AuroVist™

Nanoprobes

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Product Information and Instructions

Product Name:	**AuroVist™ Gold Nanoparticle X-ray Contrast Agent**
Catalog Number:	**1102**
Appearance:	**Brown powder/solid**
Quantity:	**40 mg Au in the form of dried biocompatible 1.9 nm gold nanoparticles**
Revision:	**1.1 (January 2009)**

The first Gold Nanoparticle X-ray Contrast Agent for in vivo* use

Introduction

AuroVist™ is a novel nanotechnology agent, the first of its kind, which enables greatly enhanced x-ray imaging of blood vessels, the kidneys, tumors, and other organs. It consists of 1.9 nm gold nanoparticles (gold core diameter) with a highly water soluble organic shell making the particles both useable at high concentrations (up to 1.5 g Au/cc) and well tolerated by animals even at high concentrations (LD_{50} > 1.4 g Au/kg). Some of the significant unique features and advantages of AuroVist™ are listed below:

- Longer blood residence time than iodine agents (1.9 nm gold core, ~50,000 Da.).
- High Contrast (> 500 HU initial blood contrast, kidneys >1500 HU).
- Clears through Kidneys.
- Low toxicity (LD_{50} > 1.4 g Au/kg).
- Can be concentrated >4 times that of standard iodine agents (up to 1.5 g Au/cc).
- Permeates angiogenic endothelium of tumors.
- Up to 10 times the contrast of standard iodine agents (Gold absorbs ~3 times more than an equal weight of iodine at 20 and 100 keV and can be ~4 times more concentrated).
- Low osmolality, even at high concentrations.
- Low viscosity, similar to water; easy to inject, even into small vessels.
- Can image using standard MicroCT, clinical CT, planar X-ray, or mammography.
- Enhances radiotherapy dose.

It is particularly useful for MicroCT animal imaging; for example, studies of kidneys, tumors, stroke, atherosclerosis and other vascular conditions.

*not approved for human use.

References

A description of some of the uses of AuroVist™ is found in these published articles:

1. Hainfeld, J. F.; Slatkin, D. N.; Focella, T. M, and Smilowitz, H. M.: Gold nanoparticles: a new X-ray contrast agent. *Br. J. Radiol.*, **79**, 248-253 (2006).

2. Hainfeld, J. F., Slatkin, D. N., and Smilowitz, H. M.: The use of gold nanoparticles to enhance radiotherapy in mice. *Phys. Med. Biol.*, **49**, N309-N315 (2004).

3. Hainfeld, J. F.; Slatkin, D. N.; Focella, T. M., and Smilowitz, H. M.: In Vivo Vascular Casting. *Microsc. Microanal.,* **11***, (Suppl. 2: Proceedings)*; Price, R.; Kotula, P.; Marko, M.; Scott, J. H.; Vander Voort, G. F.; Nanilova, E.; Mah Lee Ng, M.; Smith, K.; Griffin, P.; Smith, P., and McKernan, S., Eds.; Cambridge University Press, New York, NY, p. 1216CD (2005).

   (www.nanoprobes.com/MM05VascCast.html)

Contents

Each vial of AuroVist™ contains 40 mg of gold in the form of 1.9 nm gold nanoparticles (note this is not the total compound weight, but just the weight of gold metal). It has been freeze-dried from a water solution, so may be reconstituted in water, phosphate buffered saline (PBS), or most other buffers.

Instructions for Use

Add water, PBS, or other desired buffer to the dried gold nanoparticles. They should dissolve rapidly. Next, filter through a 0.2 micron filter; a centrifugal filter is supplied that has less loss than syringe filters. Spin at 15,000 x g for 8 minutes. To ensure maximum recovery, a second and third filtration should be performed by adding 10-20 microliters of buffer or water to the filter and re-filtering to wash the membrane. A typical amount for intravenous injection, for example into the tail vein of a mouse, is 0.2 ml. Since the LD_{50} is > 1.4 g Au/kg, a 20 g mouse could be injected with 28 mg Au. For 28 mg in 0.2 ml, one would therefore dissolve the gold nanoparticles (vial contains 40 mg Au) in 0.36 ml of buffer and inject 0.2 ml.

Vascular Imaging: Visualization of blood vessels by planar x-rays is best just after injection, since the gold clearance is biphasic with an initial more rapid decline followed by a slower rate (Fig. 1). The highest blood concentration is immediately after injection.

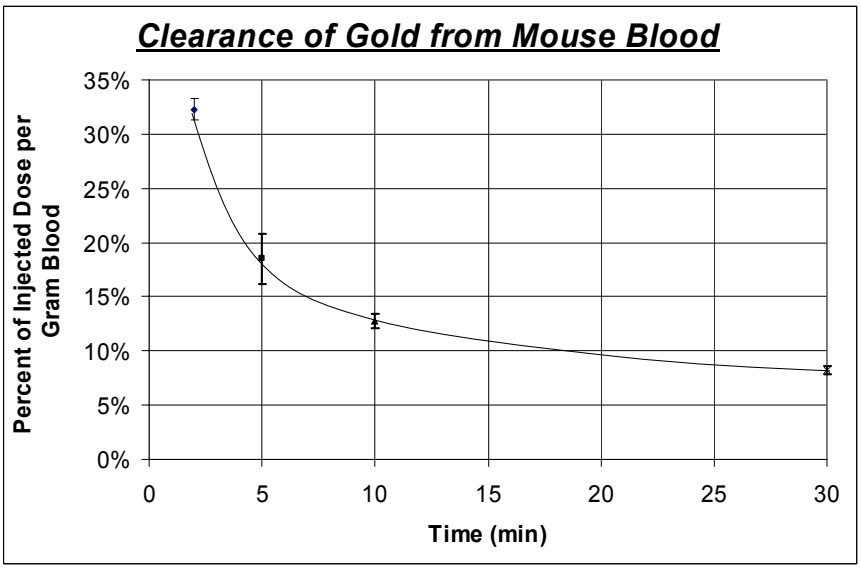

Fig. 1. Blood clearance of AuroVist™ after injection of 27 mg Au into a 20 g mouse.

Kidney and Bladder Imaging: AuroVist™ has a molecular weight of ~50 kDa, so it is rapidly filtered by the kidneys (Fig. 2).

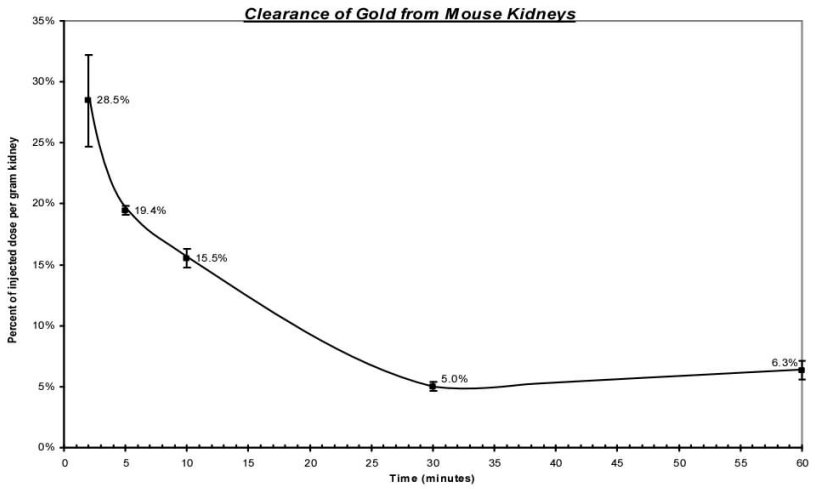

Fig. 2. Clearance of gold nanoparticles from mouse kidneys over a 1 hour period after 12 mg Au injection.

The highest amount of gold is therefore obtained just after injection. Administration of larger amounts of gold may lead to saturation of filtering capacity and longer retention times.

Tumor Imaging: AuroVist™ is large enough to not significantly leak out of normal vasculature as do standard iodine contrast agents, such as iohexol (Omnipaque®) that has a molecular weight of 821. However,

nanoparticles are documented to exit leaky tumor neovasculature. Therefore, tumor uptake can be specific (Fig. 3).

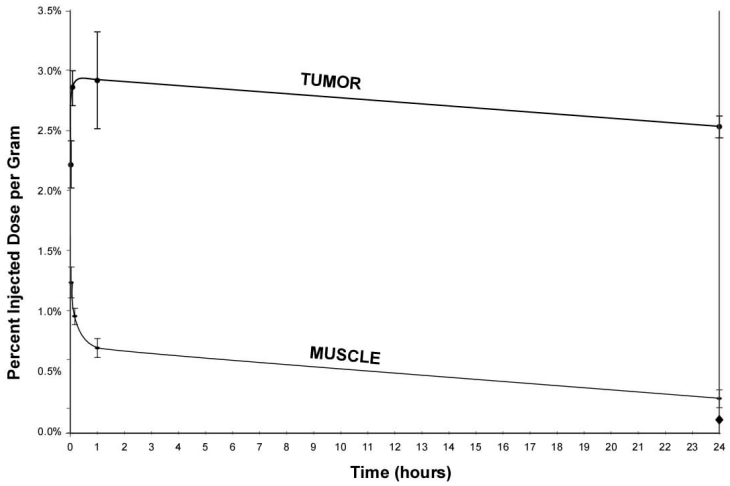

Fig. 3. Specific tumor uptake of AuroVist™ due to leaky tumor neovasculature.

X-ray Properties of Gold:

Gold (Z=79, Atomic weight = 197) absorbs x-rays as shown in Fig. 4.

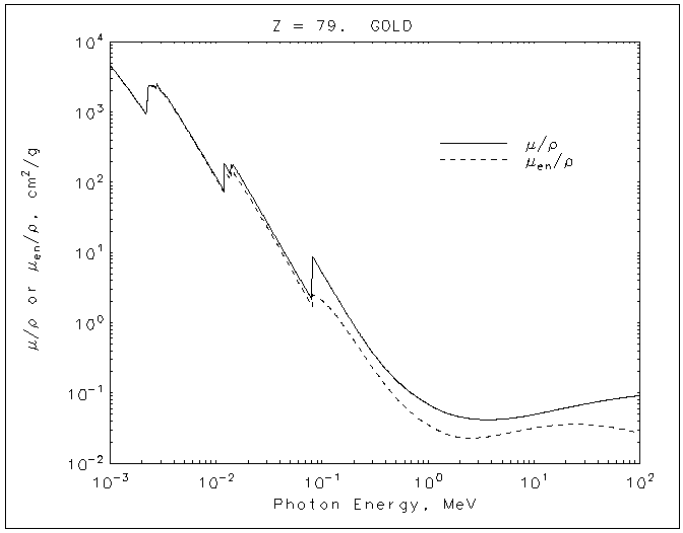

Fig. 4. X-ray absorption of gold vs. X-ray energy.

The absorption increases by a significant factor (jump ratio) above its L and K edges (Table 1).

	Energy (keV)	μ/ρ (cm^2/g)	jump ratio
	11.8	75.8	
L3	11.9	187.0	2.5
	13.6	128.3	
L2	13.7	176.4	1.4
	14.3	158.8	
L1	14.4	183.0	1.2
	80.6	2.1	
K	80.7	8.9	4.2

Table 1. X-ray absorption properties of gold.

It is therefore advantageous to image using these absorptions. MicroCT units generally allow various "kVp" energies to be selected. kVp means kilovolt peak and denotes the highest output energy. Electrons accelerated to this voltage hit a (e.g., tungsten) target and x-rays are emitted via bremsstrahlung (or stopping radiation). Actually, there is 0 intensity at the kVp and 0 at 0 kV, with a broad spectrum in between, with a median energy about 1/3 of the peak energy (kVp). Since gold has a favorable absorption at the K and L energies, one would use ~3 times this for the kVp. For L=14 keV, the unit would be set to ~ 3x14 = 42 kVp.

X-ray Instruments:

Mammography: These instruments are suitable for small animal imaging. Use of lower kVp (e.g., 22 kVp) is recommended to take advantage of the L edge Au absorptions. Exposures are typically 1 sec or less for a mouse, so live imaging is possible. Resolution can be < 0.1 mm.

Clinical CT: 80 kVp gives the greatest attenuation, but higher voltages, particularly with filtering can make use of the Au K edge. Imaging time is typically a few seconds, with resolution ~0.3 mm.

MicroCT: Here the resolution is increased (to even 2 microns), but the tube power is typically ~100 times less than a clinical unit. Fine area 2D detectors mean that many tiny pixels must each receive enough counts. This then requires a much longer imaging time (e.g., ½-2 hours) than clinical CT. Many units also slow the tube rotation down such that only 1 revolution is done in the selected imaging time (e.g., 1 hour). If the animal moves during collection of this data set, the back projection 3D reconstruction will be errant. This places significant constraints for live animal imaging, and motion must be minimized, such as breathing and heartbeat (mouse = 600 beats/min). A simple solution is to kill the animal some time after injection and then image, but live imaging has been accomplished if the region can be gated or immobilized during the imaging time.

Acute Toxicity

Certain strains of mice appear to be more tolerant of this gold. For Balb/C, the LD_{50} is ~ 3.2 g Au/kg. Nude mice and C3H mice also seem to respond about the same. Some outbred mice appear to have a lower LD_{50} of about > 1.4 g Au/kg. Until tested, it is recommended to use this lower value.

Tips on IV injection

For mice, inject into the tail vein with a volume of ~0.2 ml. At 40mg/0.2ml = 200 mg Au/ml, AuroVist™ is intensely black, and immediately during or after injection, one should see the vessels in the eye become dark, and the eyes change from pink to black; paws also become dark due to the gold in all the blood vessels. Injection should be like pushing against no resistance. If it is hard to inject or a lot of gold accumulates at the point of injection, the vein was missed; stop immediately if injection shows resistance and try again elsewhere or later. Warming the tail helps dilate the veins.

Storage

The dried gold nanoparticles should be stored frozen, at -20°C. If reconstituted in water or buffer, storage frozen is also recommended. For the product as supplied, if stored frozen, the shelf life is rated at 3 months.