HIROSAKI UNIVERSITY

Radiation Environment and Medicine 2016 Vol.5, No.1 40–45

REM
Radiat Environ Med

Review

Dual Effects of Nanoparticles on Radiation Therapy: as Radiosensitizers and Radioprotectors

Mati Ur Rehman, Paras Jawaid and Takashi Kondo*

Department of Radiological Sciences, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan

Received 9 October 2015; revised 2 December 2015; accepted 8 December 2015

Radiation therapy (RT) is an approved, most widely used strategy for the treatment and control of cancer progression but its successful application solely depends on the radiosensitivity of tumor cells and tolerance of normal tissue. To overcome this, very often radiation therapy is combined with radiosensitizing agents. Recently, due to the advancement in the field of nanotechnology, metal nanoparticles have been discovered as the novel radiosensitizers such as gold nanoparticles (GNPs). In contrast, some nanoparticles of noble metals like platinum nanoparticles (nano-Pts) act as radioprotectors and inhibit radiation-induced cell death. This review will summarize the latest findings on the 1) radiosensitizing effects of GNPs, 2) effects of platinum nanoparticles on inflammation and radiation-induced cell death.

Key words: platinum nanoparticles, gold nanoparticles, radiation, apoptosis, antioxidants

1. Introduction

1.1. Nanotechnology and nanomedicine:

In recent decades, extensive research has been focused on the nanoscience and nanotechnology. The concept of nanoscale materials has been widely applied and it holds promising features for technical, biological, industrial and biomedical applications. This field is rapidly growing due to the increased interest of researchers from academic, industry and federal sector[1, 2]. Nanotechnology deals with the features as small as 1 billion of a meter. The broad application of nanotechnology covered almost all fields of life including nanomaterials, nanoscale devices, systems, instrumentation research, and biomedical research.

The application of nanotechnology in the field of medicine is defined as nanomedicine, which deals with the biology, chemistry, engineering, and medicine. It provides help in the use of nanoscale materials and devices for diagnosis and drug delivery, without occluding needles and capillaries, improve disease prevention, diagnosis and treatment of disorders such as cancer and inflammation[3].

Recently, the use of nanoparticles in the consumer products has been widely increased. The ASTM standard defines nanoparticles as:

"Particles with lengths that range from 1 to 100 nanometers in one or more dimensions"[4].

It has been projected that by 2020 the production of nanoparticles will increase to 58000 tons[5]. Nanoparticles of different metals have been developed and found to catalyze different chemical reaction due to the large surface area of smaller particles such as nanoparticles in the form of titanium dioxide and zinc oxide have been used in the sunscreens. Nanosilver is widely used in the products for its anti-bacterial effects. Gold nanoparticles

*Takashi Kondo: Department of Radiological Sciences, Graduate School of Medicine and Pharmaceutical Science, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan.
E-mail: kondot@med.u-toyama.ac.jp

(GNPs) have been used as nano probes for transmission electron microscope (TEM) and to enhance the radiation therapy effects. Some noble nanoparticles are reducing catalyst; they may be usable as antioxidants and reduce reactive oxygen species (ROS) production in a living body[6, 7]. Nanoparticles possess great effect on the environment and human health. This review will describe the differential effects of noble nanoparticles (gold and platinum) on radiation therapy.

1.2. Radiotherapy

Radiotherapy continues to play an important role in the cancer treatment. It is non-invasive, painless and very effective treatment. It has been used as a curative, as part of adjuvant therapy regime along with chemotherapeutics. In recent times, great improvements have been made to better concentrate the dose confined to the tumor area and while sparing normal healthy tissues. Despite these advancements the use of radiation therapy is linked with serious concerns and fails to eradicate tumors, especially in case of radioresistant tumors. Therefore, in order to obtain better therapeutic effects of radiation therapy, the use of radiosensitizers has emerged as a potential combination with radiation. Nanotechnology has the potential and provides better approaches for the diagnosis, imaging, and treatment of cancer[8]. The high-Z materials induced, radiation dose enhancement has been well established in the field of cancer radiotherapy. Metal nanoparticles were used to enhance the effects of radiotherapy, due to their unique physical and chemical properties[9, 10]. GNPs are known to increase the radiotherapy effects in tumors in vitro and in vivo. However, some noble metal nanoparticles were also found to inhibit radiotherapy effects in cancer.

2. Gold nanoparticles (GNPs) and radiosensitization

It was in the 19th century when Michael Farday published the first scientific report on the synthesis of GNPs. Later on with advancements in the techniques like transmission electron microscopy (TEM) and atomic force microscopy (AFM), provides help in imaging GNPs[11, 12]. Recently, GNPs have been the subject of intensive biomedical research due to their unique physicochemical properties including surface plasmon resonance (SPR) and the ability to bind amine and thiol groups, allowing surface modification and functionalization[13]. GNPs (atomic number, Z=79) have been used as a more promising radiosensitizers because they have high Z number and a greater biocompatibility than other metals.

Theoretically the radiosensitizing effect of GNPs is based on the concept that high atomic number materials absorb low kilovoltage (kV) X-rays more efficiently and deposit the energy precisely, resulting in the enhanced-radiation dose deposition specifically to tumor cells[14, 15]. Similarly, it was demonstrated that an increased biological effective dose can be achieved with gold microspheres in cell culture or in tumor tissue exposed to kilovoltage photon beams[16]. However, it has been known that this physical dose enhancement based phenomenon on increased X-ray absorption may not be the only mechanism involved in the sensitization. Recently, the cell specific radiosensitizing effects of GNPs at megavolt X-ray energies were reported[17]. Therefore, when considering the sensitizing effects of GNPs, it is important to know the key parameters involved in the GNPs-induced radiosensitization such as GNPs size, shape, concentration, surface coating, type of cell line, and radiation energy. The pioneering study of Heinfeld *et al.* demonstrated that following intravenous injection of GNPs with the diameter of 1.9 nm resulted in the improved efficacy of radiotherapy on mammary carcinomas in mice[18]. In another study conducted by Chang *et al.*, similar improved therapeutic effects were demonstrated on a mouse model of melanoma by using 13 nm GNPs in combination with a single dose of 25 Gy[19]. In HeLa cells, 50 nm GNPs had better radiosensitizing effects than 14 and 74 nm GNPs with 220 kVp X-rays[20]. In consistent, Coulter *et al.* investigated that the uptake of GNPs with 1.9 nm occurs in a concentration-, time- and -cell type dependent manner. The GNPs were endocytosed and localized into the cytoplasmic vesicles within first few hours of exposure, reaching a plateau at 6 h[21]. This suggests that GNPs size is an important factor for the enhancement of radiation effects because the accumulation of GNPs in a tumor may depend on the size and surface properties of GNPs. Further, it has also been reported that by modifying GNPs surface properties and particle organization, localized uptake and selective binding can be achieved in the cancer cells. The GNPs conjugated with polyethylene glycol for stabilization of gold nanosol, were found to sensitize EMT-6 breast carcinoma cells and CT26 colorectal adenocarcinoma cells to various types of ionizing radiation (6.5 keV, 8.048 keV, 73 keV and 6 MeV X-rays and 3 MeV protons[22]. This PEGylated surface modification of GNPs may lead to increased bio-efficiency. Recently, the synthesis of a novel gold nano gel (GNG), consisting of a PEGylated nano gel with large payloads of GNPs (8 nm) was performed. Each PEGylated nano gel contains approximately 15 GNPs and the total diameter of GNG was 106 ± 2.68 nm. These GNG successfully incorporated into the cells, accumulated in the cytoplasm especially in the endosome/lysosome or near to nuclear membrane, however, does not localize into the nucleus. This selective uptake of GNG in endosome/lysosome showed that the radiosensitization was due to the apoptosis induction initiated by the GNG-mediated ER-stress[23].

Table 1. Some In-vitro or In-vivo studies of GNPs radiosensitization

Cell line	GNP size	surface coating/bound	GNP conc	GNP target/ distribution	Radiation	Study	Year	Experim ent
MDA-MB-231	16 nm 49 nm	Glu-Gnps Glu-Gnps	20 nM	Cytoplasm (endosomes/ lysosomes)	6-MV	Wang C *et al.*[24]	2015	*In-vitro*
HeLa	52 nm 47 nm	Folate-GNPs Pegylated-GNPs	50 µM 50 µM	N/A	X-ray 2 Gy at 180 kVp γ-raysC_o-60	Khoshkqard *et al.*[25]	2014	*In-vitro*
SCCVII A549 V79	GNP 8 nm GNG 106 ± 2.68 nm	PEGylated nanogel with large payloads of GNPs	20 ug/ml 15 ug/ml 50 ug/ml	Cytoplasm (endosomes/ lysosome) Induced ER-stress in cells	X-rays 5, 10, 15, 20 Gy at 200 kVp	Yasui H *et al.*[23]	2014	*In-vitro*
U251	N/A	surface modified with polyethylene glycol	1 mM 100 ul GNP concentrated inj. into mice tumor	N/A	4 Gy (150kVp) 20 Gy (175kVp)	Joh DY *et al.*[26]	2013	*In-vitro* *In-vivo*
EMT-6 CT26	6.1 nm	Polyethylene Glycol	400, 500 or 1000 µM	N/A	10 Gy 6.5 keV 8.048 keV 73 keV 6 MeV 3 Mev protons	Liu CJ *et al.*[22]	2010	*In-vitro*
HeLa	14.74 nm	N/A	1 nM	N/A	105 kVp 220 kVp	Chithrani DB *et al.*[20]	2010	*In-vitro*
SCCVII	1.9 nm	N/A	1.9 gkg-1	N/A	30 Gy, 42 Gy (68 keV) 40 Gy, 50.6 Gy (157 keV)	Hainfeld *et al.*[27]	2010	*In-vivo*
BAEC	1.9 nm	N/A	250-1000 µM	Cytoplasm	80 kV, 150 kV, 6 MeV Electrons 12 MeV electrons	Rahman WN *et al.*[28]	2009	*In-vitro*
B16F10	13 nm	N/A	10 nM in cells 200 nM in mice	Cytoplasm (E-R, golgi-apparatus)	25 Gy (6 MeV)	Chang MY *et al.*[19]	2008	*In-vitro* *In-vivo*

GNP, gold nanopartides, GNG, gold nano gal; N/A, not a vailable; E-R, endoplasmic reticulum

The radiosensitizing effects of GNPs may be dependent on the various factors. Table.1 summarizes some parameters studied previously, such as GNPs size, cell type, and surface modifications.

3. Platinum nanoparticles (nano-Pts)

Platinum has the similar atomic number as gold, and high atomic number materials are known to enhance the biological effects of radiation. Platinum-based drugs have long been used to treat various types of cancers. However, nanoparticles of some noble metals, including platinum (Pt), act as reducing catalysts due to the large surface area of smaller particles. Recently, platinum nanoparticles (nano-Pts) protected by polyacrylic acid (PAA) were manufactured through reduction with ethanol. These nano-Pts have gained much attention due to the fact that they may use as an antioxidants to scavenge ROS persistently and catalytically in living organisms. In fact, these nano-Pts can scavenge superoxide anions (O_2^-) and peroxides (H_2O_2), indicating that they can act as superoxide dismutase (SOD)/catalase mimetics[29]. SOD/catalase mimetics have been suggested to be co-administered with certain therapies such as radiation, to prevent ROS-mediated damage to normal tissues and thus reduced some of the adverse effects. In contrast, it was also reported that nano-Pts were able to induce lethal DNA damage and p53-mediated growth arrest. Furthermore, FePt@CoS_2 yolk shells nano-Pts were found to be more potent in killing HeLa cell than cisplatin[30].

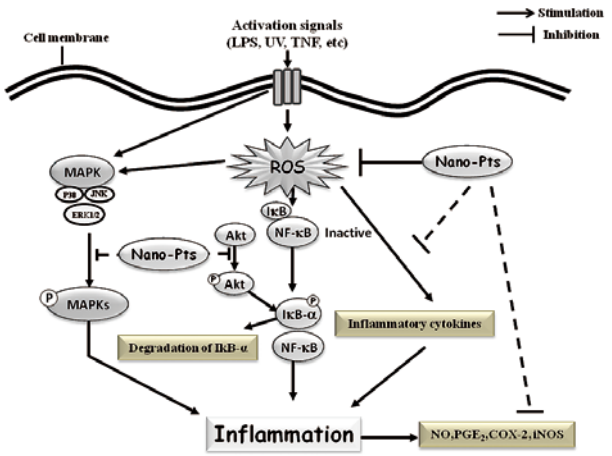

Fig. 1. Schematic view of the antioxidant or anti-inflammatory effects of nano-Pts.

Therefore, the presence of nano-Pts in a biological system can induce various effects depending upon the stimulus, and combination of nano-Pts (SOD/catalase mimetics) may result in the reversal of cell killing effects induced by different treatment modalities, such as radiation.

4. Anti-inflammatory effects of nano-Pts

The association between inflammation and cancer is not new. Most of the cancer cases are attributed by infectious agents, and inflammation is one of the key components of this chronic infection. ROS play an important role in the inflammation. Dys-regulation of ROS results in the oxidative modification of proteins, lipids, DNA, and enhances the inflammatory response, which ultimately lead to cancer progression. Macrophages produce ROS in response to phagocytosis and various stimuli like activation with lipopolysaccharide (LPS). The LPS-induced overproduction of ROS by macrophages induces oxidative damage and has been involved in the modulation of NF-$_k$B activation. Thus, ROS inhibition is a well-known therapeutic target in the treatment of several inflammatory diseases. Our findings showed that the pre-treatment with nano-Pts suppressed the LPS-induced inflammatory response in RAW 264.7 mouse macrophages in a concentration-dependent manner. It was found that the nano-Pts suppressed the LPS-induced production of both superoxide and peroxides in macrophages. This is consistent with the reported ability of nano-Pts in scavenging ROS, and thus ROS suppression by the pre-treatment of the nano-Pts resulted in the inhibition of inflammatory response.

Furthermore, as for the underlying molecular mechanisms involved, it has been known that LPS stimulation in macrophages induced an uncontrolled release of pro-inflammatory mediators, such as NO, TNF-α, and ILs. The LPS-induced expression of iNOS, COX-2, and other inflammatory mediators was decreased in the presence of nano-Pts. In addition, LPS stimulation activates the transcription factor NF-$_k$B and MAPKs, especially ERK1/2, p38, and JNK. Upon activation, MAPKs phosphorylate and control various key cellular activities, including gene expression. MAP kinases ERK, p38 and JNK are known to regulate inflammatory and immune responses and were involved in the LPS-induced expression of COX-2 and iNOS in macrophages. Increased expression of COX-2 and iNOS enhanced the production of NO and ROS, and the resulting inflammatory response. The activation of Akt and ERK was inhibited in the presence of nano-Pts, but the expression of p38 and JNK remain unchanged. Moreover, activation of NF-$_k$B was also inhibited with nano-Pts[31] (Fig. 1).

5. Effects of nano-Pts against radiation-induced cell death

Radiation has been known to induce cell death over a certain dose. The typical biological effect of ionizing radiation is supervened by two types of action that are direct and indirect action. Direct interaction of ionizing radiation such as X-rays with critical targets in the cell causing direct ionization or excitation of macromolecules, and thus initiate a chain of events that ultimately lead to biological changes. Beside this direct interaction, radiation can induce biological changes by interacting with other molecules in the cells like with water molecules, and produce free radicals such as hydroxyl radicals, which can diffuse far enough to reach, react with DNA and other critical targets in the cells. In addition, ROS generated from mitochondria also plays a crucial role in the radiation-induced cell death.

Hydroxyl radical is one of the most important ROS induced by radiation; if this ROS will not be scavenged, it reacts rapidly with cellular components and cause cell damage by inducing the DNA strand breaks and lipid peroxidation of membranes. Hydroxyl radical scavengers have been found to suppress radiation-induced cell killing as estimated by colony formation assay. However, previously our laboratory have evaluated the effects of antioxidants on the radiation-induced apoptosis in human lymphoma U937 cells, our findings showed that the prevention of radiation-induced apoptosis is not simply associated with its scavenging ability for hydroxyl radicals and that the role of hydroxyl radicals in radiation-induced apoptosis is relatively low[32]. On the other hand, the involvement of hydrogen peroxide in radiation-induced cell death has been well documented. In our study, it was demonstrated that pre-treatment with nano-Pts suppressed the radiation-induced ROS generation in human lymphoma U937 cells. The scavenging effects

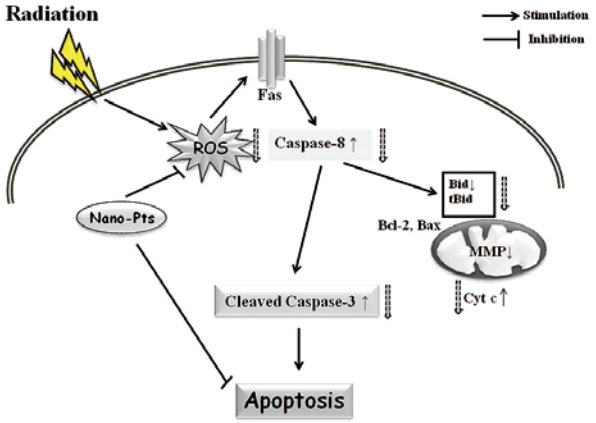

Fig. 2. Schemetic diagram of the protective effects of nano-Pts against radiation-induced apoptosis.

were found to be more pronounced on the radiation-induced hydrogen peroxide production. As for the underlying molecular mechanism is concerned, it has been well known that apoptosis is executed via pathways classified as extrinsic pathway, occurs via recruitment of death receptors such as FAS/FASL on the cellular membrane, and an intrinsic pathway, associated with loss of mitochondrial membrane potential (MMP) and disruption of mitochondrial-Bcl-2 family proteins. Both the MMP loss and FAS receptors activation are considered as the end point of apoptosis. In the presence of nano-Pts, restoration of decreased MMP and a tendency in the reduction of FAS activation was observed. Caspases are the main executors of apoptosis and activation of caspases play a cardinal role in the initiation of the apoptotic program. Moreover, the disruption of pro-apoptotic Bcl-2 family members take part in the regulation of mitochondrial apoptotic pathway, and both the pathways ultimately trigger the activation of caspase cascade that contributes to the apoptosis. Expression of t-Bid an active form of Bid, which is a member of pro-apoptotic Bcl-2 family containing BH3 domain increased after radiation treatment but decreased following pre-incubation with nano-Pts. Furthermore, radiation-induced caspase-3 activation, which is the most common executor of apoptosis, was also inhibited with nano-Pts[33]. Taken together, these results showed that nano-Pts protected radiation-induced apoptosis mainly by scavenging radiation-induced ROS, and prevented the ROS-mediated FAS receptor activation so that the activation of caspase-8 and caspase-3 was suppressed. This leads to the restoration of MMP loss and prevents the translocation of cytochrome-c from mitochondria to the cytosol, which ultimately results in the inhibition of radiation-induced apoptosis (Fig. 2).

6. Conclusion

In conclusion, it was postulated, that although gold and platinum are noble metals, with the high atomic number, which is usually considered as the efficient ability for radiosensitization. However, nanoparticles of some noble metals like platinum act as reducing catalyst due to the large surface area of smaller particles. Therefore, GNPs and nano-Pts can induce differential effects in combination with physical modalities such as radiation. The SOD/catalase mimetic activity of these nano-Pts suggests their potential as an anti-inflammatory agent, and as a potent radioprotector by providing effective protection against radiation-induced damage to normal tissue, which is another approach to improve the efficacy of radiotherapy in cancer.

Disclosure

The authors declare that they have no conflict of interest.

References

1. Szelenyi I (2012) Nanomedicine: evolutionary and revolutionary developments in the treatment of certain inflammatory diseases. Inflamm Res 61:1–9.
2. National Science and Technology Council Committtee on Technology. The National Nanotechnology Initiative: research and development leading to a revolution in technology and industry. Washington (DC): office of Science and Technology Policy: 2005.
3. Koo OM, Rubinstein I, and Onyuksel H (2005) Role of nanotechnology in targeted drug delivery and imaging: a consise review. Nanomedicine 1:193–212.
4. ASTM E 2456-06 "Terminology for Nanotechnology". ASTM International: 2006.
5. Maynard AD (2006) Nanotechnology: A research strategy for addressing Risk Washignton DC, Woodrow Wilson International Center for Scholars.
6. Esumi K, Takei N, and Yoshimura T (2003) Antioxidant-potentiality of gold-chitosan nanocomposites. Colloids Surf B Biointerfaces 32:117–123.
7. Akiyama S, Yoshimura T, and Esumi K (2005) Antioxidant activity of noble metal (gold, platinum) biopolymer nanocomposites. J Jpn Soc Colour Mater 78:112–121.
8. Sanvicens N, and Marco MP (2008). Multifunctional nanoparticles —properties and prospects for their use in human medicine. Trends Biotechnol 26:425–433.
9. Kobayashi K, et al. (2010) Enahancment of radiation effect by heavy elements. Mutat Res 704:123–131.
10. Conde J, Doria G, and Baptista P (2012) Noble metal nanoparticles applications in cancer. J Drug Deliv 2012:751075.
11. Faraday M (1857) Experimental relations of gold and other metals to light. Philos Trans 147:145–181.
12. Turkevitch J, Stevenson PC, and Hillier J (1951) Nucleation and growth process in the synthesis of colloidal gold. Discuss. Faraday Soc 11:55–75. doi 10.1039/DF9511100055.
13. Shukla R, et al. (2005) Biocompatibility of gold nanoparticles and their endocytic fate inside the cellular compartment: a microscopic

overview. Langmuir 21: 10644–10654.

14. Larson D, et al. (1989) Auger electron contribution to bromodeoxyuridine cellular radiosensitization. Int J Radiat Oncol Biol Phys 16:171–176.
15. Matsudaira H, et al. (1980) Iodine contrast medium sensitizes cultured mammalian cells to X-rays but not to gamma rays. Radiat Res 84:144–148.
16. Herold DM, et al. (2000). Gold microspheres: a selective technique for producing biologically effective dose enhancement. Int J Radiat Biol 76:1357–1364.
17. Jain S, et al. (2011) Cell-specific radiosensitization by gold nanoparticles at megavolt radiation energies. Int J Radiat Oncol Biol Phys 79:531–539.
18. Hainfeld JF, Slatkin DN, and Smilowitz HM (2004) The use of gold nanoparticles to enhance radiotherapy in mice. Phys Med Biol 49:309–315.
19. Chang MY, et al. (2008) Increased apoptotic potential and dose-enhancing effect of gold nanoparticles in combination with single-dose clinical electron beams on tumor-bearing mice. Cancer Sci 99: 1479–1484.
20. Chithrani DB, et al. (2010) Gold nanoparticles as radiation sensitizers in cancer therapy. Radiat Res 173:719–728.
21. Coulter JA, et al. (2012) Cell type-dependent uptake, localization, and cytotoxicity of 1.9 nm gold nanoparticles. Int J Nanomedicine 7:2673–2685.
22. Liu CJ, et al. (2010) Enhancement of cell radiation sensitivity by pegylated gold nanoparticles. Phys Med Biol 55:931–945.
23. Yasui H, et al. (2014) Radiosensitization of tumor cells through endoplasmic reticulum stress induced by PEGylated nanogel containing gold nanoparticles. Cancer Lett 347:151–158.
24. Wang C, et al. (2015) Thioglucose-bound gold nanoparticles increase the radiosensitivity of a triple negative breast cancer cell line (MDA-MB-231). Breast Cancer 22:413–420.
25. Khoshgard K, et al. (2014) Radiosensitization effect of folate-conjugated nanoparticles on HeLa cancer cells under orthovoltage superficial radiotherapy technique. Phys Med Biol 59:2249–2263.
26. Joh DY, et al. (2013) Selective targeting of brain tumors with gold nanoparticle-induced radiosensitization. PLoS One 8: e62425. doi: 10.1371/journal.pone.0062425.
27. Hainfeld JF, et al. (2010) Gold nanoparticles enhance the radiation therapy of a murine squamous cell carcinoma. Phys Med Biol 55:3045–3059.
28. Rahman WN, et al. (2009) Enhancement of radiation effects by gold nanoparticles for superficial radiation therapy. Nanomedicine 5:136–142.
29. Kajita M, et al. (2007) Platinum nanoparticles is a useful scavenger of superoxide anion and hydrogen peroxide. Free Radic Res 41:615–626.
30. Gao J, et al. (2007) FePt@CoS(2) yolk-shell nanocrystals as a potent agent to kill HeLa cells. J Am Chem Soc 129:1428–1433.
31. Rehman MU, et al. (2012) The anti-inflammatory effects of platinum nanoparticles on the lipopolysaccharide-induced inflammatory response in RAW 264.7 macrophages. Inflamm Res 61:1177–1185.
32. Cui Z-G, et al. (2004) Effects of antioxidnats on X-ray-or-hyperthermia-induced apoptosis in human lymphoma U937 cells. Apoptosis 9:757–763.
33. Jawaid P, et al. (2014) Effects of SOD/catalase mimetic platinum nanoparticles on radiation-induced apoptosis in human lymphoma U937 cells. Apoptosis 19: 1006–1016.