A Comparative Assessment of Mechanisms and Effectiveness of Radiosensitization by Titanium Peroxide and Gold Nanoparticles

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学 位 論 文 の 内 容 要 旨

A Comparative Assessment of Mechanisms and Effectiveness of Radiosensitization by Titanium Peroxide and Gold Nanoparticles

過酸化チタンナノ粒子と金ナノ粒子の放射線増感に関する機序と効果に関する比較検討

神戸大学大学院　医学研究科医科学専攻

Division of Radiation oncology

放射線腫瘍学分野

（指導教員 ： 佐々木　良平，教授）

Mennaallah Hassan Seddik Alsayed

Summary:

Radiation therapy is a mainstay for the treatment of many cancer types. It aims at delivering high dosages to tumor site while minimizing the dosage to the surrounding normal tissues. Despite the major technological advances in radiation therapy, normal tissue toxicity is a major limiting factor that prevent delivery of higher doses of radiation to the tumor. Radiosensitizers are agents that can deliver higher doses of ionizing radiation to the tumor site while minimizing the dose received by the surrounding normal tissues, hence, minimizing normal issue toxicity. A number of nanoparticles have been proposed as candidate agents for radiosensitizing; however, at present, no compound has been adapted for radiosensitization in a clinical setting. The development of potentially safe radiosensitizing agents is essential to enhance the treatment outcomes of radioresistant cancers.

The titanium peroxide nanoparticle (TiOxNP) was originally produced using the titanium dioxide nanoparticle, and it showed excellent reactive oxygen species (ROS) generation in response to ionizing radiation. Surface coating the TiOxNPs with polyacrylic acid (PAA) showed low toxicity to the living body and excellent radiosensitizing effect on cancer cells. Herein, we evaluated the mechanism of radiosensitization by PAA-TiOxNPs in comparison with gold nanoparticles (AuNPs) which represent high-atomic number nanoparticles that show a radiosensitizing effect through the emission of secondary electrons. In this study, we first investigated whether the TiOxNPs might be toxic or not on the normal organs of healthy mice through histological examinations of the liver, kidney, lung, and heart tissues. Subsequently, the radiosensitizing effect of TiOxNPs was evaluated in comparison with that of AuNPs, with regard to the types of ROS generated and the cytotoxic effects induced in vitro and in vivo. Moreover, we evaluated the biological responses occurring within the tumor xenografts to compensate for the increased oxidative stress. Understanding the different mechanisms of radiosensitization by different NPs enables us to choose the best radiosensitizing agent for each cancer type and tailor the treatment according to the tumor's biological behavior.

The safety of TiOxNPs after intravenous injection in healthy mice was evaluated. The liver, kidneys, lungs, and heart were evaluated grossly and microscopically after H&E staining and compared with those of the control group. The gross appearance (shape, color, surface, and weight) of liver, kidneys, lungs, and heart

were quite similar between control mice and PAA-TiOxNPs-treated mice. There were normal tissue architectures and no signs of inflammation or fibrosis.

Radiation induced cytotoxicity depends mainly on production of ROS. Therefore, we evaluated ROS generation by the nanoparticles in a cell free system, we found that PAA-TiOxNPs, in combination with x-irradiation, significantly enhanced both H_2O_2 and $HO^{\cdot}$ generation in NP concentration-dependent and radiation dose-dependent trends, while AuNPs increased only $HO^{\cdot}$ radicals. Furthermore, ROS generation in vitro in response to radiation therapy was investigated in MIAPaCa-2 cells that treated with PBS or NPs. ROS was measured using a fluorescence microscope and, carboxy-2′, 7′-dichlorofluorescein (C-H_2DCF) as fluorescent agent. Interestingly, with 5 Gy of irradiation, PAA-TiOxNPs induced a significant increase in H_2O_2 production compared with either 5 Gy alone or AuNPs (both, $p < 0.0001$).

To compare cytotoxic effects of PAA-TiOxNPs with that of AuNPs, a colony-formation assay was performed. Treatment of MIA PaCa-2 cells with PAA-TiOxNPs, at concentrations of 150 and 200 μg mL^{-1}, combined with 5 Gy significantly inhibited cell growth compared with radiation alone ($p < 0.05$ for both concentrations). In addition, treatment of MIA PaCa-2 cells with PAA-TiOxNPs, at concentrations of 400 μg mL^{-1}, combined with either 2 or 5 Gy significantly inhibited colony formation compared with radiation alone ($p < 0.05$, $p < 0.01$ for 2 and 5 Gy respectively). In contrast, AuNPs, at concentrations 2, 4, and 15 μg mL^{-1} with 5 Gy showed a decrease in colony formation more than 5 Gy alone. However, this effect was not significant at any concentration.

Tumor growth inhibitory effect was evaluated in vivo using MIA PaCa-2 xenografts mice. Treatment with TiOxNPs in combination with 5 Gy showed significantly greater radiation effects leading to higher tumor growth inhibition compared with that of 5 Gy alone or AuNPs with 5 Gy (both, $p < 0.0001$). In the same setting, the treatments were all well tolerated, as evidenced by no apparent loss of body weight and no mice died during the 55-day observation period.

In vivo cytotoxicity was evaluated by Tunnel assay. Without irradiation, both AuNPs and PAA-TiOxNPs administrations resulted in a slight increase in the number of apoptotic (TUNEL-positive) cells. With 5 Gy of irradiation, consistent with the intracellular increase in H_2O_2 production, PAA-TiOxNPs induced a significant increase in the proportion of apoptotic cells compared with those by 5 Gy alone or AuNPs with 5 Gy ($p < 0.001$ and $p < 0.01$ respectively). The above results demonstrate that PAA-TiOxNPs sensitized MIA PaCa-2 cells to radiation damage more than AuNPs due to the increased apoptosis of PAA-TiOxNPs-treated

cells. Previously, it was reported that PAA-TiOxNPs enhanced HO˙ free radical production, here in, we provided that PAA-TiOxNPs cytotoxicity is caused by both HO˙ and H_2O_2 production but mainly H_2O_2. To confirm our speculation, we evaluated redox hemostasis within the tumor tissues. Cancer cells can adapt to survive under certain levels of oxidative stress, which is called redox adaptation. Catalase (Cat) and glutathione peroxidase (GPx) are the main antioxidant enzymes for H_2O_2. In our study, redox adaptation by Cat and GPx occurred in tumors treated with PAA-TiOxNPs and radiation. Increased expression of Cat and GPx reflects the continuous production of H_2O_2 by the treating agent in vivo. Notably, Cat and GPx expressions in PAA-TiOxNPs-treated tissues were observed on day 1. They increased to a maximum on day 7 after treatment, and then decreased on day 55. Meanwhile, the expressions of Cat and GPx were not observed in AuNP-treated tissues, indicating the inability to enhance H_2O_2 production.

Identification of potentially safe radiosensitizing agents is critical for the development of variable therapeutic approaches that can improve the outcomes of various types of radioresistant tumors. This study compared the different mechanisms of radiosensitization by PAA-TiOxNPs and AuNPs. PAA-TiOxNPs showed the ability to produce H_2O_2 molecules in addition to HO˙ radicals in vitro and in vivo. In contrast, AuNPs showed a higher ability to produce HO˙ radicals only. However, the radiosensitizing effect of PAA-TiOxNPs was more effective resulting in more apoptosis and tumor growth inhibition of MIA PaCa-2 human pancreatic cancer xenografts. These findings support the important role of H_2O_2 as a mediator of PAA-TiOxNPs' radiosensitization. Moreover, administration of PAA-TiOxNPs was generally safe and nontoxic and caused no damage to the liver, kidney, lung, or heart tissue.

神戸大学大学院医学(系)研究科（博士課程）

論文審査の結果の要旨			
受付番号	甲 第 3203 号	氏　名	Mennaallah Hassan
論文題目 Title of Dissertation	A Comparative Assessment of Mechanisms and Effectiveness of Radiosensitization by Titanium Peroxide and Gold Nanoparticles 過酸化チタンナノ粒子と金ナノ粒子の放射線増感に関する機序と効果に関する比較検討		
審査委員 Examiner	主　査 Chief Examiner 副　査 Vice-examiner 副　査 Vice-examiner	岡本圭生 篠山隆司 青井貴之	

（要旨は１，０００字～２，０００字程度）

Background:

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Materials and methods:

He evaluated the mechanism of radiosensitization by PAA-TiOxNPs in comparison with gold nanoparticles (AuNPs) which represent high-atomic number nanoparticles that show a radiosensitizing effect through the emission of secondary electrons. He first investigated whether the TiOxNPs might be toxic or not on the normal organs of healthy mice through histological examinations of the liver, kidney, lung, and heart tissues. Subsequently, the radiosensitizing effect of TiOxNPs was evaluated in comparison with that of AuNPs, with regard to the types of ROS generated and the cytotoxic effects induced in vitro and in vivo. Moreover, he evaluated the biological responses occurring within the tumor xenografts to compensate for the increased oxidative stress. Understanding the different mechanisms of radiosensitization by different NPs enables us to choose the best radiosensitizing agent for each cancer type and tailor the treatment according to the tumor's biological behavior.

Results:

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Conclusions:

The radiosensitizing effect of PAA-TiOxNPs was more effective resulting in more apoptosis and tumor growth inhibition of MIA PaCa-2 human pancreatic cancer xenografts. These findings support the important role of H_2O_2 as a mediator of PAA-TiOxNPs' radiosensitization. Moreover, administration of PAA-TiOxNPs was generally safe and nontoxic and caused no damage to the liver, kidney, lung, or heart tissue.

The candidate, having completed studies on the radiosensitizing of PAA-TiOxNPs, and having advanced the field of knowledge in the area of radiation oncology, is hereby recognized as having for degree of Ph.D. (Medicine).