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DETERMINATION OF NANOGRAM QUANTITIES OF GOLD IN BIOLOGICAL TISSUES BY NONDESTRUCTIVE NEUTRON ACTIVATION ANALYSIS

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We describe a method for gold analysis in kidney and liver. The technique is simpler than other methods in that it does not require ashing or acid digestion of the sample. The tissue is dried, placed into a polyethylene vial and diluted with a 2 ml sodium chloride solution. Gold concentration is determined by neutron activation analysis. Samples are irradiated for two hours at a thermal neutron flux of 10^{12} $n \cdot cm^{-2} \cdot s^{-1}$ and are then allowed to decay for 3–4 days before counting. The detection limit (20 ng Au/ml) and precision (±6.1%) permits the accurate analysis of gold in these tissues. This technique could aid in a re-examination of gold metabolism.

Introduction

Gold salts, commonly used in the treatment of rheumatoid arthritis,[1,2] are limited by their toxicity. Toxic manifestations include renal dysfunction,[3–5] thrombocytopenia,[6] eosinophilia[7] and jaundice.[8] Studies of gold metabolism have been unable to clearly demonstrate a quantitative relationship between gold administration and the manifestation of these disorders.[9]

Neutron activation analysis[10,11] and atomic absorption spectrophotometry[12–14] have been used extensively to measure gold concentrations in plasma and urine. However, these methods have been limited in their applicability to solid tissues due to extensive sample preparation. The further evaluation of gold metabolism requires a highly sensitive, accurate and relatively simple technique for measuring gold in both biological fluids and tissues. The purpose of this study is to develop a method for gold analysis in biological tissue using nondestructive neutron activation analysis.

Experimental

Tissue preparation

To determine tissue burdens of gold in models with wide applicability, eight adult male Sprague–Dawley rats were treated with 0.25 mg Au/week (Myochrysine^R-gold sodium thiomalate) for ten weeks, while eight other rats served as controles. Kidney and liver were obtained upon sacrifice, placed into chloridometer vials (Kimble #74 400) and dried at 100–120 °C for 48 hours. Approximately 0.5–1.0 g of dried tissue was placed into polyethylene vials (used for control of counting geometry) and 2 ml deionized distilled water containing 0.16 mM NaCl was added. The NaCl solution was added to control for any background interference due to these ions. The vials were then heat sealed.

Gold standards

Standards of gold chloride were prepared from a 1000 μg Au/ml solution (Spex Industries, Inc., Metuchen, NJ 08844, lot #876). In addition, a solution of 100 μg Au/100 ml was prepared from gold sodium thiomalate by diluting 1 ml of Myochrysine^R with 252.5 ml of deionized water.

For routine analysis, a 10 μg Au/ml stock solution of gold chloride was diluted with deionized water containing 0.16 mM NaCl to provide standards of 0, 50, 100, 500 and 2000 ng Au/ml. Two ml of each standard were pipetted into polyethylene vials which were then heat sealed. This range of standards was shown to exhibit a linear correlation ($r = 0.99$) with concentration of gold. No difference was observed between the two standard sources described above.

Neutron activation

The standards and specimens were placed in the rotary specimen rack of Michigan State University's TRIGA nuclear reactor. They were irradiated for 2 hours at a thermal neutron flux of $1 \cdot 10^{12}$ n · cm^{-2} · s^{-1}. An average of 25 samples were irradiated simultaneously with the specimen rack being rotated to insure uniform irradiation of all the samples.

The samples were allowed to decay for 3–4 days before counting so that ^{24}Na, ^{38}Cl and other short-lived isotopes would decay, therefore providing higher efficiency counting geometrics by eliminating interference. The analysis system used included a high-resolution Princeton Gamma-Tech Ge(Li) detector with an active volume of 20 cm^3 and a Nuclear Data ND2200 2048-channel multichannel analyzer. One hundred second "live" counting times were used for each sample. The gold activity in each sample was determined by numerically integrating the curve in the 411.8 keV peak. This peak was approximately 7 keV wide at one-tenth

maximum height. The background for each sample was determined by averaging three channels on either side of the peak. The background was subtracted from the gross peak area to give the net peak area or activity due to gold. This activity was then decay-corrected to bring each sample to the same time base.

A comparative analysis method was used to determine the amount of gold present in the tissue specimens. A linear regression related the activity present in each standard to the amount of gold contained in each standard.

Results and discussion

Neutron activation is a technique for identifying and analyzing trace elements in a sample after inducing observable radioactivity in the sample. Radioactivity is induced by subjecting the sample to a large flux of neutrons. When the sample is placed in the neutron flux, there is a small but finite probability that a neutron will undergo an inelastic collision with a target nucleus. If this collision occurs it will transform the nucleus into the isotope with a neutron number greater by one. This new nucleus may or may not be stable; if it is unstable it will decay through the emission of radiation which can be detected and used to identify the decaying nucleus.

In analyzing for gold, the reaction we are concerned with is:

$$^{197}\mathrm{Au} + \mathrm{n} \rightarrow {}^{198}\mathrm{Au} \rightarrow {}^{198}\mathrm{Hg} + \beta^- + \gamma$$

^{197}Au is the target nucleus. It is 100% abundant in nature and has a thermal neutron capture cross-section of $98.8 \cdot 10^{-24}$ cm^2. The activated nucleus is ^{198}Au which has a half-life of 2.698 days and decays to ground state ^{198}Hg via beta decay and subsequent gamma-ray emission. During this process 95% of the gamma-rays emitted have an energy of 411.8 keV.[15] Observing the gamma-ray activity at this energy allows the identification and quantification of gold in each sample.

Linearity was observed from 20 ng Au/ml to 2 μg Au/ml, which provided us with a wide concentration range suitable for tissue analysis (Fig. 1). Initially we encountered strong interference from both sodium and chloride ions at concentrations below 100 ng Au/ml. However, sodium and chloride isotopes are relatively short-lived, and therefore these interferences became negligible after the samples were allowed to decay for 3–4 days. The addition of high concentrations of both sodium and chloride ions to the standards serves to check for any additional interferences due to these ions.

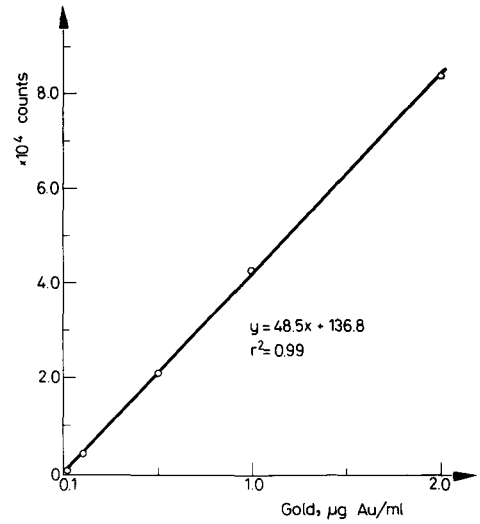

Fig. 1. Analytical working curve for gold using standards of gold chloride

This method was also evaluated for its sensitivity, precision, and recovery. The detection limit, defined as twice the background noise, was 10 ng Au/ml, which is below the range of our standard curve. The precision was determined at three concentrations and was within ±6.1% (Table 1). Recovery experiments were performed by analyzing samples to which known amounts of gold were added. The overall analytical recovery of gold was within ±6.0% (Table 2).

A simple method for determining gold concentrations in biological tissues may aid in understanding gold metabolism. We observed dramatically increased kidney (7.30 ± 0.54 µg/g vs <0.01 µg/g) and liver (4.55 ± 0.25 µg/g vs <0.01 µg/g) gold concentrations in our treated animals; demonstrating gold uptake by these organs. These organs were chosen as they are major sites of gold toxicity.[3-5,8] In addition to monitoring plasma gold levels, a close examination of specific tissue burdens of gold may help in determining at what tissue concentrations gold is toxic.

For experimental purposes, this method is accurate and relatively simple to perform. Neutron activation analysis has previously been demonstrated to be more sensitive than atomic absorption spectrophotometry for determining gold levels in plasma[11] since interferences due to various proteins or matrixes have little effect on analysis. We have currently demonstrated that nondestructive neutron activation analysis is extremely sensitive, accurate, and easy to perform on kidney and liver tissues. Furthermore, we believe this method could be used for a variety of other

Table 1
Reproducibility of ten determinations
at three different concentrations

Au, ng Au/ml	Range, ng Au/ml	Mean, ng Au/ml	Coefficient of variation
20	17.2– 19.9	18.4	6.1
100	92.1–108.2	102.0	5.3
500	481.8–511.2	499.7	2.1

Table 2
Analytical recovery of gold based on
six determinations at each concentration

Au present, ng	Au added, ng	Recovery, %
50	50	98–103
50	100	94–101
200	50	96–102
200	500	97–104

tissues. The expense and availability of adequate facilities prohibits the use of this technique by many laboratories; however, for those investigators interested in examining the total metabolism of gold, neutron activation analysis might be the most sensitive and accurate method to determine gold concentrations in both plasma and tissue.

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