TiO2 Nanoparticle as a Sensitizer Drug in Radiotherapy: in Vitro Study

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Abstract

Background: Radiosensitizer drugs are used to enhance the efficiency of radiotherapy. Some nanoparticles can be considered as radiosensitizers, because they enhance cytotoxicity due to oxidative stress and increase free radical yield, especially ROS, within cells resulting to cell death.

Methods: In this study, synergistic effect of TiO2 nanoparticles was evaluated in presence of 60Co gamma rays on human breast cancer (MCF-7) and gastric cancer (MKN-45) cell lines. After cell culture, cells were exposed to several doses of gamma rays and a dose of 2Gy was selected due to survival analysis. Next, several doses of nanoparticle from each type was applied and cell survival was analyzed from which a dose of 30µg/ml was selected for the remainder of study. Finally, synergistic effect of gamma rays and nanoparticles was evaluated in two time delay groups using MTT assay.

Results: Viability of cells in presence of gamma radiation and nanoparticles, significantly reduced compared to viability of cells exposed only to radiation or nanoparticle, alone (P-value≤0.05). The effect was dependent on nanoparticle type, time between addition of nanoparticle to cells and exposure to gamma rays and also cell dependent.

Conclusion: TiO2 increased sensitivity of cancer cells to gamma radiation, due to an increase in ROS production and cytotoxicity. Anatase crystals have more severe effects than Rutile crystal because of having a larger surface area and creation of more free radicals. Therefore, this nanoparticle has the potential to be used as a radiosensitizer and further studies should be considered on other cell lines and in vivo.

Keywords: Titanium dioxide; Breast cancer; Gastric cancer; Radiation-sensitizing agents radiotherapy

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Introduction

Radiotherapy is one of the major cancer treatment in which ionizing radiation is used to destroy cancerous cells [1]. In this treatment a high radiation dose should be delivered to tumor in which in some cases due to existence of some functional and healthy tissues, it is not possible to increase radiation dose practically [1] and to increase efficacy of this treatment, some chemicals are used to increase radiosensitivity of tumoral cells or reduce radiosensitivity of healthy cells [2]. Mechanism of induced radiosensitivity by most of these sensitizers is free radical and specifically ROS production. There exist a wide variety of literature on different materials have been used as sensitizers [3, 4]; along which, nanomaterials have been considered markedly which include: gold nanoparticles [5, 6], carbon nanotubes [7, 8] and metallic nanoparticles [9]. It has been shown that simultaneous exposure to gamma-rays and nano-C60 causes a reduced survival in tumor cells and this agent increases gamma-ray effect by induction of damage in cell membrane which may be used in radiotherapy; but some problems such as low sensitizing effect, short half life and side effects should be studied [7]. There are several studies on mechanism of toxicity of nanomaterials which have shown that oxidative stress, lipid peroxidation and reaction of DNA with ROS has an important role on DNA damage, destruction of membrane and finally cell death [8, 10-12]. TiO2 is a biocompatible material which in nano size causes some inflammatory effects and confirms the idea that

nanomaterials have different properties [8]. Some evidence show that nano-TiO2 causes H2O2 and hydroxyl free radical formation which result to cell toxicity in mammals [13-15]. Nanomaterials play an important role in DNA damage, membrane destruction and finally cell death via oxidative stress and lipid per-oxidation [13] which can develop effective modalities to destroy tumor with least side effects [14]. Among nanomaterials, TiO2 is a biocompatible agent which causes inflammation in nano-domain [13] and leads to cell toxicity by super-oxide, H2O2 and free hydroxyl radical formation in mammalians [14]. Increasing free radicals due to induction of oxidative stress activates necrosis and apoptosis reactions and finally leads to cell death [12,15].

In this study, due to potential of TiO2 nanoparticle in free radical formation -specially ROS- and also initial passive accumulation of nanoparticles in tumor cells because of increased angiogenesis [16], probability of increasing radiosensitivity of tumoral cells in presence of nanoparticle was assessed to reach a similar therapeutic efficiency using lower doses of radiation.

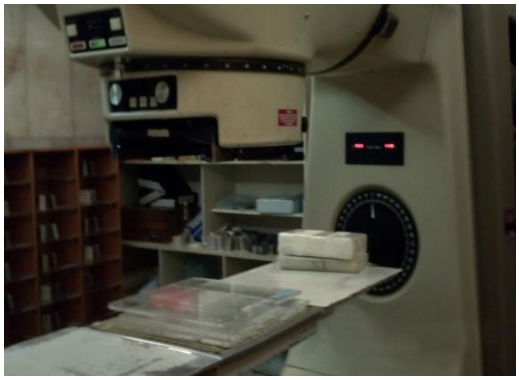
Materials and Methods

Cell culture

In this study MCF-7 and MKN-45 cell lines were purchased from Iran Pasteur Institute. Cells were cultured in DMEM (Gibco, Invitrogen GmbH, Darmstadt, Germany) containing 10% FBS (Gibco, Invitrogen GmbH, Darmstadt, Germany), 100unit/ml Penicillin and 100 µg/ml Streptomycin and incubated at 37°C and 5% CO2.

Irradiation condition

Cells were irradiated using a 60Co therapeutic unit (AECL Theratron, Canada) at the radiotherapy department of Shohada Hospital (Tehran, Iran). Samples were placed in a 15×15cm2 field of at a Source to Surface Distance (SSD) of 80cm and were irradiated. In order to find out response of selected cell lines to radiation, they were irradiated with 1, 2 and 3Gy 60Co gamma rays and their survival was calculated in percents. Within three irradiation groups, 2Gy exposure was selected for the remainder of study in which survival was reduced to 20% and 37% for MKN-45 and MCF-7, respectively. Cell survival curves for these three groups are presented in Figure 1.



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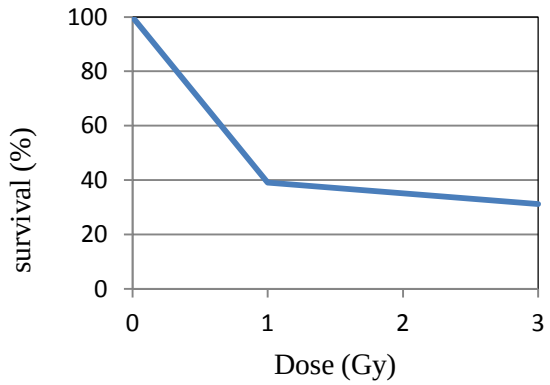

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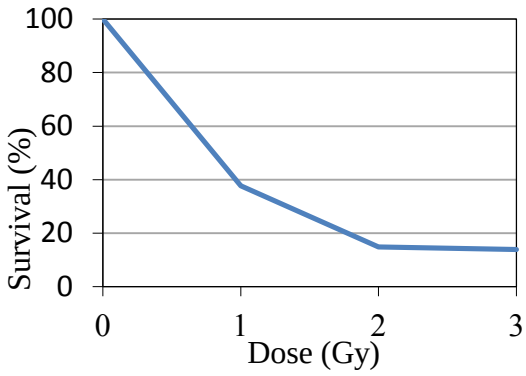


C

Figure 1. A) Irradiation set up, **B)** *MCF-7* survival curve under ^{60}Co irradiation, **C)** *MKN-45* survival curve under ^{60}Co irradiation

TiO2 nanoparticle

To obtain effect of TiO2 nanoparticles on cells, TiO2 Anatase and Rutile nanoparticles (Grafen Chemical Industries, Ankara, Turkey) were added to DMEM cell culture separately. Required concentration was obtained from the pilot study. After 24 hours from initial cell culture in 96 well plates, cell culture medium was replaced with cell culture medium including nanoparticle and after 7 days, MTT assay was done. In order to find out required concentration of nanoparticles of each type, several concentrations of nanoparticle in culture media was added to two cell lines and survival percentage was obtained by MTT assay.

Cytotoxicity assessment using MTT assay

To analyze cell survival, MTT assay was implemented. To do so, a flask of cell with concentration of 60% was trypsinized and after cell counting, cells were moved to a 96 well plate flask to embed 5000 cells in 200 µl of cell culture medium. After 24 hours, experimental groups were exposed to nanoparticle and gamma rays and were incubated at 37°C for 7 days. On the day of assessment, 20 µl of MTT solution was added to each well and after 3 hours incubation, contents of wells were replaced with 100 µl of DMSO to solvate formazon crystals. In order to run assay, 3 similar samples were obtained and their absorbance was read at 570 nm with Rayto software of Elisa reader system. Finally, survival percentage was calculated as the ratio of percentage optical density (mean light absorbance) in experimental group (Odets) to control group (ODcont) as below:

$$\text{Survival Percentage} = \mathrm{OD}_{\text{test}} / \mathrm{OD}_{\text{cont}} \times 100$$

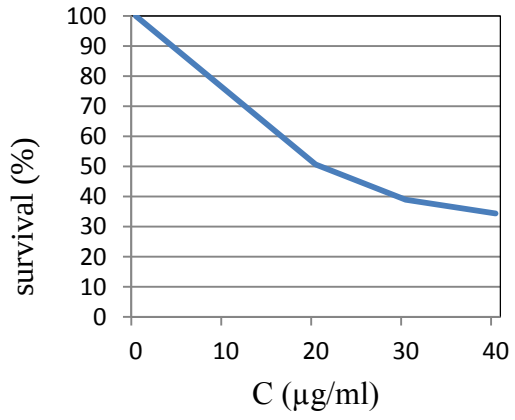
Statistical analysis

Each experiment was repeated three times and data were presented as Mean (Standard Deviation). Statistical analyses were performed with SPSS v.17 (SPSS/PC Inc., Chicago, IL, USA) and graphs were prepared using Microsoft Excel 2007. After verifying normality and homogeneity of variables, analysis of variance (ANOVA) was performed with a 95% confidence interval (P-value≤0.05); Tukey was used for multiple comparisons.

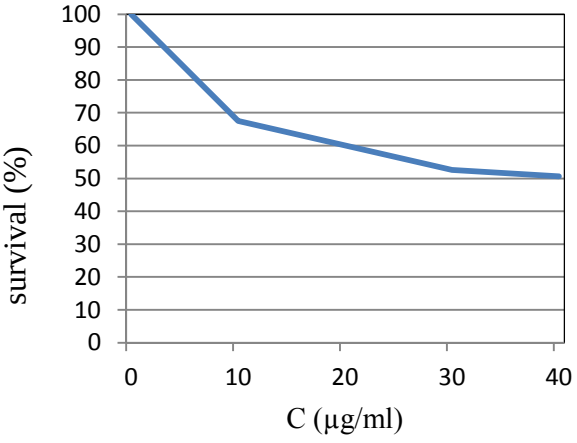
Results

Cytotoxicity induced by nanoparticles

Figure 2 shows the effect of nanoparticle concentration on cell survival. It was shown that in the case of Anatase, a concentration of 30µg/ml was resulted to a 60% and 90% reduction in survival relative to non-exposed control groups for MCF-7 and MKN-45, respectively. Besides, in the case of Rutile a concentration of 30 µg/ml resulted to a 50% reduction in survival for both cell lines. Figure 3 shows microscopic images of nanoparticle exposed cell lines. As it is obvious, Rutile nanoparticle produced less cytotoxicity compared to Anatase and within two cell lines; MKN-45 was more sensitive to Anatase than MCF-7. This might be due to their different physical structure and chemical properties (Figure 2).



2A

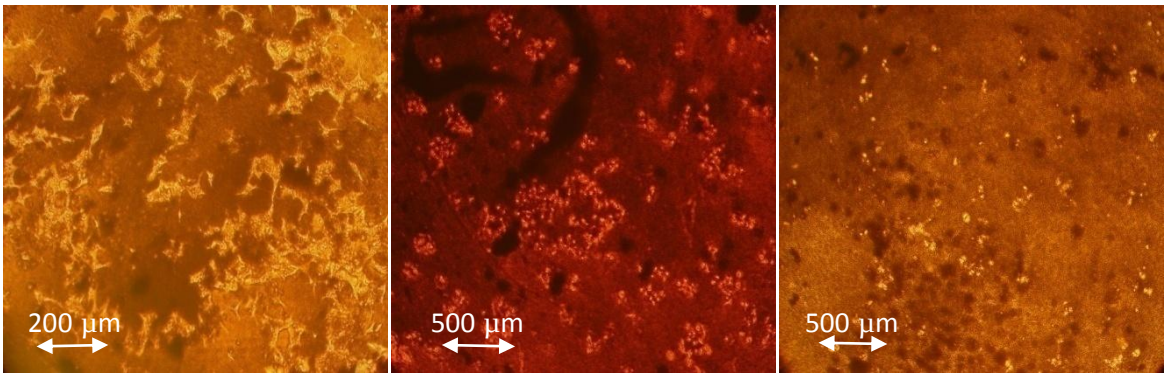


2B

Figure 2. A) Effect of several concentrations of Anatase on *MCF-7* cell line, **B)** Effect of several concentrations of Rutile on *MCF-7* cell line

Microscopic study

In order to analyze cell morphology and observe effect of nanoparticle and radiation on cell structure in vitro, study was run under several groups. These groups included: absence of nanoparticle and radiation, presence of nanoparticle with no radiation, absence of nanoparticle with radiation and presence of nanoparticle and radiation. The obtained results revealed that nanoparticle induces some morphological changes in cells (Figure 3).



A **B** **C**

Figure 3. A) MCF-7 cell line in the presence of Anatase with concentration of 30µg/ml, **B)** MKN-45 cell line in the presence of Anatase with concentration of 30µg/ml, **C)** MKN-45 cell line in the presence of Rutile with concentration of 30µg/ml

Radiosensitivity induced by Anatase nanoparticle

MCF-7 cell line

After addition of 30µg/ml Anatase nanoparticle to MCF-7 cell line and under 2Gy of gamma-irradiation with 10 hours and 72 hours time delay, survival reduced to 12% and 14%, respectively (Figure 4). As it is obvious, presence of Anatase nanoparticle in cell culture medium alone had a lower effect on survival than radiation and simultaneous application of nanoparticle and radiation with both 10 hours and 72 hours time delays and did not reduce cell survival significantly compared to control group (P-value≤0.05).

MKN-45 cell line

Addition of Anatase with concentration of 30µg/ml to MKN-45 cell line and exposing cells to a dose of 2Gy from gamma rays with 10 hours and 72 hours time delay, reduced cell survival to less than 8%. This might be due to higher sensitivity of this cell line to radiation(Figure 5).

Radiosensitivity induced by Rutile nanoparticle

MCF-7 cell line

In the case of Rutile nanoparticle, after addition of nanoparticle with a dose of 30 µg/ml and irradiation with a dose of 2Gy, 10 hours and 72 hours later, survival was reduced to 35% which compared to radiation alone, had no significant difference (P-value≤0.05) (Figure 6).

MKN-45 cell line

A concentration of 30µg/ml from Rutile nanoparticles was added to MKN-45 cell line and a dose of 2Gy from gamma rays was applied to them with a 10 hours and 72 hours time delay. Our findings showed that cell survival was reduced to less than 30% in both groups which effect was less than Anatase nanoparticle, and had no significant difference with gamma irradiated cells (Figure 7).

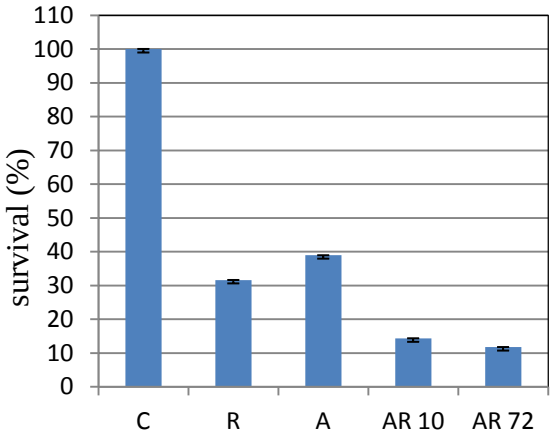


Figure 4. Survival percentage in MCF-7 cell line in experimental groups; **C:** Control, **R:** Radiation, **A:** Anatase nanoparticle, **AR10**: Anatase nanoparticle and irradiation with 10 hours time delay and **AR72:** Anatase nanoparticle and irradiation with 72 hours time delay

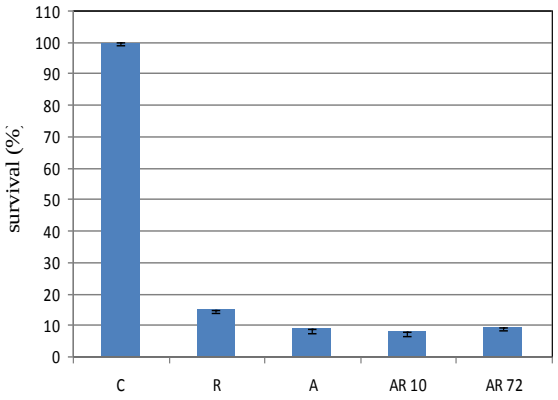


Figure 5. Survival percentage in MKN-45 cell line in experimental groups; **C:** Control, **R:** Radiation, **A:** Anatase nanoparticle, **AR10**: Anatase nanoparticle and irradiation with 10 hours time delay and **AR72:** Anatase nanoparticle and irradiation with 72 hours time delay

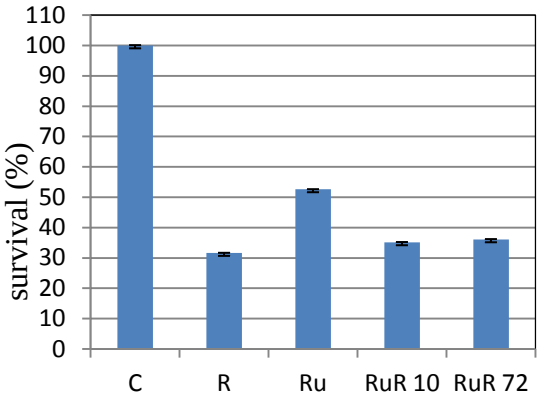


Figure 6. Survival percentage in MCF-7 cell line in experimental groups; **C:** Control, **R:** Radiation, **Ru:** Rutile nanoparticle, **RuR10:** Rutile nanoparticle and irradiation with 10 hours time delay and **RuR72:** Rutile nanoparticle and irradiation with 72 hours time delay

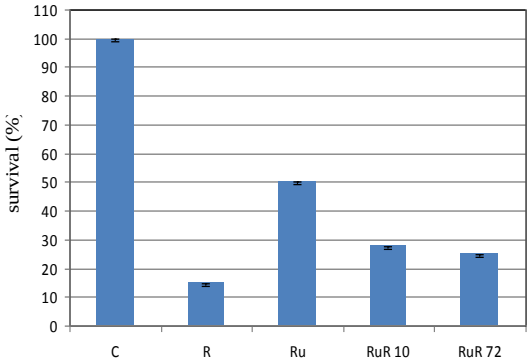


Figure 7. Survival percentage in MKN-45 cell line in experimental groups; **C:** Control, **R:** Radiation, **Ru:** Rutile nanoparticle, **RuR10:** Rutile nanoparticle and irradiation with 10 hours time delay and **RuR72:** Rutile nanoparticle and irradiation with 72 hours time delay

Discussion

With increasing development of nanotechnology and using different aspects of nanomaterials, a new scope has been formed in all scientific fields. This technology has found application in several fields from industrials, home appliances and specially medicine [17]. In the field of medicine, using nanomaterial properties in both fields of diagnosis and therapy of disease has profoundly studied [18-20]. TiO2 is a member of metallic nanomaterials and has been considered widely due to its specific chemical properties. This nanoparticle has different forms with different physical and chemical properties and the most important forms are Anatase and Rutile. Rutile has a higher stability and larger size than Anatase [21]. This nanoparticle has some photocathalitic characteristics which in higher concentrations, stimulates with UV radiation and produces free radicals [22-24]. This nanoparticle interacts with water molecules in cell medium and using electron capture pathway, produces free radicals specially ROS [17]. The exact mechanism of free radical production by TiO2 has not determined yet, but several studies have shown that Anatase crystals aggregate in mitochondria and cause some defect in electron chain and destruct its function. This leads to more and more production of free radicals. But in the case of Rutile, the problem is different. Rutile crystals place in cell sparsely and don't enter in mitochondria [25]. Our findings support this problem when Anatase alone has a higher effect than Rutile on the breast cancer cell line. There exists a wide variety of literature on cytotoxicity induced by TiO2. These include fibroblast and epithelial cells [26, 27], kidney cells [28], neuroblast cells [27] and endothelial cells [29]. All of these studies relate this toxicity to ROS production. Some studies consider synergistic effect of UV radiation and TiO2 nanoparticles on several cells such as CHO [24], glioma [22] and HeLa [23]. These studies relate the enhanced cytotoxicity to the capability of this nanoparticle to react with water molecule in cell medium and yield ROS via electron capture pathway. This capability provides the potential for this particle to be considered as a radiosensitizer. According to production of ROS by TiO2 nanoparticle and their aggregation in tumoral cells via active or passive targeting because of their high angiogenesis [30], it has been implemented in photodynamic therapy as a photosensitizing agent [23, 24, 31]. UV and laser light that are used as external stimuli in this treatment has low penetration capability, therefore this treatment is only effective for surface tumors [23]. Besides, we observed more radiosensitivity induced by Anatase compared to Rutile. Our results showed that by increasing the concentration of Anatase nanoparticles, survival percentage was reduced, but the amount of this reduction was not as severe as lower concentrations which might be due to high toxicity induced in this lower concentration. In addition, the amount of reduction in survival was more in MKN-45 compared to MCF-7; it was due to faster cell cycle of MKN-45 which caused a higher sensitization. In the case of Rutile nanoparticle, it had a lower effect on cell survival compared to Anatase nanoparticle in both cell lines. This was in a manner that simultaneous application of nanoparticle and radiation resulted to a significant reduction in Anatase groups' survival in both cell lines (P-value$\leq$0.05), but Rutile did not cause a significant reduction in cell survival when simultaneously exposed to gamma rays (P-value$\geq$0.05). The different observed effect might be due to the higher surface to volume ratio in Anatase compared to Rutile which makes it more effective than Rutile. So, it produces more ROS and because Anatase has higher aggregation in mitochondria than Rutile and causes more disorder on electron transfer chain function and mitochondria [32]. This phenomenon might be the reason for different radiosensitization induced by two nanoparticles. There were no published work on the radiosensitization of TiO2, but several studies has showed the different effect of these two types and higher ROS production in Anatase compared to Rutile [21, 24, 26, 28, 32-34].

Results of statistical analysis showed that time delay between application of nanoparticles and exposure to gamma rays doesn't induce any significant difference between groups (P-value$\geq$0.05). This might be due to fast and stable effect of nanoparticle, so any increase in time delay doesn't change their effect, but it is necessary to design more studies.

Conclusion

Our findings showed that the toxicity of Anatase have been increased in presence of gamma irradiation therefore it is possible to consider this particle as a radiosensitizer drug; but it is necessary to assess this in vivo and examine its possible side effects. The effect of this nanoparticle is both dose and cell type dependent. In the case of Rutile, it is not a good candidate for a radiosensitizer drug but it is recommended to assess the effect of particle size on cell survival, because in nano domain, size and surface to volume ratio play an important role in observed effects. Besides, we recommend different types of radiations and energies to be considered in combination of this nanoparticle.

Acknowledgment

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Conflict of Interest

The authors have no conflict of interest in this study.

Authors' Contribution

Mostafa Rezaei-Tavirani, Hadi Hasanzadeh and Samaneh Sadat Seyyedi designed the study, gathered and analyzed the data and wrote the paper. Elham Dolat and Vahid Semnani contributed to study design, sample collection and indentation. Sara Sobhi helped in writing and overall correction of the manuscript.

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