Radioactive gold nanoparticles in cancer therapy: therapeutic efficacy studies of GA-\(^{198}\)AuNP nanoconstruct in prostate tumor–bearing mice

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Abstract

Biocompatibility studies and cancer therapeutic applications of nanoparticulate \(\beta\)-emitting gold-198 \((^{198}\text{Au}; \beta_{\text{max}} = 0.96\text{ MeV}; \text{half-life of 2.7 days})\) are described. Gum arabic glycoprotein (GA)–functionalized gold nanoparticles (AuNPs) possess optimum sizes (12–18 nm core diameter and 85 nm hydrodynamic diameter) to target individual tumor cells and penetrate through tumor vasculature and pores. We report the results of detailed in vivo therapeutic investigations demonstrating the high tumor affinity of GA-\(^{198}\)AuNPs in severely compromised immunodeficient (SCID) mice bearing human prostate tumor xenografts. Intratumoral administration of a single dose of \(\beta\)-emitting GA-\(^{198}\)AuNPs (70 Gy) resulted in clinically significant tumor regression and effective control in the growth of prostate tumors over 30 days. Three weeks after administration of GA-\(^{198}\)AuNPs, tumor volumes for the treated animals were 82% smaller as compared with tumor volume of control group. The treatment group showed only transitory weight loss in sharp contrast to the tumor-bearing control group, which underwent substantial weight loss. Pharmacokinetic studies have provided unequivocal evidence for the optimum retention of therapeutic payload of GA-\(^{198}\)AuNPs within the tumor site throughout the treatment regimen with minimal or no leakage of radioactivity to various nontarget organs. The measurements of white and red blood cells, platelets, and lymphocytes within the treatment group resembled those of the normal SCID mice, thus providing further evidence on the therapeutic efficacy and concomitant in vivo tolerance and nontoxic features of GA-\(^{198}\)AuNPs.

From the Clinical Editor: In this study, the biocompatibility and cancer therapeutic applications of glycoprotein (GA) functionalized gold nanoparticles containing \(\beta\)-emitting Au-198 are described in SCID mice bearing human prostate tumor xenografts. Intratumoral administration of a single dose of \(\beta\)-emitting GA-\(^{198}\)AuNPs (70 Gy) resulted in clinically significant tumor regression and effective control in the growth of prostate tumors over 30 days. Three weeks after administration of GA-\(^{198}\)AuNPs, tumor volumes for the treated animals were 82% smaller as compared with tumor volume of control group. The treatment group showed only transitory weight loss in sharp contrast to the tumor-bearing control group, which underwent substantial weight loss. Pharmacokinetic studies have provided unequivocal evidence for the optimum retention of therapeutic payload of GA-\(^{198}\)AuNPs within the tumor site throughout the treatment regimen with minimal or no leakage of radioactivity to various nontarget organs. The measurements of white and red blood cells, platelets, and lymphocytes within the treatment group resembled those of the normal SCID mice, thus providing further evidence on the therapeutic efficacy and concomitant in vivo tolerance and nontoxic features of GA-\(^{198}\)AuNPs.

Key words: Radioactive gold nanoparticles; Prostate tumor; Therapeutics; Tumor vasculature; Intratumoral

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Approximately 192,280 men are diagnosed with prostate cancer every year, resulting in loss of life for 27,360 men in the United States and 288,000 men worldwide if not treated in its early stages. It is the second most deadly cancer in men, after lung cancer. Currently accepted diagnostic procedures for prostate cancer start with a screening test—either a digital rectal exam during which a doctor palpates the prostate to check for irregularity or a blood test to check the level of prostate-specific antigen (PSA), or both. The most recent study involving 77,000 North American men showed that regular PSA screening did not save a meaningful number of lives over 10 years. The early detection of prostate tumors by various imaging modalities is more complex, because the prostate is located deep within the pelvis and is thus more difficult to access. These clinical impediments would continue to hamper accurate and early detection of prostate cancer, resulting in more cases of androgen-dependent and hormone-independent prostate cancers. These statistical data imply the inevitability of more men succumbing to this debilitating disease; therefore, therapy of prostate cancer poses some of the most vexing questions in medicine. In fact, one of every six men in the United States will need therapeutic intervention for prostate cancer. Treatment recommendations have ranged from expectant observation to radical prostatectomy, external-beam radiation therapy, and brachytherapy. The goal of surgical resection of prostate cancer is to remove the entire tumor with clear margins. Unfortunately, in primary and metastatic cancer this is not possible because of tumor size or location with respect to vital structures adhering to the tumor, which cannot be removed. Additionally, resection of metastatic disease entails considerable morbidity, especially in patients already weakened by their metastatic cancer, chemotherapy, or radiation therapy. The ability to reduce the size of the tumor before surgical resection or complete tumor resolution without surgery would be an important breakthrough. Prostate tumors are currently treated using low-dose brachytherapy agents whereby radioactive seeds of iodine-125 ($^{125}$I; half-life 60 days; photon energy 27 keV) or palladium-103 ($^{103}$Pd; half-life 16.99 days; photon energy 20–23 keV and Auger electrons) are placed permanently into the prostate gland. These agents deliver a low dose of radiation over a period of several months. Gold-198 ($^{198}$Au), because of its higher energy of emission ($p_{\text{max}} = 0.96$ MeV; half-life 2.7 days), has been used as a permanent implant either alone or as an adjunct to external-beam radiation therapy. Brachytherapy implants of $^{198}$Au provide the important advantage of rapid delivery of radiation at a very high dose rate, thus avoiding some of the radiological problems associated with $^{125}$I. Because of the considerable heterogeneity of radioactive seeds, oncologists have developed a consensus that a majority of patients receiving low- or high-energy brachytherapy will experience post-treatment symptoms ranging from adverse side effects to severe clinical complications. Complications include proctitis, cystitis, incontinence, and rectal bleeding. Following implantation of radioactive seeds patients commonly experience problems with urination for a few months. Additional clinical complications reported as a result of heterogeneity of implanted seeds include pelvic pain, especially when seed placement is too far to one side; loose, frequent stools from rectal mucosal irritation; and reduction in ejaculate volume with time as the prostate gland becomes more fibrous. Therefore, homogeneously dispersed therapeutic modalities not based on “seeds” that result in effective delivery with optimum therapeutic payload and higher tumor retention would provide a major oncological advance in the treatment of prostate cancer.

Nanotechnology has the potential to provide a paradigm shift in the way diagnostic and therapeutic drugs are delivered to achieve target specificity and increased retention for considerable improvement in the overall treatment of the prostate and various inoperable tumors. Nanoparticles possess extraordinary capabilities to detect, image, or treat cancers at the cellular and molecular levels. Among various metallic nanoparticles, gold nanoparticles (AuNPs) have extraordinary tumor retention capabilities because of their natural affinity to leaky tumor vasculature, which is manifested in angiogenesis of tumor growth. Recently Balogh and co-workers have made groundbreaking contributions toward the fabrication of poly $^{198}$Au radioactive gold dendrimer composite nanodevices of sizes between 10 nm and 29 nm and demonstrated their utility in targeted radiopharmaceutical dose delivery to tumors. Their studies have shown that single intratumoral injection of poly $^{198}$Au$_{4} = 22$ nm composite nanodevices in phosphate-buffered saline (PBS) delivering a dose of 74 $\mu$Ci, after 8 days, resulted in a statistically significant 45% reduction ($P = 0.0245$) in tumor volume when compared with untreated groups and those injected with the “cold” nanodevice therapeutic modalities.

We proposed that biocompatible $^{198}$AuNPs will serve as a new generation of therapeutic agents with potential to eliminate serious clinical impediments associated with existing heterogeneous (seed-based) brachytherapy agents for treatment of various types of human cancers. We performed extensive in vitro and in vivo investigations to validate this proposal using gum arabic glycoprotein (GA)-coated nontoxic AuNP formulations. GA is a plant extract approved by the US Food and Drug Administration for use as a food additive in a variety of foods including yogurts, chocolates, soup mixes, candies, etc. Our research efforts have demonstrated that the complex polysaccharides and protein structures within the GA backbone can effectively and irreversibly bind AuNPs on the protein matrix to produce nontoxic gold nanoparticulate constructs (GA-AuNPs) that are stable under in vitro and in vivo conditions for potential applications in therapeutic use in nanomedicine.

We have recently reported the synthesis of a library of biocompatible AuNPs using biologically benign and green nanotechnology approaches for therapeutic and molecular imaging applications. As part of our ongoing efforts in cancer research and nanomedicine, we now discuss our results on the therapeutic efficacy of GA-$^{198}$AuNPs involving direct intratumoral injections of the construct in human prostate tumor—bearing severely compromised immunodeficient (SCID) mice. Herein we present (1) fabrication and characterization of therapeutic GA-$^{198}$AuNPs, (2) detailed in vitro biocompatibility analysis including platelet aggregation and complement activation of the corresponding nonradioactive GA-AuNP surrogate, and (3) therapeutic efficacy of GA-$^{198}$AuNPs in prostate tumor—bearing SCID mice.
Methods

Synthesis and characterization of GA-AuNPs and GA-198AuNPs

University of Missouri Research Reactor irradiation facilities were used for the production of 198Au. The radioactive and nonradioactive GA-conjugated AuNPs were synthesized using synthetic procedures already established in our laboratory.31,41 Briefly, H198AuCl4 in 0.05 M HCl was added to aqueous solutions of GA followed by the addition of trimeric alanine conjugate, P(CH2NHCH(CH3)COOH)3. The color change from yellow to a red-purple was observed to yield nanoparticles stabilized by GA. The solution was characterized by ultraviolet-visible spectrophotometry, which showed a plasmon absorption band around 540 nm for 198AuNPs, characteristic of nanoparticle gold formation. The nonradioactive analogue, GA-AuNPs, were synthesized from HAuCl4 using similar protocols for synthesizing radioactive nanoparticles and used for establishing the stability and biocompatibility properties of the conjugates before in vivo application of the GA-198AuNPs for therapy. Transmission electron microscopy (TEM) of GA-AuNPs provided the metallic-gold core size of the nanoparticles, which is in good agreement and in the range of 12–18 nm. Dynamic light scattering (DLS) revealed that the hydrodynamic diameter of GA-AuNPs is 85 nm and the zeta potential is $-24.5 \pm 1.5$ mV. These data provide crucial information on the stability of nanoparticulate dispersion in aqueous solution (Figure 1). Furthermore, the stability studies of GA-AuNPs in biologically relevant solutions (10% NaCl, 0.5% cysteine, 0.2 M histidine, 0.5% human serum albumin (HSA), and buffers of different pH values) were performed.31,41

Biocompatibility assessment of GA-AuNPs

The biocompatibility of GA-AuNPs was established by hemocompatibility, platelet aggregation, and complement system activation analyses (see Figure S1 in the Supplementary Material section in the online version of this article). Specimens from healthy human volunteers were drawn under National Cancer Institute–Frederick Protocol OH99-C-N046. Blood was collected in Becton-Dickinson vacutainer tubes (BD Diagnostics, Franklin Lakes, New Jersey) containing lithium heparin (hemolysis test) or sodium citrate (platelet aggregation test) as anticoagulant. Specimens from at least three donors were pooled. The hemocompatibility assay involved direct exposure of GA-AuNPs to a freshly drawn human blood cell suspension for 3 hours at 37°C. After exposure the blood suspensions were centrifuged to separate the free hemoglobin (produced by lysed cells) from the unlysed cells, and the oxidized form of hemoglobin (cyano-hemoglobin) was quantified by colorimetric assay as shown in Figure 2. For platelet aggregation, GA-AuNPs were reconstituted in culture medium (RPMI Invitrogen Corporation, Grand Island, New York), and the studies were carried out using the platelet-rich plasma (PRP). The plasma was obtained from freshly pooled human whole blood and incubated with control or test sample for 15 minutes at 37°C. The extent of platelet aggregation in PRP was analyzed using a Z2 COULTER COUNTER cell and particle counter (Beckman Coulter, Brea, California) to determine the number of active platelets (Figure 3).

The qualitative determination of complement system activation by GA-AuNPs was performed by following the exposure of AuNPs to human plasma. The activation and subsequent cleavage of complement system protein C3 was studied by western blot analysis (Figure 2). Hemocompatibility, platelet aggregation, and subsequent complement system activation assays were performed by following the reported procedures.52

Therapeutic efficacy of GA-198AuNPs

Animal studies were approved by the Institutional Animal Care and Use Committees of the Harry S. Truman Memorial Veterans Hospital and the University of Missouri, and were performed in accordance with the Guide for the Care and Use of Laboratory Animals. Female ICRSC-M SCID mice (4–5 weeks of age; Taconic Farms, Hudson, New York) were housed in a temperature- and humidity-controlled pathogen-free barrier facility. Animals were maintained on a 12-hour light-dark cycle and had free access to sterilized standard chow and water. Animals were allowed to acclimate for 7–10 days before initiation of work. Human prostate cancer cell line PC-3 was obtained from the American Type Culture Collection (ATCC; Manassas, Virginia) and cultured according to ATCC recommendations by the University of Missouri Cell and...
Immunobiology Core facility. Mice received ear tag identifiers under inhalational anesthesia (isoflurane-oxygen) followed by unilateral, subcutaneous hind flank inoculations of $5 \times 10^6$ PC-3 cells (passage 20) suspended in 0.1 mL of sterile Dulbecco’s PBS (DPBS) and Matrigel (2:1, vol/vol; BD Biosciences, San Jose, California). Solid tumors were allowed to develop over a period of 3 weeks, and animals were randomized (day 0) into control and treatment groups ($n = 7$) having no significant difference in tumor volumes ($P = 0.64$; Student’s t-test) or body weights ($P = 0.17$). Tumor volumes were estimated from caliper measurements using the formula $V = \text{length} \times \text{width} \times \text{depth}$. On day 8 animals in the treatment group received intratumoral injections of GA-198AuNPs (408 μCi) in DPBS (30 μL) while under inhalational anesthesia. Similarly, control animals received 30 μL of DPBS intratumorally. No significant difference ($P = 0.93$) in tumor volume or body weight ($P = 0.21$) was noted between the groups. Tumor volumes, body weights, and health status were then determined twice each week. At the end of the study (day 31) mice were euthanized by cervical dislocation, and blood samples were collected by cardiac puncture. Samples of tumors, liver, and other organs of interest from the treatment group were also harvested, weighed, and analyzed for radioactivity in comparison to a sample of the injected dose using an automated γ-counter (Wallac Wizard Model 1480, Perkin Elmer Inc., Waltham, Massachusetts). A third group of SCID mice ($n = 7$) received experimental manipulations and were maintained through the end of study for determination of normal blood cell and platelet counts for comparison to the control and treatment groups. Complete blood counts were determined by the University of Missouri Research Animal Diagnostic Laboratory (RADIL) using an

Figure 2. Biocompatibility of surrogate GA-AuNPs. (A) Hemocompatibility assay showing the nonhemolytic nature of GA-AuNPs, wherein amounts of total blood hemoglobin in negative control (NC; 4% polyethylene glycol solution) and in test samples were below the lower limit of quantification (BLOQ) at two different GA-AuNP subtoxic concentrations tested. Triton X-100 was used as a positive control (PC) for hemolysis. (B) Western blot showing qualitative complement activation and subsequent C3 cleavage in PC but not in NC and the three independent replicates of complement proteins exposed to GA-AuNPs (lanes 1, 2, and 3).

Figure 3. Platelet aggregation in the presence of (A) GA-AuNPs and of (B) collagen and GA-AuNPs. PBS was used as negative control (NC), and collagen served as positive control (PC). Platelet aggregation ≥20% was considered positive.
Abbott Cell-Dyn 3500 analyzer (Abbott Laboratories, GMI Inc., Ramsey, Minnesota) on blood samples obtained by cardiac puncture and treated with tripotassium ethylenediaminetetraacetate (K₃-EDTA) (Vacuette MiniCollect; Greiner Bio-One Monroe, North Carolina).

**Results**

*Synthesis and characterization of GA-¹⁹⁸AuNPs*

The synthesis and characterization of GA-¹⁹⁸AuNPs and GA-AuNPs were performed by following the procedure established in our laboratory.³¹,⁴¹ Physicochemical properties such as size, charge, and morphology of GA-AuNPs were determined by TEM and DLS. TEM images of GA-AuNPs indicate that the nanoparticles are spherical with core size range of 12–18 nm. DLS measurements revealed that GA-AuNPs have the hydrodynamic diameter of 85 nm; these data suggest that AuNPs are wrapped with glycoprotein matrix present in GA. The zeta potential of GA-AuNPs was −24.5 ± 1.5 mV and provides crucial information on the stability of nanoparticle dispersion (Figure 1). As an indication of repulsive forces that are present, the magnitude of measured zeta potential can be used to predict the long-term stability of the nanoparticulate dispersion. The stability of GA-¹⁹⁸AuNPs was also checked in 10% NaCl, 0.5% cysteine, 0.2 M histidine, 0.5% HSA, and various pH values by monitoring the plasmon wavelength (λ<sub>max</sub>) and plasmon band width (Δλ<sub>λ</sub>) over a 24- to 48-day period. The plasmon wavelength and width in all the above media shifts ~10 nm, indicating that the AuNPs are intact and thus demonstrating high in vitro stability of GA-AuNPs in biological fluids at physiological pH.

*Biocompatibility assessment of GA-AuNPs: hemolysis, complement activation studies, and platelet aggregation studies*

GA-conjugated AuNPs were synthesized for the pursuit of our overall goals of their use in molecular imaging and therapy.³¹,⁴¹ Their biocompatibility was assessed in terms of hemocompatibility, platelet aggregation, and complement system activation techniques (Figures 2 and 3, and Figure S1 in the Supplementary Material section in the online version of this article). Hemocompatibility studies were used to assess the compatibility of nanoparticles by estimating their hemolytic properties upon direct contact with blood. The test procedure was carried out under static conditions. A hemolytic index (percentage hemolysis) was determined using the optical density readings of the sample and data obtained from a hemoglobin standard curve, and the hemolytic index was used to evaluate the acute hemolytic properties of nanoparticles. As shown in Figure 2, A, the hemolytic index of GA-AuNPs is below a detectable level of equivalence, indicating no hemolysis, as compared to the positive control, Triton X-100. In addition, complement activation assays reveal that GA-AuNPs are highly stable and are biocompatible under in vitro and in vivo profiles (Figure 2, B). Further biocompatibility tests for GA-AuNPs have involved platelet aggregation investigations. Platelets or thrombocytes are the cell fragments circulating in the blood that are involved in the cellular mechanisms of primary hemostasis leading to the formation of blood clots. These studies were carried out using PRP obtained from freshly pooled human whole blood and incubated with control or test sample for 15 minutes at 37°C. The PRP was analyzed using a Z2 COULTER COUNTER cell and particle counter (Beckman Coulter, Brea, California) to determine the number of active platelets. Percentage aggregation was calculated by comparing the number of active platelets in the test sample to the number in the control baseline tube. For ideal in vivo applications the nanoparticles should neither lead to nor inhibit platelet aggregation. The results, as outlined in Figure 3, clearly demonstrate that exposure to GA-AuNPs did not result in any platelet aggregation. It is also important to recognize that GA-AuNPs displayed inhibition of collagen-induced platelet aggregation. These results point to the optimum biocompatibility of GA-AuNPs and their potential use as a therapeutic agent for in vivo administration.

*Therapeutic efficacy of GA-¹⁹⁸AuNPs*

For therapeutic efficacy and pharmacokinetic studies we used SCID mice bearing a flank model of human prostate cancer derived from a subcutaneous implant of 5 × 10⁶ PC-3 cells. For a therapy study, unilateral solid tumors were allowed to grow for 3 weeks, and animals were randomized at day 0 into control and treatment groups (n = 7) with no significant differences in tumor volume (see Methods section). On day 8, 30 μL of GA-¹⁹⁸AuNPs (408 μCi) were injected directly into the tumor to deliver an estimated dose of 70 Gy. Control SCID mice received 30 μL DPBS. Tumors were then measured twice each week. Figure 4, A shows results from the single-dose radiotherapy study of GA-¹⁹⁸AuNPs in human prostate cancer–bearing SCID mice. Within 1 week (day 14), tumor growth in the treated animals appeared to be slowing (P = 0.413) in comparison to controls. Nine days after GA-¹⁹⁸AuNP administration (day 17), tumor volumes were ~50% lower (P = 0.0051) for treated animals compared with controls. This significant therapeutic effect was maintained throughout the 30-day study. Three weeks after GA-¹⁹⁸AuNP administration, tumor volumes for the control animals were fully fivefold larger than those in the radiotherapy group (P < 0.0001; 0.86 ± 0.08 vs. 0.17 ± 0.02 cm<sup>3</sup>). On days 16 and 26 one animal from the control group had to be euthanized because of excessive weight loss (~20%). On day 28 the five animals remaining in the control group were euthanized because of continued weight loss, deteriorating overall health status, and risk of tumor ulceration. By contrast, none of the seven animals in the treatment group reached early-termination criteria. They did exhibit a transient weight loss that peaked at −17.6 ± 2.4% on day 17 but recovered to −10.6 ± 2.9% by day 31.

Tumors harvested from the treatment group consisted largely of necrotic tissue, indicating extensive tumor cell kill. These residual tumor tissue samples still contained 19.9 ± 4.2% of the injected dose (ID) of GA-¹⁹⁸AuNPs. Liver contained 0.91 ± 0.26% ID, kidney 0.13 ± 0.01% ID, and small intestines 0.09 ± 0.00% ID. Levels of radioactivity noted for blood, heart, lung, spleen, stomach, and pancreas were barely distinguishable from background, and the remaining carcass contained 18.5 ± 4.6% ID. Insignificant or no radioactivity in liver, intestine, and various nontarget organs unequivocally established that the
therapeutic payload had remained within the tumor site throughout the 30-day treatment regimen.

Blood parameters were compared between the treatment and control groups with baseline levels obtained from a third group of SCID mice that received no manipulations. Analysis of variance followed by a post hoc Dunnett’s test was employed. The mean white blood cell (WBC) count for GA-198AuNP-treated animals (1.40 ± 0.21 × 10^3 WBC/μL) was not significantly different (P > 0.05) from the baseline WBC count (1.25 ± 0.13 × 10^3 WBC/μL) of the normal SCID mice. By contrast, the WBC count for the untreated tumor-bearing group (2.20 ± 0.31 × 10^3 WBC/μL) was significantly elevated by 75% in comparison with baseline values (P < 0.05). Reduction in tumor volume has a direct bearing on the efficiency of a therapeutic agent in reducing the size of tumors before surgical resection and possibly even reducing or eliminating the need for surgical resection in certain circumstances. To establish the overall

Discussion

As part of our long-standing interest in the development of nanoparticle-based therapeutic agents, synthetic protocols for stabilizing AuNPs via labeling with biocompatible vectors (including GA glycoprotein) have been optimized.\textsuperscript{31-41,45-48,51} Traditional methods use NaBH\textsubscript{4} (and other reducing chemicals) for the production of AuNPs at macroscopic levels. However, such methods fail when used at tracer levels to produce 198Au nanoparticulate radioactive gold. We have recently demonstrated that nontoxic trimeric alanine conjugate (\textit{P}CH\textsubscript{2}NHCH(CH\textsubscript{3})\textsubscript{3})\textsubscript{COOH}\textsubscript{3}, upon mixing with reactor-produced 198AuCl\textsubscript{4}, results in the formation of nanoparticulate gold of well-defined particulate size (15–20 nm).\textsuperscript{31} In vitro stability studies of GA-AuNPs have clearly shown that the glycoprotein part of GA provides exceptional stability for extended periods. Addition of 10% NaCl, 0.5% cysteine, 0.2 M histidine, 0.5% HSA, or buffers of various pH values to GA-AuNP solution caused no aggregation or decomposition of nanoparticles. When new nanomaterials are fabricated their biocompatibility under in vivo conditions is of paramount importance for biomedical applications. We had already demonstrated the nontoxic nature of GA-AuNPs.\textsuperscript{31} Assessment of the hemolytic properties of GA-AuNPs relative to human blood components is also essential in determining their safety for in vivo applications. The GA-AuNPs caused no detectable hemolysis upon exposure to human blood as compared with the Triton X-100 positive control. Indeed, GA-AuNPs displayed hemolytic stability similar to that of the negative control polyethylene glycol. In the platelet aggregation study with freshly pooled human blood, GA-AuNP exposure did not result in any platelet aggregation. It is also important to note that GA-AuNPs displayed inhibition of collagen-induced platelet aggregation (Figure 2, B). The complement activation assay confirmed the high stability and biocompatibility profile of GA-AuNPs.

The therapeutic efficacy of GA-198AuNPs was determined in prostate tumor–bearing SCID mice models. The reductions in tumor volume were monitored for a period of 30 days post treatment. The overall reduction in tumor volume reached an unprecedented 82%, 3 weeks after single-dose intratumoral administration of GA-198AuNPs (408 μCi). There was no uptake of therapeutic payload in nontarget organs, because the small amount (2% to 5%) of the injected dose released from the tumor was subsequently cleared through the renal pathway. Reduction in tumor volume has a direct bearing on the efficiency of chemotherapy and immunotherapy, and has shown indications of a natural immune response. In this context the significant reduction in tumor volume (82%), as shown by GA-198AuNPs in prostate tumor–bearing SCID mice, is an important clinical development showing the potential for clinical translation of this agent in reducing the size of tumors before surgical resection and possibly even reducing or eliminating the need for surgical resection in certain circumstances. To establish the overall

Figure 4. Tumor therapy using GA-198AuNPs. (A) Therapeutic efficacy of GA-198AuNPs in prostate tumor–bearing SCID mice. Subcutaneous tumors were generated in SCID mice by PC-3 engraftment. Mice bearing palpable tumors were randomized for treatment (n = 7) and control (n = 7) groups followed by intratumoral (IT) injections of GA-198AuNPs (408 μCi per animal) or DPBS, respectively. Graph represents mean tumor volume following 30 days of treatment. (B) TEM image showing uptake of GA-AuNPs in prostate cancer cells.
therapeutic response we have also monitored body weight loss and other important blood parameters for both the treated and control groups of animals for the entire treatment period. The treatment group showed only transient weight loss, with recovery to normal weight without any early terminations. These data stand in sharp contrast to the data for the untreated control group, which showed continued weight loss and deterioration of health status leading to the early death of two animals. As shown in Figure 5, the measurements of white and red blood cells, platelets, and lymphocytes within the treatment group resembled those of the normal mice without tumors. This observation provides further evidence on the therapeutic efficacy and concomitant in vivo tolerability and nontoxic features of GA-AuNPs. Although hormone therapy is widely used for treating metastatic lesions, the radioactive therapy presented in this article can have a profound influence in the control or ablation (or both) of metastatic lesions. Metastasis of prostate cancer to distant organs occurs via the leakage of tumor cells to blood. It is widely accepted that many currently available clinical treatment modalities fail to effectively remove tumor with clear margins. Therefore, micrometastases cannot be controlled by the currently available clinical modalities. The gold nanoparticle agent as discussed here has a hydrodynamic size of \( \sim 85 \text{ nm} \) and has shown effective penetration in prostate cancer (PC-3) cells (Figure 4, B). Therefore, it is conceivable that the GA-\(^{198}\text{AuNPs} \) therapeutic agent can control micrometastases by effectively targeting prostate cancer cells under in vivo profiles.

Therapeutic agents that are used in current clinical practice include \(^{125}\text{I} \text{ or } ^{103}\text{Pd} \text{ radioactive seeds and, ytrrium-90 immobi-}

lized glass microspheres. Even though these agents are effective, the limited natural affinity of these agents toward tumor vasculature coupled with considerably larger sizes (50–100 \( \mu \text{m} \)) as compared to the pore size of tumor vasculature (150–300 nm) results in limited retention and marked leakage of therapeutic dose away from the tumor site. Such clinical problems have resulted in decreased efficacy, acute toxic side effects, and lower tumoricidal activity of these therapeutic agents. It is important to note here that the hydrodynamic diameter of GA-\(^{198}\text{AuNPs} \) (85 nm) would be a perfect fit to penetrate into the porous tumor vasculature. The size match of GA-\(^{198}\text{AuNPs} \) to tumor vasculature allows the injected therapeutic dose to homogeneously disperse within the tumor with consequent easy passage across the endothelium at tumor sites, and thus is effective in targeting to appropriate endocytosed cancer-marking epitopes.

Recent results of Khan et al provide new pathways for the encapsulation of radioactive gold within a dendrimeric composite to fabricate a therapeutic nanodevice.\(^{28}\) Although this approach has considerable efficacy in terms of treating cancer through an intratumorally administered treatment regimen, the \(^{198}\text{GA-AuNPs} \) as described here provide a direct pathway toward capitalizing on such important characteristics as high affinity toward tumor vasculature and the optimum hydrophobicity of the GA matrix for effective penetration across tumor membranes. Further studies are warranted to realize the considerable potential offered by the two approaches for the ultimate benefit of the patient community.

Our therapeutic efficacy studies have demonstrated an unprecedented 82% reduction in tumor volume after a single-dose administration of GA-\(^{198}\text{AuNPs} \) (408 \( \mu \text{Ci} \)). The uptake of

![Figure 5. Comparison of blood parameters including (A) red blood cells, (B) white blood cells, (C) platelets, and (D) lymphocytes counts between the treatment and control groups with baseline levels obtained from a third group of SCID mice that received no manipulations (normal).](image-url)
therapeutic payload in nontarget organs is insignificant, and 2% to 5% of the injected dose is released from the tumor and cleared through the renal pathway. Oncologists rely heavily on the concept that for prostate and pancreatic cancer patients, even incomplete tumor volume reduction will increase the efficiency of some systemic therapies as well as of natural healing mechanisms and thus may improve the quality of life. Oncological evidence implies that partial tumor volume destruction will reduce the release of hormones from tumor cells, thus improving the overall systemic condition of cancer patients. Reduction in tumor volume also has a direct impact on slowing the process of invasion and metastasis. Therefore, the efficiency of chemotherapy and immunotherapy, has shown reduction in tumor volume also has a direct impact on slowing the process of invasion and metastasis. Therefore, the efficiency of chemotherapy and immunotherapy, has shown indications of a natural immune response, and is associated with the efficiency of chemotherapy and immunotherapy.

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