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Gold Nanoparticle Contrast in a Phantom and Juvenile Swine: Models for Molecular Imaging of Human Organs using X-ray Computed Tomography

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Abstract

The purpose of this study was to demonstrate the application of gold nanoparticles (AuNP) as a contrast agent for a clinical x-ray computed tomography system using a phantom and using juvenile swine. A tissue-mimicking phantom with spherical inclusions containing known concentrations of gold was scanned. Swine were injected with gum Arabic stabilized gold nanoparticles (GA-AuNP), up to 85 mg kg⁻¹ body weight. CT scans were performed prior to and after the injections. Changes in HU values between pre- and post- injection scans were evaluated and compared to post-mortem determinations of gold uptake. Average uptake of GA-AuNP in the liver of the swine was 380 µg per gram of liver and 680 µg per gram of spleen. Concentrations of gold in tissues increased the CT numbers in liver by approximately 22 HU per mg Au concentration at 80 kVp and 27 HU per mg Au concentration at 140 kVp. These data were consistent with HU changes observed for similar concentrations in the phantom. Conclusion: AuNP based contrast agents may be useful in x-ray based computed tomography. This study provides data for determining concentrations of AuNP in comparison to other contrast materials.

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Introduction

Contrast agents are used in radiographic imaging to provide additional information about vascular anatomy, to depict vascular flow and more recently, to determine the degree of perfusion. The most commonly used contrast materials are iodinated blood pool agents and physiologic information is limited to the vascular system and its extension into major organs. The goal of molecular imaging¹ is to accomplish image contrast enhancement in which the signal producing or enhancing agents are targeted to various types of cells or biological mechanisms by means of biochemical processes. As a clinical tool, molecular imaging has been realized only in part for one modality; research efforts continue to explore its potential.

While most of these efforts have been directed toward radioisotope modalities (SPECT and PET), MRI and ultrasound, some investigators have chosen to investigate the potential of nanoparticle based agents to provide contrast enhancement in x-ray imaging and more specifically, in x-ray computed tomography^{2–6} (CT). Nanoparticles offer the potential for the attachment of targeting peptides or antibodies that would be specific towards the cellular membrane with receptors for these molecules. CT offers high spatial and temporal resolution in comparison to the other modalities and in this, there is incentive to develop these types of contrast agents.

To be a successful imaging agent (and especially a molecular imaging agent), the following criteria must be met: 1) physical characteristics that increase the localized image signal (positive or negative) and thus resulting in improved image contrast, 2) little or no toxic effects on the patient, 3) high uptake specificity to the targeted tissue, and 4) low background uptake. In this study, we have focused primarily on first criteria. To some extent in this work and to a larger extent in other publications^{7, 8}, we have addressed the second. Efforts are well underway in our group as well as others with respect to the last two criteria.

With regard to an x-ray contrast agent, the concentrations of high-Z materials are of principle concern. While this may seem straightforward at first glance, the realized image signal resulting from a certain concentration of a material (e.g. gold) in tissue is complicated by the energy dependence of attenuation (principally photoelectric effect) and the energy spectrum utilized in CT imaging.

In this article we will address the issue of how gold nanoparticle concentrations can affect the local image signal. We intend to demonstrate that low concentrations of AuNP are capable of providing contrast enhancement using a clinical CT system. The elemental properties of gold (at certain levels of concentration) are the basis for x-ray contrast^{8–10}. In this, we will show initial data on the concentrations necessary to achieve some level of contrast. To a limited extent, we also demonstrate that biocompatible formulations of gold nanoparticles^{11, 12} at a relatively high dose have limited or no toxic effects on the animal model used.

Methods and Materials

Tissue-mimicking AuNP phantom

A tissue mimicking phantom (prostate tissue) was formed in a cylinder 95 mm in diameter and 50 mm high, using an agarose formulation described by D'Souza, *et al*¹³. Varying concentrations of GA-AuNP nanoparticles were cast in agarose spheres (9.5 mm and 6.35 mm in diameter). These spheres were embedded in fixed positions, thus simulating "AuNP uptake" against a "tissue background". In order to simulate the appropriate thickness of a human subject, a 20 cm diameter plexiglass holder was constructed. This was in the shape of an annular right circular cylinder and the volume of the holder was filled with water. Thus, the simulated

“patient” used for the CT study has the cross-sectional attenuation characteristics of a 20 cm subject. This configuration was scanned using a Siemens Definition CT scanner using both 80 kVp and 140 kVp beams with an exposure technique of 105 mAs. The slice thickness used was 1 mm with a 275 mm field of view.

Swine model

Juvenile swine (Chinn Farms, Clarence, Missouri) were kept in individual stainless-steel metabolism cages. Animals were fed an age-appropriate commercial swine diet once daily at ~5% body weight, and water was provided ad libitum. The swine were delivered between six and eight weeks of age and weighed between 8 and 12 kg. All animals were allowed a ~1-week acclimation period and maintained on a 12-hour light/dark cycle. The animal room was kept at an appropriate ambient temperature for the size of the animals, ~80°F (26°–27°C). Humane care of the swine used in these experiments and the administration of AuNP dose were carried out in compliance with the University of Missouri Animal Care and Use Committee approved protocol.

AuNP contrast agent production

Production of non-targeted, non-specific AuNP has previously been described by Kattumuri, et al.⁸ Concentrated solutions of gum Arabic stabilized AuNP (GA-AuNP) were produced through a combination of centrifuge and evaporation. The mean diameter of the AuNP, determined by optical absorption and transmission electron microscopy was 20 nm. Gold concentrations in these solutions ranged from 300 µg Au ml⁻¹ up to 5 mg Au ml⁻¹, dependent upon the extent of processing. Prior to intravenous administration of the GA-AuNP in the swine model, the solution was adjusted to a normal physiological pH using a concentrated phosphate buffer.

Animal Dose Protocol

For AuNP administration, the animals were anesthetized using face-mask induction with isoflurane. Using jugular vein access, a central venous catheter was placed in the cranial vena cava and secured to the skin for the duration of the study. GA-AuNP were administered in solution to the juvenile swine through the intravenous route at a rate of 4 ml min⁻¹. Heart rate and respiration were monitored during the dosing. GA-AuNP doses were administered at 24 hour increments over five to seven days during the course of the study. (In the study that lasted 29 days, all of the GA-AuNP dose was administered within the first seven days). The juvenile swine were dosed to between 86 mg AuNP kg⁻¹ to 99 mg AuNP kg⁻¹ body weight. Cases performed early in the study used GA-AuNP solutions that had a relatively low Au concentration. The later cases were given a dose of GA-AuNP that had been concentrated to a higher Au density. The total dose was limited by the volume tolerance for IV fluid of the subject. In all cases, the body weight used was that at the conclusion of the study. As a result of weight gain, the dose normalized to body weight is lower for the individual subject held in the 29 day study. Serum samples were submitted to the University of Missouri Veterinary Medical Diagnostic Laboratory to be analyzed using a large-animal Maxi profile. Glucose, blood urea nitrogen, creatinine, sodium, potassium chloride, carbon dioxide, anion gap, albumin, total protein, globulin, calcium, phosphorous, magnesium, total bilirubin, direct bilirubin, aspartate aminotransferase, gamma-glutamyl transpeptidase and creatine phosphokinase levels were determined.

CT Imaging Protocol

Juvenile swine were imaged under a protocol approved by the University of Missouri Animal Care and Use Committee. For each imaging procedure, the swine were anesthetized with isoflurane using face-mask induction and had normal respiration during the scan. The animals

were secured on the scanner table using a vacuum cushion (Vac-Lok, Civco Medical, Orange City, IA).

CT imaging was performed at the following time-points in the study: a) after 1 week of the animals acclimation period and prior to injection of GA-AuNP, b) within 24 hours of first GA-AuNP injection, c) within 24 hours of final GA-AuNP injection and d) between 7 and 14 days following the final injection. Due to scheduling problems, the “b” and “d” scans were not performed for one of the subjects. The total duration of each study varied with the number of doses administered to the animals and the elapsed time to the final image acquisition. CT images were acquired using one of two scanners; 1) Siemens Somatom 4 or 2) Siemens Definition. The latter became available for use during the course of the study. In addition to performing a faster scan, this unit was chosen for the ability to acquire simultaneous images at 80 kVp and 140 kVp

In the case of scanner 1, the protocol was as follows:

- a. position animal
- b. AP projection scout view through whole body
- c. Helical acquisition through whole body (about 600 mm table travel) at 80 kVp, 1 mm slice thickness, FOV ~ 20 cm.
- d. Repeat scan with identical acquisition through whole body at 140 kVp.

In the case of scanner 2, the protocol was as follows:

- a. position animal
- b. AP and LAT projection scout views through the whole body
- c. Helical acquisition through whole body (about 600 mm table travel) with A tube operating at 140 kVp and B tube operating at 80 kVp. 1 mm slice thickness, FOV ~ 20 cm.

Standard clinical reconstruction (medium-soft kernel) methods were used. Voxel dimensions were approximately $0.5 \times 0.5 \times 1.0$ mm. Except for adjustment of the field of view to the size of the pig, there was no attempt to modify the beam hardening correction algorithms on the CT scanners. Images were uploaded to a PACS server dedicated for research and were reviewed using a standard image viewer application (Osirix, www.osirix-viewer.com). Regions of interest (ROIs) were manually drawn to determine mean Hounsfield Unit (HU) values for various organs. The ROIs were carefully drawn to exclude extraneous tissues. In the liver, however, the large hepatic vessels were included in the ROIs for both pre-dose and post-dose scans. A volume calculation was performed using all of the ROIs within the organ; this also calculated a mean HU value for the entire organ and this was the value recorded. Here the 2nd CT scanner provided an advantage as the anatomic positioning of the 140 kVp/80kVp images were identical and the ROIs could be copied to the identical series.

Prior to each scan, the CT scanner was calibrated using the on-board calibration (in air calibration) to insure the proper scaling of CT numbers. Thus, the evaluation of changes as a result of gold uptake could be reliably measured. As a further check to determine that the Hounsfield unit values did not change during the period, the mean HU of an ROI within the longissimus dorsi muscle of each subject was measured. Since the muscle tissue does not take up GA-AuNP, each animal served as an internal control. For all scans, and with both kVp's (80 and 140), the mean HU value of the longissimus dorsi did not vary by more than 2 HU for any individual subject over the course of the study.

Biodistribution of AuNP

Following the last CT image acquisition, the animals were euthanized and tissues were collected for subsequent analysis for gold concentrations. Tissue sample collection and biodistribution of GA-AuNP were performed according to procedures described by Fent, *et al.*⁷ The tissue Au concentration data are represented in units of mass of Au per wet mass of tissue. Samples of liver tissue (the organ with the highest uptake) were preserved for TEM imaging. In addition, a small sample of the injected solution was also preserved for analysis by atomic absorption spectroscopy. These data were multiplied by the volume of solution delivered to determine the total dose of AuNP received by each individual.

Results

Results from the phantom images are seen in Figure 1a. Images of the phantom are shown in Figure 1b-d. There is a linear relationship between Au concentration and the shift in Hounsfield units from the background. The spherical inclusions with AuNP concentrations may be seen in the axial slice and the three-dimensional renderings. These data indicate that even at very low AuNP concentrations, there are discernable attenuation changes visible in the CT image.

A total of three juvenile swine were used in this study. The duration of the study for twenty-nine days for one subject and lasted ten days for two others. In the former, the study was performed over a long duration to investigate the build-up and retention of GA-AuNP. The average dose given to the pigs was 91 mg kg⁻¹ body weight (determined at the time of necropsy). During the administration of GA-AuNP solution, there was no obvious physiologic change. Heart rate and respiration remained within a normal range during the administration. The pigs were monitored daily and experienced no apparent physiologic changes - blood panels remained normal throughout the study. The pigs seemed to thrive, with appropriate appetite and weight gain associated with their age. No abnormalities were noted in the biochemical analysis or on gross or microscopic examination of the tissues. In this study and in previous experiments, GA-AuNP distribute rapidly from the bloodstream to the following tissues, in order of predominance: liver, lung, kidneys, spleen.

As measured by AAS, the average uptake in the liver was 380 µg Au per gram of wet tissue and average uptake in the spleen was 684 µg Au per gram of wet tissue. The summary of results in Table 1 shows the change in Hounsfield units for each case; dividing by the uptake mass of Au, there was a change of +22.3 HU (per mg Au uptake in 1 cm³) at 80 kVp and +26.7 HU (per mg Au uptake in 1 cm³) at 140 kVp in liver tissues. Data from the spleen demonstrated HU values changing by +9.7 HU (per mg Au uptake in 1 cm³) at 80 kVp and +10.1 HU (per mg Au uptake in 1 cm³) at 140 kVp. The uptake of AuNP may also be expressed in terms of molar concentration. For example, the average uptake in the liver is equivalent to a molar concentration of 1.92 nM/L and the average uptake in the spleen is 3.5 nM/L.

By reviewing the images, it appears that the distribution of nanoparticles in each of these organs was fairly uniform. Figure 2a is example of this distribution in the liver imaged using 140 kVp. Aside from the obvious growth of the pig during the 29 days of study, it also appears that GA-AuNP have been retained and distributed uniformly through the liver. Figure 2b is a TEM image, showing AuNP present in the Kupffer cells of the liver, with relatively uniform distribution. Figure 3 is of the same subject, with axial slices positioned at the level of the spleen. Again, it appears as though the distribution of nanoparticles in the spleen is uniform.

Discussion

Early efforts to use nanoparticles to enhance computed tomography have utilized iodine as the primary agent¹⁴. More recently, a dual-modality agent using gold and gadolinium chelates for

CT and MRI has been proposed, but this agent appears to remain in the blood pool until it is excreted and will need specific targeting to be useful as a molecular agent⁵. Prior publications on the application of AuNP for x-ray contrast have indicated promise^{2, 3}, but may have been over-enthusiastic about the degree of contrast attained¹⁵. Claims of high attenuation coefficients were made using comparisons attenuation at single photon energies (e.g. at 100 keV) rather than considering the entire spectrum of x-ray energies. Images presented in this article were acquired using a mammography system operating at 22 kVp - for this spectrum, the principle x-ray photon interaction with gold will be photoelectric effect. Comments on this article questioned the dosage and relevant toxicity¹⁵. In a similar fashion, the claim by Kim, *et al*³ that 33 mg ml⁻¹ of PEG-coated AuNP has a contrast effect equivalent to 189 mg ml⁻¹ of Iodine is difficult to evaluate, given the lack of description as to how these data were acquired. In another article, Jackson and co-authors have presented theoretical and empirical evidence on the benefits of gold nanoparticles as contrast agents, particularly for higher energy techniques such as CT¹⁶.

The purpose of this study was to perform a study of how AuNP affect the attenuation in a tissue-mimick phantom and in tissues. CT imaging of the phantom showed that for very low concentrations, on the order of several hundre micrograms of Au per gram of background material (agarose), the high Z and density of the AuNP was able to produce a change in the x-ray attenuation sufficient to change the HU value. This was not an extensive study of the contrast/detail/noise aspects of this material as a contrast agent, however, in the proof-of-concept, it is a successful proof of the hypothesis that AuNP might be useful as an x-ray contrast agent. In the concentrations seen here, there are approximately 10¹² 20 nm AuNP per milliliter of volume. At the cellular volume (10⁻¹⁰ cm³ per cell), this means sufficient attenuation change would occur with several hundred AuNP per cell¹⁷.

A prior AuNP biodistribution study indicated that these gum Arabic stabilized gold nanoparticles (GA-AuNP) preferentially locate in the lung and the liver⁷. A smaller percentage locate in the spleen, however, due to its small size, the concentration of gold is significant. Therefore the liver and spleen were our target organs. The purpose was not to establish a statistically proven outcome and therefore it was limited to a small number of subjects. However, we did make observations about the condition of the subjects in light of the relatively high dose rate of AuNP administered. This particular agent (gum Arabic AuNP) is also taken up by the lungs, however, since respiration was not inhibited during the scans, it is difficult to evaluate the change in CT number due to the wide range of HU values for lung from inspiration/expiration.

The ability of AuNP to enhance contrast in organs has been demonstrated. The usefulness of these as molecular imaging agents will be dependent upon factors that are beyond the scope of this report. This is because the nanoparticle constructs used here were localized non-specifically (e.g., they distributed homogeneously throughout the organ). Microscopic examination of the tissues indicates that the majority of Au present in the tissues was located within endothelial cells and macrophages.

As expected, the degree to which enhancement occurs is proportional to the administered dose, and the resultant proportional uptake in tissues. There may be some individual variation in AuNP uptake to particular organs and differences in the dose response, but a linear relationship between concentration and HU was established using the agarose phantom results.

The ongoing question with regard to the ability of AuNP to act as molecular imaging agents is to be answered in future work. As is true for radioisotope imaging, this will be utterly dependent upon the target selectivity and the background distributions of the agent. Biological half-life may also play a role; in this particular study, elimination of the AuNP agent did not

occur. However, unlike a radiopharmaceutical, physical half-life is not an issue - for example, the extended duration of one subject to 29 days.

The sensitivity of computed tomography with these contrast agents will be photon (or dose) limited as well as being related to the size of the object. In other words, a higher dose to the subject will result in a lower level of contrast detection for a similar size lesion. Hence, one may choose to increase the number of photons used in imaging following Rose model criteria. Another approach would be to utilize only a certain portion of the x-ray spectrum, based upon energy-discriminating detectors. One group has already explored the theoretical boundaries of such an approach for gold and other high-Z elements¹⁸. Answers to these questions will have to be addressed in future work.

In this study, there was no specificity of the agents, hence the relatively large ($>80 \text{ mg kg}^{-1}$ body weight) dose of GA-AuNP; the biodistribution occurred homogeneously over each organ. How this will be realized in a clinical application sometime can be imagined if we borrow some concepts from current molecular imaging. For purposes of this discussion, let's assume that we have a targeted AuNP probe, peptide specific to a lesion, approximately 1 cm in diameter. If we assume that 2% of the injected hybrid-AuNP agent dose will be accumulated into this lesion. Furthermore, given practical limitations in CT dose, if one assumes that +40 HU change will be sufficient to visualize the target against a background, a 10 nM/L uptake in this lesion is required for an observer to detect it against the background. In order to achieve this mass concentration of 1.96 mg cm^{-3} using the 2% i.d. uptake, the dose to a 70 kg subject would be about 0.732 mg Au per kg body weight (51.25 mg total). This represents a 115 times lower dose rate than what was administered in our study, which had no deleterious effects on the subjects. Comparing with a previously published^{2, 15} LD₅₀ for AuNP of 3.2 g Au kg^{-1} ; this dose is then approximately 4×10^{-3} times lower than the LD₅₀.

Biodistribution, pharmacokinetics and toxicity of GA-AuNP have been studied and reported in the literature^{8, 19}. Biocompatibility assessment of GA-AuNP has been accomplished in terms of hemocompatibility, platelet aggregation and complement system activation techniques in a standards laboratory setting. The hemolytic index of GA-AuNP is below a detectable level compared to a positive control. Exposure of freshly pooled human blood to GA-AuNP did not lead to nor inhibit platelet aggregation. These tests as well as the successful dosing of both swine and mouse models lead to confidence that GA-AuNP constructs are quite biocompatible. Gum Arabic, a common food additive, and gold are both relatively non-toxic, however, consistent with any development of imaging agents, further tests to determine the LD₅₀ dose, or at minimum, the LOEL dose will be required in the future.

We note that there is a small difference in the contrast achieved at 80 kVp versus 140 kVp. This result is consistent with the earlier discussion about the energy spectrum for a clinical CT scanner. The attenuation of x-rays, integrated over the range of the spectrum, does not change appreciably between these two operating points. Considering the elemental and density advantages of gold over the traditional iodinated contrast agents requires that one consider the effect on the entire spectrum. At 80 kVp, AuNP do not have a considerable advantage, primarily because the k-shell electrons are not contributing to photoelectric absorption. However, at 140 kVp, AuNP do have a distinct advantage over iodine, since a great majority of the photons are above the k-edge of iodine.

Future development of computed tomography technology may also have a positive bearing on the potential of AuNP based contrast agents. One possible advance would be advancements in dual energy CT methods. For the present time, the x-ray spectra of current clinical CT scanners are more favorable to the detection of iodine than to gold. However, this may be remedied by extending the kVp at the high end to favor the k-edge energy (80.7 keV) of gold. As of now,

most CT scanners are limited to 140 kVp, however, extending this to higher energies would have this benefit while also increasing penetration (lowering patient dose).

In addition to the use of energy discriminating detectors²⁰, the extension of dual energy methods into triple energy methods and advanced reconstruction algorithms using iterative methods may contribute to increasing the usefulness of AuNP constructs for CT imaging. In all probability, these innovations will be available first in pre-clinical systems. Even now, theoretical considerations of the imaging problem have been noted in the literature¹⁷. However, as these technical developments occur there will be a harder push to develop labeled AuNP agents with various targeting moieties²¹.

Summary

AuNP based contrast agents can be useful in x-ray based computed tomography. In order to meet the criteria for effective contrast agents, nanoparticles must be targeted with specificity to pathologies that are being imaged.

Summary

In this work, the authors have used an agarose-based phantom and juvenile swine as a model for evaluating the x-ray contrast properties of gold nanoparticles (AuNP) at low concentrations. This serves to advance our understanding of the effects of these agents and their potential for application to molecular imaging using targeted, hybrid nanoparticle agents.

This work is unique in that we have performed our studies employing the higher energy x-ray spectrum used in clinical computed tomography equipment. This provides a more realistic challenge for the gold-based nanoparticle agents as a lower photoelectric interaction is in effect at these higher energies than has been reported in earlier published results. This work also is testing the effect of relatively low concentrations of AuNP and provide some framework for expectations of the required uptake and dose to make molecular imaging possible using AuNP with targeting moieties.

This article contributes the following advances in knowledge. 1) Knowledge that gold nanoparticles are able to enhance the visualization of tissue using a clinical computed tomography system. 2) Knowledge about the concentration of gold and how that relates to CT number changes.

Acknowledgments

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Figure 1.

a) plot of ΔHU versus Au concentration in agarose phantom. b) axial slice of agarose phantom with spherical inclusions bearing AuNP. c) and d) 3D volume renderings showing AuNP bearing spheres in the agarose background.

Figure 2.

a) change in brightness of liver seen in coronal images shown at the same window/center. In the pre-dose CT image, the mean ROI value is 69 HU; in the post-dose CT, the mean ROI value is 76. b) a microscope image of the liver from one of the subjects showing uptake of AuNP in Kupffer cells.

Figure 3.

Coronal images of the spleen for the same subject, showing an increase in the brightness of the spleen due to uptake of AuNP. In the pre-dose image, the mean CT value in the ROI is 48.5; in the post-dose image, the mean CT value in the ROI is 57.8.

Summary of CT results from Swine Subjects
A Summary of results for the subjects used in this study. Mean voxel values for each organ (measured in Hounsfield Units) generally increased in proportion to the gold concentration determined following necropsy. ΔHU values are determined from differences in images acquired at the beginning of the study (Day 0) and at the end of the study (# Days in study).

Table 1

Subject	Days in Study	Dose (mg Au kg ⁻¹)	Liver				Spleen			
			AuNP uptake (μmol)	ΔHU @80 kVp	ΔHU @140 kVp	AuNP uptake (μmol)	ΔHU @80 kVp	ΔHU @140 kVp	AuNP uptake (μmol)	ΔHU @140 kVp
A	29.00	86.00	264.21	12.50	13.3	425.26316	9.1	10.9		
B	10.00	88.00	148.42	5.70	9.3	297.89474	2.24	2.79		
C	10.00	99.00	187.37	7.23	7.87	356.84211	8.5	7		