constant by doses, moreover the later parameter 'y' quantified the concentration of lesion occurred, which was significantly expressed at the 40 keV irradiation with AuNPs. By Andisheh et al., 2013, optimization was carried out by PLQ model by became smoothly disappearing the quadratic term by using the binomial expansion for $(1+\gamma D)^{-1}$ showed the additional of $\gamma$ generated from LQ model, which given advantage at high doses range. Moreover, PLQ model also susceptible by explaining the intermediate doses (Andisheh et al., 2013).

According to SER results, The same result were also published by other researchers on the maximum dose enhancement that can achieve at range within 40 - 50 keV (Mesbahi et al, 2013, Brun et al., 2009, Roeske et al., 2007 and Rahman et al., 2014). Study by Srinivasan and friends explained in detail about the probability of the interactions that may happen by lower energies irradiation. The energy less than the K-edge absorption contributes to the formation of Auger electron and photoelectrons shower result from the effective photoelectric interaction. Due to low energy, the dose enhancement interaction may occur at L-shell, even the energy not strong enough to eject electrons at K-edge shell yet somehow significant enough to the occurrence of auger, delta electron and photoelectrons with short coverage will enrich localized ionization at specific treated area (Srinivasan et al., 2019). As noted from the SER results obtained, low energy of 70 keV produced higher SER values, as proven by low energy of conventional radiotherapy also expressed promising of SER increment. However, slightly different values engaged by different radiobiological model fitting, thus visualised the precision of each model approach (Rashid et al, 2018).

The finding concluded that a physical interaction causes the least enhancement effects as compared to the more complex chemical-biological processes. However, it played a role in the determination of the energy dependence of the dose enhancement. In fact, the effective role in DNA damage most related through both direct and indirect processes. One of the processes, the formation of oxidative cellular response such as ROS may cause mechanism of mitochondrial depolarization (McQuaid et al., 2016).

## CONCLUSION

SER obtained indicate AuNPs induced radiosensitisation effects up to 2 fold, especially at low energy. The model's parameters show correlation with radiosensitisation effects. LQ model may explain the radiosensitization effects but only at low dose. However PLQ model may give advantage at high doses range. Theoretically well-founded radiobiological models could prove usefulness in predicting the radiosensitisation effects of AuNPs combined with kilovoltage synchrotron beam.

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