NANOPARTICLES AS RADIOSENSITIZERS IN PHOTON AND HADRON RADIOTHERAPY

NANOCZĄSTKI JAKO RADIOUCZULACZE W RADIOTERAPII FOTONOWEJ I HADRONOWEJ

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ABSTRACT

The article presents the possibility of utilizing nanoparticles as radiosensitizers both in X-ray and hadron therapy. Local reinforcement of the effect of the dose can be obtained by means of gadolinium, platinum, and gold nanoparticles, which are able to intensify the emission of photoelectrons and Auger electrons after the X-ray irradiation or increase the generation of low-energy electrons during hadron therapy. Such electrons induce water radiolysis in the vicinity of nanoparticles and create radicals that damage cancer cells. In addition to the presentation of the mechanisms responsible for radiosensitizing properties of nanoparticles, selected animal, cell culture and simulation experiments are mentioned. Attention is also drawn to the most important problems, which must be solved before clinical application of nanoparticles. The broadly defined biocompatibility is the basic feature required from radiosensitizing agents injected into the patient's body. The effective delivery of nanoparticles to the target and obtaining the proper concentration and distribution within the tumor volume present a biggest challenge.

Keywords: nanoparticles, radiosensitizers, Auger electrons, radiotherapy, hadron therapy, photon radiotherapy

STRESZCZENIE

Artykuł prezentuje możliwość wykorzystania nanocząstek jako radiouczulaczy zarówno w radioterapii fotonowej jak i terapii hadronowej. Lokalne wzmocnienie efektu dawki może być uzyskane dzięki nanocząstkom gadolinu, platyny i złota. Są one zdolne do zintensyfikowania emisji fotoelektronów i elektronów Augera przy napromienieniu fotonami X albo do zwiększenia emisji niskoenergetycznych elektronów podczas terapii hadronowej. Takie elektrony indukują radiolizę wody w sąsiedztwie nanocząstek, a generowane rodniki niszczą komórki nowotworu. Oprócz omówienia mechanizmów odpowiedzialnych za radiouczulające właściwości nanocząstek, przedstawiono wybrane eksperymenty symulacyjne oraz doświadczenia na hodowlach komórkowych i zwierzętach. Zwrócono również uwagę na najważniejsze problemy, które muszą zostać rozwiązane przed zastosowaniem nanocząstek w praktyce klinicznej. Szeroko zdefiniowana biokompatybilność jest podstawową własnością, jaką muszą posiadać środki radiouczulające, wstrzykiwane pacjentowi. Wciąż dużym wyzwaniem pozostaje efektywne dostarczanie nanocząstek tak, aby uzyskać ich wymagane stężenie i równomierny rozkład w objętości nowotworu.

Słowa kluczowe: nanocząstki, radiouczulacze, elektrony Augera, radioterapia, terapia hadronowa, radioterapia fotonowa

1. Introduction

Although radiotherapy is the primary method for cancer treatment, it utilizes ionizing radiation, which does not interact selectively and damages not only cancer cells but also normal tissues. Megavoltage X-ray beams often used in conventional teletherapy have unfavorable depth dose distribution with short build-up region and subsequent exponential decrease in the energy deposition with increasing depth in tissue. During the treatment session, few beams delivered from different directions and overlapping in target volume are used to raise the dose delivered to the target and minimize the dose received by surrounding tissues [1]. Kilovoltage X-ray beams have even worse characteristics with maximum dose occurring very close to the surface, so they are applicable to intraoperative radiation therapy or treatment of skin cancers [2]. In comparison with X-rays, γ-rays and electrons, the main advantage of protons and carbon ions is different depth dose distribution with Bragg maximum at the end of the particle range. Therefore, it is possible to give them such initial energy, that maximum energy transfer will take place precisely at the area of the tumor [3]. Many hadron therapy centers use the passive beam forming system, which utilizes the superposition of Bragg peaks, called Spread-Out Bragg Peak (SOBP), in order to cover the total length of the tumor. Unfortunately, SOBP also results in an increased entrance dose compared to pristine Bragg peak [4, 5]. Such a high dose for tissues situated in front of the tumor reduces the advantage of hadron therapy over conventional radiotherapy [5]. However, the effectiveness of both mentioned forms of cancer therapy can be improved by radiosensitization of tumor cells. Local enhancement of the effect of the dose can be obtained by means of high-Z nanoparticles, which are capable of intensifying the emission of photoelectrons and Auger electrons after the X-ray irradiation or reinforcing the particle-induced radiation during hadron therapy [4, 6]. Gadolinium (Z = 64), platinum (Z = 78) and gold (Z = 79) nanoparticles are frequently tested as radiosensitizing agents [7, 8, 9, 10, 11, 12].

2. Nanoparticles in X-ray radiation therapy

The mechanism of action of high-Z nanoparticles as radiosensitizers in photon therapy is associated with their higher photoelectric cross section at kilovoltage energy in comparison to soft tissue [10, 13]. Photoelectrons, which have a kinetic energy corresponding to the difference between the energy of incident photons and the electron binding energies, are generated when nanoparticles are irradiated. The gold K-shell electrons have a binding energy of 80.7 keV whereas K-edge of gadolinium is 50.24 keV and for platinum 78.4 keV [7, 14, 15]. The CSDA (continuous-slowing-down approximation) range of electrons in water and soft tissue can be found on the webpage of The National Institute of Standards and Technology [16]. For example, the irradiation of gold atoms with 150 MeV photons results in the emission of K-edge photoelectrons (70 keV) with a range in water of about 78 μm. When the primary photoelectrons are produced inside the nanoparticle they range is several orders of magnitude larger than the radius of this particle [17]. It is worth mentioning that the size of circulating tumor cells is 10–20 μm [18]. The emission of photoelectrons causes vacancies, which are subsequently filled with electrons from higher energy level. The electronic reorganization of atoms results in the release of energy carried by either fluorescence X-rays or Auger electrons [7]. For high-Z elements the likelihood of the Auger electron yield (slightly dependent on the atomic number) is considerably lower than the probability of radiative emission (proportional to the fourth power of the atomic number) [19]. Probabilistic model of the electron and photon emission from gold nanoparticles with different radius (10, 20 and 50 nm) irradiated by X-ray photons has been developed recently [17]. It indicates that the main part (80%) of the energy transferred to the nanoparticle by the incident photon is carried outside the nanoparticle by photoelectrons and the rest is transformed via Auger cascade. This cascade is understood as process of multiple transitions between the subshells, which are associated with subsequent electron or photon emissions and last until the energy excess created by the photoionization is evacuated [17]. Auger electrons have short range, so when their source is located external to the cell nucleus the probability of direct interaction with DNA is low and the damage of cell membrane or mitochondria may occur more likely [7, 19]. However, the electrons emitted as a result of photon interaction with nanoparticles are responsible for the increased generation of reactive oxygen species (ROS) [20]. Radicals cause damage to DNA and other cell structures.

Many research has confirmed the ability of high-Z nanoparticles to cause the local enhancement of the effect of the dose. The experiment performed *in vitro* and *in vivo* with human head and neck squamous cell carcinoma cell lines has proven the radiosensitizing effect of ultrasmall (2.9±0.2) nm gadolinium-based nanoparticles irradiated with 250 kV photons and shown that the cell death caused by such treatment is characteristic of mitotic catastrophe followed by late apoptosis [20]. Another study with glioma cells has demonstrated that the gadolinium nanoparticles (3.0±1.0) nm have better radiosensitizing properties than gadolinium molecules of contrast agent [7]. The increased death rate of the HeLa cancer cells containing gold nanoparticles (47 and 52 nm) has been observed *in vitro* after their irradiation with orthovoltage photon energies (from 120 to 250 kVp) [14].

The dose enhancement effect of nanoparticles was also tested by means of Monte Carlo simulations. Simulations concerning the influence of a flattening filter present in the X-ray beam's path on the radiosensitizing properties of gold nanoparticles of different size and concentrations indicated that dose enhancement factor calculated for unflattened beam was always higher compared to flattened beam [21]. Monte Carlo study of gold nanoparticles irradiated with X-rays (mean energy 55 keV) has demonstrated that dose enhancement factor increases nonlinearly with the concentration of nanoparticles and additionally rises with target depth in the phantom volume [22]. The dose reinforcement effect for capillary endothelium and surrounding tumor volume has been also tested assuming that the blood vessel is located at the centre of soft tissue [22]. Another work utilizing Monte Carlo simulations considers five mono-energetic X-ray sources (50–250 keV) and presents dose enhancement factors for different incident photon energies as a function of distance from gold nanoparticles [23]. One should remember that the radiosensitizing effect of nanoparticles strongly depends on the energy of photons used in therapy [7, 23]. It is believed that the main contribution to dose enhancement originates from photoelectric effect [6, 10, 22]. Better results obtained for kilovoltage X-ray sources confirm this theory. However, the positive outcomes have been also observed for megavoltage X-ray beams. One can cite the in vitro experiment with 50 nm gold nanoparticles incorporated into HeLa cells and irradiated with a clinical 6 MV beam. Statistically significant dose enhancement, which depends on the delivery mode and increases with depth of cell embedding in clinical solid water, was observed [24]. Megavoltage radiation dose-reinforcing effect of PEGylated rod-shaped gold nanoparticles has been also demonstrated in vivo, in mice with subcutaneously implanted prostate cancer cells [25]. The explanation of megavoltage radiosensitization still bases on hypothesis.

3. Nanoparticles as radiosensitizers in hadron therapy

Radiosensitizing properties of high-Z nanoparticles more and more often hold scientists' interest. Several experiments were aimed at presenting the ability of metallic nanoparticles to improve the efficiency of hadron therapy. The increased mortality of human prostate carcinoma cells containing internalized gold nanoparticles has been observed in vitro after the proton irradiation [26]. The amplification of radiation-induced cell death by gadolinium-based nanoparticles has been demonstrated in the experiment with carbon (270 MeV/u) and helium (150 MeV/u) ion beams [8]. Greater reduction of tumor volume as a result of metallic nanoparticles assisted proton treatment has been observed *in vivo* utilizing mouse colon cancer model [5]. Further study has shown the significant increase in long-term survival of animals and complete tumor regression in comparison with standard proton treatment [4].

The mechanism of the enhancement of the effect of the dose in hadron therapy is worth to consider. Initially, it was assumed that metallic nanoparticles activated with a high-energy proton beam have the ability to release localized X-rays and such particle-induced X-ray emission was called PIXE effect [5, 26]. However, this concept met with wide criticism [27, 28, 29]. In fact, the energy transferred to the cells by photons from PIXE is negligible and never leads to measured effects [28]. The major contribution to the reinforcement of local dose is made by low-energy electrons, particularly single Auger electrons [9]. The secondary electrons produced along the primary tracks of the incident ions have the ability to cause the inner shell ionization of the atoms in nanoparticles and the relaxation of the excited cores occurs mainly by Auger process [8, 27, 29]. Also ionisation of outer shells cannot be ignored when considering the amplification of the electron emission [8]. Generally, for particle fluences used in hadron therapy (up to 10^9 particles per cm^2) the likelihood that ion directly traverses the nanoparticle and induces inner ionization resulting in the Auger cascade is lower than the probability of

photon induced ionizations in conventional therapy [9]. Nevertheless, the Monte Carlo simulations have proven the potential utility of Pt, Au, Gd, and Ag nanoparticles as radiosensitizing agents in proton therapy and linked the local dose reinforcement to the increased generation of low-energy electrons [9]. The estimation of the dose distribution around gold nanoparticles in terms of ejected electrons was performed in other Monte Carlo experiment and indicated that the dose reinforcement occurs not only in depth but also in the radial direction from the proton beam axis [30]. Additionally, the new explanation of the enhancement of the low-energy electron production by nanoparticles irradiated by fast ions has been proposed recently [31]. It suggests that considerable increase in the number of emitted electrons originates from two different types of collective electron excitations: plasmon type excitations of delocalized valence electrons in whole metal nanoparticle and the collective excitation of d electrons in individual atoms in a nanoparticle (atomic giant resonances). First mentioned excitation results in the yield of 1–10 eV electrons, the second one in the production of higher energy electrons (of about 10–30 eV) [31]. Regardless of the mechanisms of the emission of low energy electrons from nanoparticles it is important that such electrons induce radiolysis of water molecules present in the close neighborhood. The reactive oxygen species, e.g. hydroxyl radicals ($HO^{\bullet}$), which might be produced even by electrons with the energy < 20 eV, cause damages and consequently death of cancer cells [8, 29]. The key role of free radicals in the radiosensitization effect has been experimentally confirmed. In comparison with proton-alone treatment, the dose-dependent increase in the generation of intracellular reactive oxygen species has been observed when proton therapy was combined with metallic nanoparticles [4]. Furthermore, the experiments utilizing free radical scavengers (e.g. dimethyl-sulfoxide) have shown that the radiation effect decreases strongly when the scavengers are used [12, 27]. Therefore, one can firmly state that the part of the mechanism of the enhancement of the effect of the dose in hadron therapy is free radical mediated.

4. Problems and limitations

Although the efficiency of high-Z nanoparticles as potential radiosensitizers has been proven in animal and cell culture experiments, their clinical application is still associated with overcoming many problems. Among the most important things one should mention the need for ensuring the biocompatibility of nanoparticles injected into the patient's body, the necessity for development of methods for effective delivery of nanoparticles to the target and obtaining the proper concentration and uniform distribution within the tumor volume. It is worth to take a closer look at these issues.

4.1. Biocompatibility and stability of nanoparticles

To fulfill their function, nanoparticles must be delivered to the tumor. In most cases, direct intratumoral injection is not possible and intravenous administration is the primary way to introduce them into the body. Therefore, the broadly defined biocompatibility is the basic feature that is required from nanoparticles. Such biocompatibility is understood not only as the lack of toxicity but also as the ability to maintain intended function in the biological environment [32]. The potential agglomeration of injected nanoparticles can influence their biodistribution, cellular interactions, toxicity and pharmaco-kinetic profile [33]. Particles, which have large surface area to volume ratio, have a tendency to agglomerate, because they strive to reduce their surface energy [34]. An appropriate polymer coating modifies the surface properties of nanoparticles core and thus improves the stability of nanoparticles (both in aqueous solutions and in the bloodstream), prevents agglomeration and also provides the surface hydrophilicity [32]. Additionally, the protective shell has a strong impact on the interactions between nanoparticles and their environment. The capability to prolong the circulation time of nanoparticles injected into the bloodstream by preventing their opsonization and reducing the uptake by macrophages is a great asset of coatings formed of poly(ethylene glycol) [35]. Chitosan, which is another popular coating material, due to cationic character and mucoadhesive properties, is able to enhance the nanoparticles interaction with the mucus layer covering different epithelial surfaces [36]. The selection of a coating material from a plurality of tested polymers and determination of appropriate grafting density of polymer chains on the nanoparticle surface is also important in terms of the radiosensitizing properties of nanoparticles. Some studies indicate that coating can attenuate the low-energy electrons

released from gold nanoparticles and thus diminish the radiosensitization effect, so the use of the shortest possible ligands is recommended [37]. Electrons emitted due to the photoelectric effect may be scavenged by the coating before they manage to react with water and produce radicals. It has been observed that the functionalization of gold nanoparticles reduces the generation of hydroxyl radicals ($HO^{\bullet}$) after X-ray irradiation to an extent, which depends on the number of coating atoms [38]. The significantly reduced abundance of reactive oxygen species may be also associated with the fact that they are scavenged by specific chemical groups attached to the surface of nanoparticles [38]. The optimization of polymer shells in order to protect nanoparticles during their transport in bloodstream and achieve the proper radiosensitizing effect after the delivery to the target still requires further study.

4.2. Targeting and accumulation of nanoparticles in the tumor cells

The sufficient concentration of nanoparticles within a target volume is the basic condition for achieving the considerable enhancement of the effect of the dose. Many articles concerning the radiosensitizing properties of nanoparticles are based on cell cultures experiments and do not consider the fundamental problem of providing such amount of nanoparticles to tumor cells in human body. In experiments performed on mice nanoparticles were administered intravenously in large quantities e.g. 300 mg/kg of body weight, which is definitely too high for clinical application in human [4, 5, 39]. Additionally, it was found that only less than 1% of injected dose was captured by cancer tissue [4, 39]. One should remember that small nanoparticles can penetrate the tumor tissue and passively accumulate thanks to the enhanced permeability and retention (EPR) effect [21]. Tumor vasculature is leaky. Because of defective, irregular endothelial cells, vessel walls frequently exhibit the loss of integrity and have wider fenestrations in comparison with normal blood vessels [40, 41]. Moreover, in case of brain tumors it was observed that radiotherapy can further increase the permeability of the blood-brain barrier and improve the accumulation of gold nanoparticles in cancer tissue [40]. After extravasation nanoparticles must cross the tumor interstitium and enter cells, probably via endocytosis [8, 41]. Even if nanoparticles penetrate the cancer cells located in the vicinity of the blood vessels there is a problem with reaching the poorly differentiated hypoxic cells situated apart from blood pathways. It seems highly improbable to obtain homogeneous distribution of nanoparticles within the tumor, which microvasculature exhibits disorganization resulting in heterogeneity of blood flow [42]. The effectiveness of a passive targeting utilizing EPR effect is poor, only small fraction (up to 10% depending on the size and coating material) of injected nanoparticles reaches a tumor [43]. Therefore, there is a strong need to improve the efficiency of nanoparticle delivery. The enhancement of the uptake of nanoparticles by cancer cells can be can be reached via active targeting. Specific ligands, which selectively bind receptors or molecules overexpressed on tumor cells, can be attached to nanoparticles [43]. Monoclonal antibodies, epidermal growth factor (EGF), folic acid, and transferrin are often proposed as such ligands [41]. As an example, one can mention the experiment with gold nanoparticles which were tested as radiosensitizing agents. Due to the over-expression of folate receptors on the membrane of HeLa cancer cells, the internalization of folate-conjugated nanoparticles into these cells was higher in comparison with ligand-free nanoparticles, which translated into a better radiosensitizing effect [14]. Also functionalization of gold nanoparticles with PEGylated trastuzumab has been proposed in order to target them at human epidermal growth factor receptor-2 (HER-2) positive breast cancer cells [44]. Such nanoparticles (with a core diameter of 30 nm) in combination with 300 kVp X-rays had the ability to significantly increase the induction of DNA double strand breaks in cancer cells [44]. The advanced tumor targeting methods are promising, but they are still under development and require a lot of testing before clinical application. An example of prospective use of gold nanoparticles as radiosensitizers during proton therapy of breast cancer is illustrated in Fig. 1. Nanoparticles assisted radiotherapy opens up new perspectives in the fight against cancer.

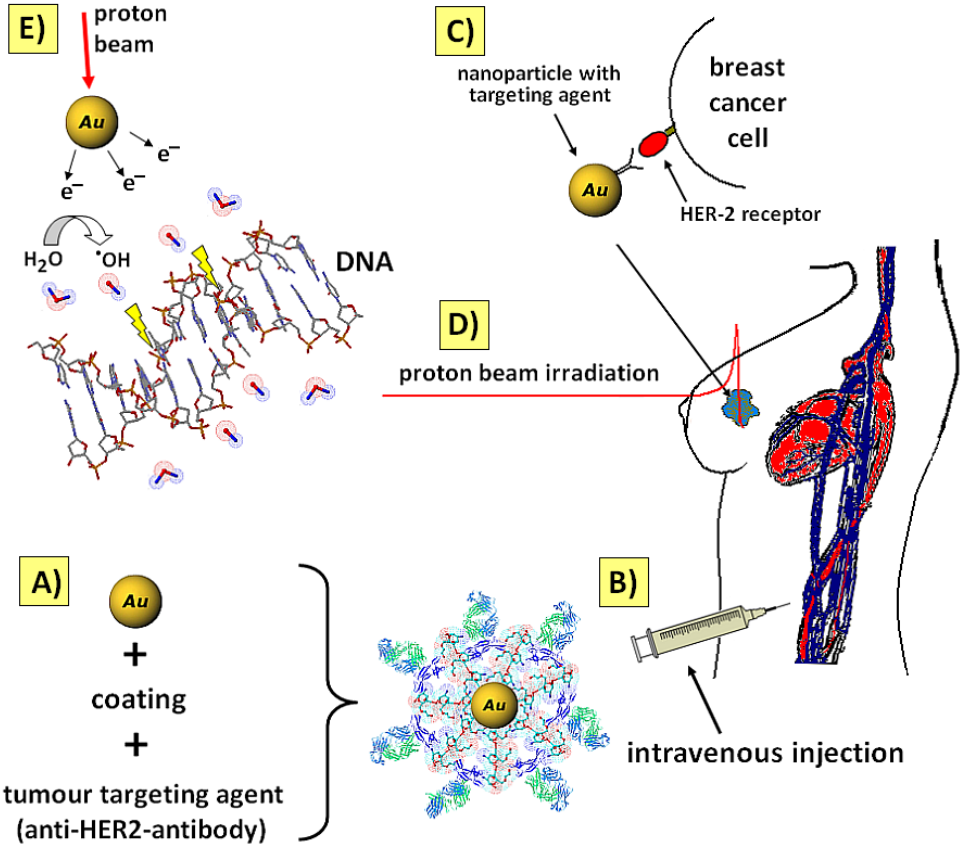

Fig.1. An example of prospective use of gold nanoparticles as radiosensitizers during proton therapy of breast cancer. A) Gold nanoparticles are covered with biocompatible polymer and conjugated with tumor targeting agent (anti-HER-2 antibodies). B) Such functionalized nanoparticles are injected into bloodstream. C) HER-2 receptor mediated internalization AuNPs occurs in the target area, because HER-2 is overexpressed on breast cancer cells. D) Tumor is irradiated by proton beam. E) Low energy electrons emitted by nanoparticles induce water radiolysis and create radicals that damage cancer cells

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