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Toxicity and Attenuation of Gold Nanoparticles as a cancer theranostic agent

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ABSTRACT

The size of nanoparticles (NPs) is very smaller than the human cells and also, is comparable to the subcellular organelles, and then they can pass via cell membrane and apply their influences on the different organelles. Aim of nanomedicine is the investigation on the using nanostructures to design new different applications at the diagnostic and treatment of the diseases. Among different kinds of NPs, gold nanoparticles (GNPs) are highly regarded because of the low cytotoxicity and the special properties of their surface. This review focuses on the cytotoxicity of GNPs and uptake of them by different cell lines and animal models. According to some studies reports it is obvious that there is not specified rules predicting the toxicity and the amount penetration of GNPs into the cells. Different parameters like size, shape, and kind of material coated on the GNPs surface can be influence on the toxicity and their penetration rate into the cells or tissues. In the animal models there are additional parameters like that administration routs should be taken into consideration. The all parameters effects have been concerned in this review.

1. Introduction

Nanotechnology is defined as a science that can design and build structures in nanometer scale in the range of 1-100 nm [1,2]. These dimensions are smaller than the human cells and are comparable to subcellular organelles and biological molecules; they can enter to cells and act with different subcellular organelles and molecules both on the surface and inside the cells [3,4] Because these unique properties different kind of nanoparticles (NPs) have been investigated for medicine application, in the term of nanomedicine, in diagnostic and treatment modalities. Early cancer detection and treatment with more therapeutic ratio is the one of fields that the nanotechnology has been attended [5].

In recent years, many studies have been done on the application of different designed organic and nonorganic NPs in cancer diagnostic and treatment [6]. Between all kind of NPs, Gold nanoparticles (GNPs) are highly regarded because of special properties such as facile synthesis in different shape and size, different methods for characterization, nontoxic nature, biocompatibility, and optical and chemical surface properties [6-8]. Different studies indicate that the GNP can be used in computed tomography and magnetic resonance imaging to improve the image quality for early cancer detection [4,9]; and they can be used for drug delivery and in the combination of photodynamic therapy, photothermal therapy and radiotherapy to improve the efficiency of cancer treatment [10-12].

For all of the applications of GNPs, before the use of them in real clinical setting as drug, the toxicity and health impact of designed nanoparticle on targeted and normal tissue must be investigated. In this article the cellular and animal studies of the toxicity and attenuation of GNPs is reviewed. Different studies have been shown that the toxicity and uptake of GNPs by different cell lines and animal model is dependent to several parameters such as size, shape and surface properties of GNPs. For investigation of different studies, we categorized them in two parts: cellular and animal studies.

2. Cellular studies

2.1. Size of GNPs

NPs can pass from cell membrane via endocytosis phenomena in vesicles within 300-500 nm diameters [13]. The reports indicate that the uptake of GNPs by cells is dependent to distance and number of endocytosis membrane receptors on the cell surface and it is different from cell to cell [2,14,15]. Although the best size for more uptake GNPs by mammalian cells is reported about 50 nm, but there are several studies that have reported different optimum size for kind of cells [13,14,16]. According to the study of Xing et al. MCF-7 breast cancer cells uptake the 100 nm sphere GNP significantly more than the other sizes tested of 5-100 nm [17]. Pancreas cancer cell lines uptake the 20 nm GNPs higher compared to the 5-50 nm GNPs [18]. It is shown that the size of GNPs is an important factor for uptake of them by different cells and it can be different for kind of cell lines.

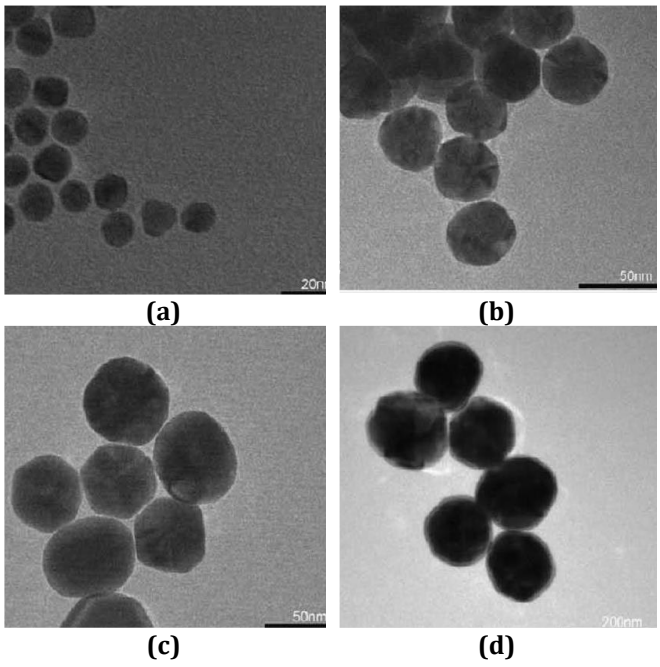

Fig. 1. TEM of gold nanoparticles a) 10 nm, b) 30 nm, c) 40 nm, d) 50 nm (ref 17).

2.2. Shape of GNPs

In addition to size, the shape of GNPs is an important factor. Different studies have shown that the NPs uptake is dependent to shape and the sphere shaped NPs have more uptakes by cells. Chitrani et al. reported the optimal GNPs for Hella cells is 50nm nanosphere and the uptake of rod shaped GNP is lower than the spherical one [19].

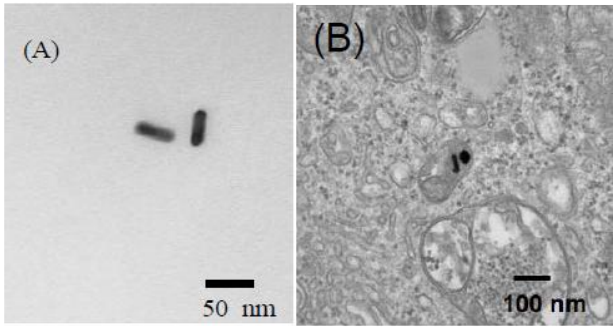

Fig. 2. TEM images of rod-shaped gold nanoparticles (A) and rod-shaped gold nanoparticles trapped inside the vesicles of the Hela cells (B) (ref 19).

2.3. Surface coating of GNPs

Because of surface properties of GNPs and for selective uptake of them by cells, different kind of cancer targeting molecules such as antibody, thioglucose, nucleic acid aptamer and small targeting molecules can be coated on the surface of GNPs. These coating can decrease the cytotoxicity of GNPs, in addition to increase of their uptake by selective cells [19,20]. One of the materials that can be used for stability increase, toxicity decrease and circulation time increase of GNPs for in vivo studies is Polyethylene glycol (PEG) [21]. Although the Hella cells have optimum uptake of 50 nm GNPs, but the Zhang et al. indicated that the PEGylated GNPs have different quite performance of the naked GNPs. They showed 15 nm PEGylated GNPs had more uptake that the 45 nm PEGylated GNPs [22]. The influence of shape, size and surface properties of GNPs on cellular uptake of PC-3 cell line at three type of GNPs (spherical, PEGylated spherical, PEGylated rods) have been evaluated. The results showed the spherical GNPs were taken up much higher than PEGylated particles and the size of 30 and 50 nm were taken up higher than the size of 90 nm [23].

Several studies have shown the coat of glucose on the surface of GNP can enhance the uptake of cells based on the metabolism of cancerous cells [24-26]. For A549 and MDA-MB-231 cells, it is reported that glucose coated GNPs can be taken up 2 folder more than naked GNPs [24]. Even exposure to glucose coated GNPs results in a three time increase of GNP uptake in prostate cancer cells [27].

Li et al coat the surface of GNPs with folic acid, glucose, and both of them. The folate receptors are over expressed in kind of cancerous cells such as brain, ovarian, renal, and breast

cancer. The folic acid coating can be used as small targeting molecules for these kinds of cancers. In the study of li et al showed the uptake of GNPs by KB cells (over expressed folate receptor) with both coating of glucose and folic acid was increased 3.9 and 12.9 folds compared with folic acid and glucose coating alone, respectively [20].

For the assessment of size, shape and surface coating effect of GNPs on cell uptake, different size of sphere and cubic GNPs with three different kind of coating (PEG, antibody anti-HER2, and poly allymine hydrochloride (PAA)) have been investigated in SK-BR-3 cells. As a targeting molecules, the antibody HER2 was selected because this cells are known to overexpress HER2 receptors. The results of the study showed the cells have more uptake spherical particles than cubic particles and there is not any obvious uptake difference between PEGylated or anti-HER2 coated nanoparticles, while the nanoparticles with the PAA coating have the lower uptake [28].

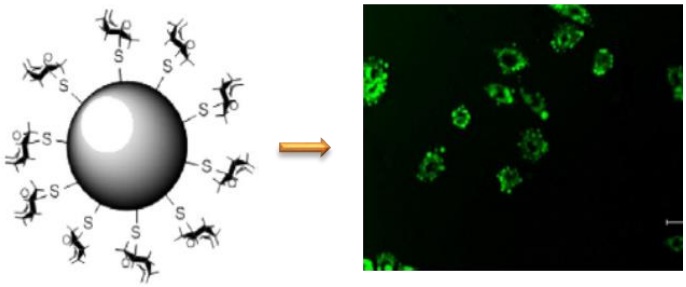

Fig. 3. The schematic of GNPs coated with thio-glucose and After culturing with Glu-GNPs for 24 h, SK-OV-3 cancer cells were treated with 6 MV irradiation (8 Gy) + 5 nM Glu-GNPs (ref 26).

2.4. Used concentration of GNPs

After the investigation of GNPs uptake by cells, is important to evaluate their toxicity. Although most studies reported that GNPs have nontoxic nature and the toxicity comparison of different NP (silver, gold, and platinum) showed that the GNP are non-toxic and the silver nanoparticles are most toxic, but some have shown that they can induced toxicity at special condition [29].

Some cytotoxicity studies of gold nanoparticles in human cells have indicated that they can be nontoxic up to 250 mM [30]. But, more studies confirmed the usage of low GNPs concentration. Zhang et al. reported the concentration of 75 mg/ml had not any obvious toxicity. However, the concentration of 150 mg/ml could indicate the slight decrease viability of K562 cells [31].

Measuring the cytotoxicity of GNPs of 3-100 nm in Hella cells indicated that GNP without regardless of their size had nontoxic nature in the concentration of 0.4 mM [32]. In our previous studies, we investigate the toxicity of glucose coated GNPs in two cancerous MCF7 and QUDB cell lines. We did not see any obvious toxicity up to 0.1 mM for both cell lines [33,34].

2.5. Zeta potential of GNPs surface (cationic or anionic)

Studies showed that zeta potential of GNPs is an important factor for entrance of them into cells and toxicity of them. Goodman et al reported because of interaction of cationic GNP with negative cell membrane, they are more toxic than anionic GNPs within the same size [2]. Kong et al. compared the toxicity of cysteamine and glucose coated GNPs at 10.8 nm in MCF7 and MCF-10A cells. Pictures from the TEM indicated that cysteamine capped GNPs are mostly bounded to the cell membrane, while the glucose coated GNPs can enter to cells and distributed in the cytoplasm that it is due to difference in zeta potential of two kinds of GNPs. The toxicity evaluation did not any toxicity for both of them [35].

2.6. Kind of cell or tissue

Beside these parameters, cytotoxicity of GNPs also can be influenced by cell type. GNPs with same concentration was found to be nontoxic to hamster kidney and human hepatocellular liver carcinoma cells, but toxic to a human carcinoma lung cell line [31]. In the other study the viability of Hela cells reduced by 20%, but for murine fibroblastoma cells this decreasing was about only 5% with the same kind and GNPs concentration [36]. At the same concentration of 1.9 nm GNPs, a kind of prostate cancer cell line showed more toxicity compared to MDA-231-MB cells [37].

According to all studies that mentioned and the different results of them, it is clear that we cannot define a rule for the prediction of cytotoxicity and uptake of GNP by cells. The surface properties of GNP, size, shape, kind of cell, and even the method of synthesis determine the uptake and cytotoxicity of gold nanoparticles process by cells. Real biological systems act different from in vitro systems and has own complexity that an in vitro system cannot replicate that or provide quite data about the response of a physiological system to an agent. Because that, the biodistribution, cytotoxicity and the effect of GNP in animal models have been investigated as follow.

3. Animal studies

A whole organism and real biological systems are more complex than the cells outside the body. Therefore, for assessment the biodistribution and safety of nanoparticles as drug, more toxicological studies are required [38].

3.1. Size of GNPs

The size of nanoparticles is the key parameter that determines the biodistribution of NPs in different body organs. Pharmacokinetic research at NPs showed the NPs smaller than the 50 nm can pass through cell membrane and enter into cells. When the nanoparticles are injected, it is important that they can pass through the vessels and reach to cells. Studies showed the NP smaller than 20 nm in diameter can pass through blood vessel endothelium [35,39]. Another study reported the NPs with the size of 12 nm and smaller can pass the blood brain barrier and inter to brain tissue [38].

In the study of De Jong et al. the distribution of GNPs with the sizes of 10-250 nm was investigated 24 hours following intravenous injection within rats. The GNPs with the size of 10 nm more widely distributed in different organs but the bigger GNPs accumulated in liver and spleen [40]. In the other study by Schmid et al., GNPs of 1.4-200 nm has been used for the assessment of biodistribution in female rate 24 hours after intravenous injection. The result of this article is similar to previous one and all size of GNPs except the 1.4 nm, cumulate between 90-100% in the liver tissue [41].

There are more several articles that confirm the results similar to above. We can say a rule about the biodistribution of GNPs in the biological and physiological systems; NPs around 10 nm and smaller can enter and cumulate in different tissue even brain, but the bigger size remains more in blood or absorb by liver and spleen. In fact, there is a direct relation between size of NPs and uptake of liver and spleen that as the particle size increase, increase in concentration observe in liver and spleen whereas the concentration of them in the other organs, especially brain, decrease. Actually small NPs of 5-15 nm have wider organ distribution than the bigger. On the other hand, if the organs like liver, spleen and kidney are the treatment target in clinical situation, the bigger NPs are more suitable [42-45].

The biodistribution of GNPs in female C57BL/6 mice with a tumor have been investigated with 13 nm GNPs. The atomic absorption detection showed the ratio of GNPs concentration in the tumor and tumor surrounding muscle was 6.4/1. 24 hours after injection, the ratio of gold (micro gram) to tissue weight (milligram) for liver, tumor and tumor surrounding tissue have been reported 147, 74.24, and 11.5 respectively. This results showed, although the uptake of GNPs is much more for liver but the tumor can uptake the GNPs with good concentration in comparable with surrounding healthy tissue, that it can help to improve image quality and the efficiency of cancer treatment [46].

3.2. Shape of GNPs

In addition to size, the shape of gold nanoparticles can be influence on biodistribution of them. In the study of Arinda et al. with animal models, the effect of GNPs shape on biodistribution of PEGylated gold nanosphere and nanorode has been investigated. The result showed the gold nanosphere has shorter circulation time than rod and can be accumulated by liver faster than rod [47]. In the other study, in vivo biodistribution of sphere, rod, and cubic shaped GNPs revealed that the sphere shaped have the best biocompatibility, and rod shaped were more toxic than sphere and cubic shaped of GNPs. These results showed that the in vivo biodistribution and cytotoxicity of GNPs are shape dependent too [48].

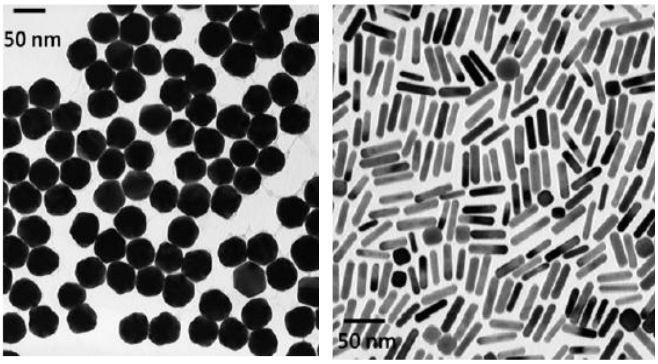

Fig. 4. TEM images of PEGylated gold nanospheres (a) and rods (b). PEGylated gold nanospheres were uniform in size and shape, whereas there was 6% shape discrepancy for nanorods (ref 47).

3.3. Surface coating of GNPs

Coating of nanoparticles is an important factor that can change them biodistribution and cytotoxicity. biodistribution and pharmacokinetic effect on the performance of an intravenous injection [49]. With the aim of GNPs usage as a radiosensitizer drug, Geng et al. were coated them with glucose and PEG layer. The result of the study indicated the circulation time of 20 nm GNPs with combination of 2 layers is more than for glucose coated and naked nanoparticle. The biodistribution assessment showed that the concentration of GNPs with 2 layers in the tumor mass was about 20 times higher than the surrounding normal tissues, although the amounts of them was fined in liver, spleen, lung, and kidney. They reported that this kind of GNPs (combination of 2 layers coating with glucose and PEG) provide excellent in vivo stability and tumor targeting. Their data indicates the 20 nm nanoparticles size is the optimal diameter for tumor targeting and reduce cytotoxicity for healthy tissues [49].

The investigation of in vivo cytotoxicity of GNPs with 1.9 nm in diameter showed normal hematology and blood chemistry [50]. In the other study, the naked GNPs with the sizes of 3-100 nm were injected intraperitoneally to BALB/C mice. The results indicated GNPs in the range of 8 to 37 nm induced sever affects in mice such as weight loss, loss of appetite, and various degrees of abnormality in liver, lung, and spleen [32]. The other size did not show obvious harmful effect. 13 nm sized GNPs coated with PEG layer were

found to have long circulation time in blood about 30 hours. The investigation of organs in 7th day after injection showed GNPs accumulated in the spleen and liver and induced inflammation and apoptosis in the liver tissue [51].

In the other study with PEG-coated GNPs with sizes of 4.8, 12.1, 27.3, and 46.6 nm, the pathology and blood chemistry investigations indicate that this kind of GNPs cannot cause damages of spleen and kidney, but they can induce liver damage. Among them 46.6 nm PEG-coated GNPs had the best biocompatibility. According to the results, they reported that the cellular toxicity of GNPs is in acceptable level for all sizes of GNPs, but the 12.1 nm GNPs have the highest concentration in the tumor [22].

Zhang et al. reported that the cytotoxicity of GNPs coated with PEG layer is complex and there is not the definite relationship between size and toxicity. In other word, we cannot say the smaller ones are more toxic. In their study the cytotoxicity of PEGylated GNPs with sizes of 10 and 60 nm was higher than the 5 and 30 nm sizes. The toxicity appeared in decreasing in weight, but without any statistically differences and no abnormal behaviors and clinical signs were seen [30].

3.4. Administration routs for animal studies

According to the different result that are mentioned above, the tissue biodistribution and cytotoxicity of GNPs are dependent to size, shape and surface coated layer of them. In addition, that, several studies appear that the cytotoxicity of them can be influenced by exposure routs. Because that, the assessment of tissue distribution and cytotoxicity of GNPs following different exposure routs such as the lungs, gastrointestinal tract, skin, intravenous and intraperiotoneal injection has been done by different groups. In the study of Zhang et al. the toxicity of 13.5 nm GNPs was investigated at three exposure routs of oral, intraperiotoneal, and tail vein injection. They reported the oral and intraperitoneal injections induced obvious toxicity and slight decrease in body weight, while the vein injection induced low toxicity [45]. Hillyer et al. reported that the oral administration of GNPs had strong absorption effect on gastrointestinal system [52].

In order to assess the distribution of GNPs in the rat body from the respiratory system, Behnke et al. used from radiolabelled GNPs in the sizes of 1.4 and 18 nm. About 24 hours after administration, 99.8% of 18 nm GNPs remained in lung, but for 1.4 nm GNPs it was 91.5% and 8.5% found in the blood and liver. It is obvious that the passage of GNPs from air-blood barrier of the lung is size dependent. They also have reported 24 hours after intravenous injection of 18 nm GNPs, they located in the spleen and liver and remove perfectly from the blood. However the 3.7% of 1.4 nm GNPs remained in the blood after 24 h and the lower amount accumulated in liver and spleen in comparable with larger size [43].

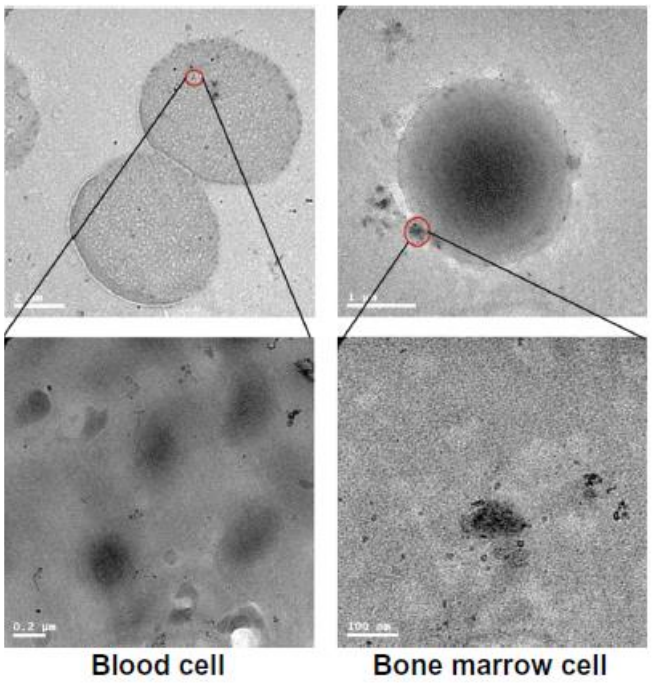

Fig. 5. Transmission electron microscopy figures for gold nanoparticles in bone marrow and blood cells 14 days after oral administration at 2200 µg/kg (ref 45).

For investigation the permeation of GNPs through skin, sonavane et al used the different size of GNPs of 15, 102 and 198 nm in diameter with rat. They reported the permeation of NPs is dependent to size and time. The 15 nm GNPs had a higher efficiency for penetration and reaching to deeper layer of skin, whereas the other particles remained in surface layers of skin, dermis and epidermis [53].

4. Conclusion

According to different results of above studies that mentioned, the toxicity and the uptake of GNPs by different cell lines or tissues is dependent to several parameters. Although the more studies reported that the GNPs have nontoxic nature but on the other hand, there are several studies that they indicated a degree of toxicity for GNPs. We can conclusion the toxicity and uptake of GNPs must to be investigated by regarding all of the below parameters:

1. Size of GNPs
2. Shape of GNPs
3. Surface coating of GNPs
4. Zeta potential of GNPs surface (cationic or anionic)
5. Used concentration of GNPs
6. Kind of cell or tissue
7. Administration routs for animal studies

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