Pharmacokinetic study of PEGylated plasmon resonant gold nanoparticles in tumor-bearing mice

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ABSTRACT

Advances in plasmonic nanoparticle synthesis and functionalization open new possibilities for biomedical applications, including enhanced cancer therapy by selectively gaining access to tumor due to their small size and modifiability. Herein, we report results encompassing the synthesis and PEGylation of gold nanorods and silica/gold nanoshells and an *in vivo* pharmacokinetics study of functionalized nanoparticles in tumor-bearing mice.

Keywords: nanoparticles, pharmacokinetics, biodistribution, cancer therapy

1 INTRODUCTION

Gold nanoparticles conjugated to biospecific macromolecules are very important for current nanobiotechnology [1]. Their use for analytical purposes [2-4], cellular-structure visualization [5-8] and imaging [9], targeted drug delivery [10-16], and photothermal cancer therapy[17-23] is based on a combination of biological recognition (the probe molecule + the target molecule) and resonance absorption or scattering of light on frequencies corresponding to the excitation of localized plasmons [24]. The spectral tuning of the single-particle plasmon resonances and the change in the ratio between absorption and scattering efficiencies are achieved by varying particle size, shape and structure [25-28].

Pharmacokinetic and biodistribution properties of functionalized nanoparticles are of great interest from clinical point of view because of potential human toxicity. After the initial injection of nanoparticles into animal, the systemic circulation distributes the particles towards all organs in the body. To evaluate the distribution of absorbed nanoparticles inside the body over the various organ systems and within the organs, one needs systematic studies on various animal models.

Several publications have shown distribution of particles to multiple animal organs including spleen, heart, liver, and brain. For instance, Hillyer and Albrecht [29] observed an increased distribution to other organs after oral administration of metallic colloidal gold nanoparticles of decreasing size (from 58 to 4 nm) to mice. The highest amount of gold particles was obtained for smallest particles (4 nm) administered orally.

For 13-nm colloidal gold nanoparticles the highest amount of gold was observed in liver and spleen after intraperitoneal administration [30]. Niidome et al. [31] showed that after intravenously injection of gold nanorods these particles accumulated predominantly in the liver within 30 min. The functionalization of gold nanorods with PEG-thiols resulted in a prolonged circulation.

De Jong et al. performed a kinetic study to evaluate the gold nanosphere size (10, 50, 100 and 250 nm) effects on the in vivo tissue distribution of nanoparticles in the rat. Rats were intravenously injected in the tail vein with gold nanoparticles, and after 24 h the gold particle distribution was measured quantitatively. The authors showed that the tissue distribution of gold nanoparticles is size-dependent with the smallest 10 nm nanoparticles possessing the most widespread organ distribution.

Katti and co-workers [32] published detailed *in vitro* analysis and *in vivo* pharmacokinetics studies of of AuNPs within the nontoxic phytochemical gum-arabic matrix in pigs to gain insight into the organ-specific localization. Pigs were chosen as excellent animal models because of their similar physiological and anatomical characteristics to those of human beings.

Earlier we have reported antitumoral effect of laser "microexplosions" of different carbon nanoparticles against animal tumors [33]. However, carbon nanoparticles are removed from a blood by reticuloendothelial system very quickly after intravenous injection and they do not accumulate preferably in tumor. Here we report results of pharmacokinetic study of PEGylated plasmon-resonant gold nanoparticles in tumor-bearing mice. Two types of nanoparticles have been used in our study: gold nanorods and silica/gold nanoshells.

2 MATERIALS AND METHODS

2.1 Reagents

The following reagents were used in synthetic procedures: tetraethyl orthosilicate (TEOS, Aldrich), tetrakis(hydroxymethyl)phosphonium chloride (THPC, Fluka), 3-aminopropyltrimethoxysilane (APTMS, Aldrich), PEG-thiol (Nektar), absolute ethanol, tetrachloroauric acid (TCAA, Aldrich), potash (Reachim Co., Russia), and formaldehyde (Serva) were of research grade; 25% aqua ammonia was of analytical grade. Ethanol was purified by additional distillation.

2.2 Gold Nanoparticles Synthesis

Nanoshells have silica cores 80 nm in diameter with 25 nm thick gold shells. Nanorods have length of 50 nm and diameter of 15–20 nm. These parameters have been chosen to provide plasmon resonance in red region of the spectrum where ruby laser radiates (Q-switched ruby laser can be used for laser "microexplosions" of these nanoparticles). Then poly(ethylene glycol) molecules have been attached (using end thiol groups) to surface of nanoparticles to provide their defense from reticuloendothelial system.

Silica-gold nanoshells were fabricated as described in [34], with minor modifications concerning the reagent concentrations. First, silica nanoparticles were grown by reducing TEOS with NH4OH in absolute ethanol. To do this, we mixed 10 ml of absolute ethanol, 0.6 ml 25% aqua ammonia and 0.3 ml TEOS. For the preparation of gold seeds, 220 μl of 1 M aqueous NaOH and 6 μl of 80% THPC were added to 20 ml of triply distilled water. The solution was vigorously agitated on a magnetic stirrer at 1000 rpm, and 880 μl of a 1% TCAA solution were added. Next, aminated silica particles were added to the gold-seed dispersion. Gold particles adsorb to the amine groups on the silica surface, resulting in silica nanoparticles covered with the gold colloid. Gold nanoshells were then grown by reacting HAuCl4 with the silica−Au colloid particles in the presence of formaldehyde at room temperature. In this process, additional gold reduces on the adsorbed metal particles, which act as nucleation sites. The nanoshells were centrifuged and sonicated in HPLC-grade water before use.

Gold nanorods were prepared by two step growth method [35]. Seeding solution of gold nuclei was first prepared by mixing 0.10 ml of 0.02 M $NaBH_4$ with 10.0 ml of an aqueous solution containing 0.5 mM $HAuCl_4$ and 0.1 M CTAB. After vigorous stirring for 30 min, 12 μl of seeded nanoparticles' solution were added to a growth solution at room temperature. The latter was prepared by addition of 0.10 ml 0.08 M isoascorbic acid to a mixture of 10 ml aqueous 0.1 M CTAB solution, 0.5 ml 0.01 M $HAuCl_4$, and 0.2 ml of 4 mM $AgNO_3$ without stirring. The formation of gold nanorods proceeded over a period of one hour.

The following PEGylation protocol was used for silica/gold nanoshells. The nanoshells' solution was centrifuged at 7000 *g* for 20 min to pellet the particles, decanted, and then nanoshells were resuspended in the same amount of water. One hundred microliters of 2 mM potassium carbonate and 10 μl of 1 mM PEG-thiol solution were added per 1 ml of the nanoparticles' solution. The mixture was stored overnight at room temperature, then it was centrifuged, decanted and prepared pegylated nanoshells were resuspended in water several times to remove the PEG-thiol excess.

Gold nanorods were pegylated according with procedure described in [36].

2.3 Animals

For in vivo experiments, BDF_1 male mice bearing Lewis lung carcinoma were used. Aqueous suspensions of nanoparticles were injected in tail vein on the 7^{th} day after tumor transplantation. Mice were decapitated through various time intervals after injection, and samples of blood, liver, tumor and muscles have been taken.

2.4 Gold Analysis

To prepare samples for analysis, two methods of mineralization (i.e., acid decomposition) – in open and enclosed systems – were used. The mixture of two acids, HNO_3 and HCl, with the addition of small amount of H_2O_2 was used for complete mineralization of the sample organic components. The strong gold complex formation in the solution analyzed was achieved by the use of 2 N HCl. The duration of sample mineralization using the open vessel was varied from 3 to 8 hours. This procedure of mineralization causes the formation of coagulated organic particles in blood and liver samples. This precipitates were separated and exposed to the further decomposition in enclosed system (autoclave). At the same time, analyzed samples (0.1–0.6 g) were completely mineralized in autoclaves at 240°C for 1.5 h. The gold analysis in solutions obtained was carried out using 3030 Z Perkin-Elmer spectrometer and graphite furnace HGA-600. Note that the presence of NaCl in solutions analyzed does not influence the results of gold determination in mouse blood and organs.

3 RESULTS

Gold concentrations for nanorods and nanoshells in blood, liver, tumor, and muscle of mice after i/v injection are shown in Tables 1 and 2, respectively.

Very good effect of "enhanced permeability and retention" (EPR) was observed in case of PEGylated nanorods that collect in tumor even better than in liver and much more than in muscles (Table 1). Such high accumulation contrast (35:1) can be applied for optical

diagnostics of tumors and for targeted drug delivery without use of specific antibodies.

Good but some lesser contrast (9:1) was found for PEGylated nanoshells (Table 2). Probably this is due to their larger sizes. But their advantage is several times higher cross-section of light absorption and light scattering than for nanorods.

Time interval after injection	Gold concentration, mg/kg			
	Blood	Liver	Tumor	Muscles
5 min	14	3.5	3.3	0.13
15 min	13	2.5	6.0	0.16
1 h	14	2.6	9.0	0.26

Table 1: Gold concentration in mice blood and organs through various time intervals after intravenous injection of gold nanorods

Time interval after injection	Gold concentration, mg/kg			
	Blood	Liver	Tumor	Muscles
5 min	11	4.1	0.33	0.12
1 h	9.5	9.0	0.57	0.41
3 h	6.2	9.7	1.1	0.15
6 h	2.7	11	1.3	0.14
24 h	0.075	10	1.6	0.29

Table 2. Gold concentration in mice blood and organs through various time intervals after intravenous injection of gold nanoshells

4 CONCLUSIONS

In this work, we have prepared PEGylated nanorods and nanoshells to study their pharmacokinetics and biodistribution in mice. We have found that the distribution of gold nanoparticles is shape and size-dependent, the smaller nanorods particles showing the most widespread organ distribution compared with silica/gold nanoshells. Keeping in mind published results on the size-dependent distribution effects, we believe that further pharmacokinetic and toxicokinetic studies are required to extend our understanding the plasmon-resonant particle behavior in vivo.

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