Prevention of Hepatic Metastases by Intrahepatic Radioactive Gold

Antonio E. Alfonso, Ali Hassan, Bernard Gardner, Sidney Stein, Joseph Patti, Nathan A. Solomon, Joseph McCarthy, and Joseph Steinbäum


ABSTRACT

This investigation was designed to determine whether liver tumor growth can be prevented by localized radioactivity through radioactive gold (198Au) infusion. Colloidal 198Au is phagocytosed by Kupffer cells and emits β- and α-radiation. Measurement of α-radiation showed marked 198Au liver concentration. Liver chemistries, histology, and isolated hepatocyte function studies that measure 14Cacetate incorporation into bile acid and cholesterol microaggregation, becomes rapidly phagocytosed by reticuloendothelial cells in the liver (Kupffer cells), and can provide a selective temporary homogeneous field of hepatic radioactivity without systemic radioactive isotopic spillover. Radioactive colloidal gold was also ideally suited for our investigation since it emits both β- and α-radiation and possesses a relatively short half-life. β-Radiation from 198Au has an ionizing tumoricidal range of 8 mm in tissue and water, and its concomitant α emission permits measurement of the isotope distribution in other organs of the body.

INTRODUCTION

Successful treatment following an adequate wide-field colon cancer resection has often been frustrated by the subsequent occurrence of hepatic metastases (14). Unfortunately, once overt metastases become firmly established in the liver, current treatment modalities to date have yielded extremely poor overall results. The likelihood of the development of hepatic metastases reflects by the advanced stage of the local tumor at the time of initial surgical resection can be predicted in a majority of cases. Such patients could be greatly benefitted if there were a practical and effective method of preventing liver "tumors" without the significant local and systemic side effects of external or internal hepatic radiation and systemic or infusional chemotherapy.

Recent interest has focused on the prevention of clinical liver metastases that utilized adjuvant modalities of therapy (1-3, 17). Previous studies demonstrating that radioactive colloids injected into either a splanchic or peripheral vein selectively concentrate in the liver (5, 13, 18, 21, 22) have prompted our interest in utilizing prophylactic internal radioisotopic therapy to prevent clinical liver metastases. Considerable data have already been accumulated concerning the biochemical properties, tissue distribution, effects, and metabolism of radioactive colloidal gold (198Au) after systemic administration in both humans (16) and experimental animal models (19). After i.v. administration 198Au remains insoluble in serum, does not cause capillary microaggregation, becomes rapidly phagocytosed by reticuloendothelial cells in the liver (Kupffer cells), and can provide a selective temporary homogeneous field of hepatic radioactivity without systemic radioactive isotope spillover. Radioactive colloidal gold was also ideally suited for our investigation since it emits both β- and α-radiation and possesses a relatively short half-life. β-Radiation from 198Au has an ionizing tumoricidal range of 8 mm in tissue and water, and its concomitant α emission permits measurement of the isotope distribution in other organs of the body.

Preliminary studies were performed to establish the minimum effective i.v. dose of 198Au that would prevent liver tumor "takes" in rats after introduction of tumor cells into the portal circulation, to determine tissue distribution of the isotope following either splanchic or peripheral vein injection, and to observe for associated hepatic toxicity of the proposed treatment regimen. The main experiment was designed to evaluate the efficacy of internal hepatic radioisotopic therapy with respect to reducing the incidence of hepatic "metastases" after either splanchic or systemic injection of radioactive colloidal gold.

MATERIALS AND METHODS

All experiments were performed on male Sprague-Dawley rats weighing 200 g and kept under standard laboratory and diet conditions. Operations were performed under i.m. sodium pentobarbital anesthesia, 0.05 mg/g body weight, and with sterile techniques. Tumor cells were prepared from homogenates of solid i.p. Walker 256 carcinoma implants. The homogenates were appropriately diluted with sterile 0.9% NaCl solution, and separated cells were obtained to provide a constant dose of 10,000,000 cells/injection. Cells were counted in a hemocytometer, viability was confirmed by trypan blue exclusion (>90%), and subsequent growths of aliquots were implanted s.c. Previous results from our laboratory have consistently confirmed an 80 to 85% liver tumor "take" after this dose of Walker 256 carcinoma was given through a portal vein. Radioactive...
colloidal gold obtained from Amersham/Searle Corp., Arlington Heights, Ill., with an average fairly uniform particle size (20 to 40 nm), and calibrated to 25 mCi/ml was used. Colloidal 198Au after it had been allowed to decay for at least 60 days to negligible levels of radioactivity ("cold" colloidal gold) was used for the control animals.

RESULTS

Minimal Effective Dose of 198Au. Twenty-four rats received either "cold" colloidal gold (8) or 100 (4), 200 (4), 300 (4), or 400 (4) μCi of 198Au through a mesenteric vein tributary 5 to 15 min after intraportal introduction of 10,000,000 Walker 256 carcinoma cells. All animals were sacrificed at 28 days and examined for liver tumor growths.

As shown in Table 1, there was complete Walker 256 sarcoma tumor hepatic growth inhibition at 28 days following a 400-μCi dose of 198Au. Dosages of 100 and 200 μCi were completely ineffective, while the 300-μCi dose prevented liver tumor growth in only 2 of 4 rats. In the 8 control animals that received "cold" gold colloid, a 100% liver tumor "take" was observed.

Isotope Tissue Distribution. Thirty-six rats were divided into equal groups. In 18 rats 400 μCi of 198Au were injected intraportally through a mesenteric vein tributary. The remainder had a similar dose administered systemically through the femoral vein. Paired rats from each group were sacrificed at 4 h and 1, 2, 3, 5, 6, 7, 10, and 15 days. The following organs were dissected out, weighed, and prepared for counting: liver, spleen, lung, brain, and gastrointestinal tract. A sample of muscle and bone with marrow was also taken. The specimens were immediately placed in appropriately shielded α-scintillation counters, and individual radioactivity in terms of cpm/g weight of tissue was determined. These data obtained on tissue distribution of 198Au as measured by the decay of α emissions at sequential time intervals from 4 h to 14 days are presented in Charts 1 and 2. Confirming previous work by others with radioactive colloidal gold particles, the liver and spleen preferentially concentrated the gold particles after introduction through either an intraportal or systemic route. Other phagocytic tissues, like lung, bone, and marrow, were significantly less active in the removal of gold particles from the blood. Muscle, brain, and intestines did not significantly concentrate the isotope. Interestingly, transient higher concentration of intestinal 198Au followed intraportal injection as compared with systemic administration.

Toxicity. Following intraportal 198Au administration in increasing dose increments (400, 1200, and 2000 μCi), serum levels of bilirubin, alkaline phosphatase, SGOT, and SGPT were determined and liver histological examinations were performed in 18 rats that were sequentially sacrificed at 7 and 21 days. Ten other rats consisting of pairs that received 400, 1200, 2000, 3000, and 6000 μCi 198Au were observed clinically for 6 months and then autopsied. All livers were examined microscopically for morphological alterations. Bile acid secretion from isolated hepatocytes

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* The abbreviations used are: SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase.

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**Table 1**

<table>
<thead>
<tr>
<th>198Au dose (μCi)</th>
<th>No. of rats</th>
<th>Positive for tumor</th>
<th>%</th>
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<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>8</td>
<td>100</td>
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<tr>
<td>100</td>
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<tr>
<td>400</td>
<td>4</td>
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**Chart 1. Distribution of 198Au in different organs after portal (mesenteric vein) injection.**

**Chart 2. Distribution of 198Au in different organs after systemic (femoral vein) injection.**
of 400, 1200, and 2000 μCi (Table 2). None of the animals observed for 6 months developed any clinical signs of cirrhosis. At autopsy gross liver nodularity, hyperemia, and hepatomegaly were observed in both rats that received 6000 μCi. These were accompanied by distortion of lobular pattern, hepatic cell ballooning, and necrosis on histological examination. Similar microscopic alterations were also observed at 6 months in rats that received 3000 μCi. None of the livers of animals that received dosages of 2000 μCi or less showed either gross or histological alterations at 6 months. The average total bile acid secretion rates in mol/g liver cells from isolated hepatocyte cultures from 2 rat livers exposed to 400 μCi at 2 hr (18.71) were comparable to a pair that received "cold" colloidal gold and to values derived from normal rat hepatocytes (19.15).

Efficacy of 198Au. For establishment of the effectiveness of 198Au-induced hepatic local radioactivity in preventing experimental liver tumor "takes," 393 rats were divided into 179 control and 214 experimental animals. All animals received tumor injections of 10,000,000 Walker carcinoma cells introduced into a mesenteric vein portal tributary. All experimentally treated animals received 400 μCi of 198Au. Five to 10 min after tumor administration, a comparable volume of "cold" colloidal gold was injected either through a separate mesenteric vein (149 rats) or the femoral vein (30 rats). After a similar time interval, experimental rats received 198Au colloid through either a mesenteric vein (123 rats) or a femoral vein (25 rats) injection. In 66 rats the 198Au mesenteric vein injection was delayed for 2 weeks following tumor introduction. All animals either died of tumor after 2 weeks or were sacrificed at 28 days. At autopsy all livers were examined grossly and microscopically for tumor growths. A summary of our results is recorded in Table 3. In 149 control rats that received "cold" colloidal gold through a mesenteric vein 5 min after tumor injection, 83% developed bilateral massive liver tumors within 28 days. This significantly decreased to 15% or 19 of 123 animals in the treatment group that received intraportal 198Au 5 min after tumor administration. In the 30 control animals that received "cold" colloidal gold through a systemic (femoral vein) injection 5 min after tumor injection, there was a 93% liver tumor "take" as compared to 8% in the systemically treated group of rats that received 198Au via a peripheral vein 5 min after the tumor cells. When 198Au administration via the portal route was delayed by 2 weeks after tumor infusion, the tumor "take" was 54% as compared to 83% in the comparable control group.

DISCUSSION

Previous experimental and limited clinical experiences with the use of β-emitting 90Y tagged to ceramic microspheres have demonstrated that localized interstitial hepatic radiation can be successfully produced with minimal systemic spillover and result in effective growth prevention of virulent tumors without significant systemic or liver toxicity (6, 7, 10, 12). Localization of organ radioactivity, however, relies on the trapping of microspheres in the size of 50 to 100 within the precapillary vessels by a process of microembolism and requires operative placement of a hepatic artery catheter.

Other radioactive colloidal compounds that are known to be selectively concentrated by the reticuloendothelial cells of the liver after i.v. administration have been studied. Grady et al. (5) and Ackerman et al. (1) have separately reported on encouraging results with the use of radiocolloid suspensions of 32P-tagged chromic phosphate in preventing liver "takes" of Walker 256 carcinoma and the Novikoff hepatoma injected into the rat portal vein to simulate liver metastases. A disadvantage of the use of Cr32PO4 has been its reported high bone marrow uptake after systemic i.v. injection. A radiocolloid suspension of 198Au was chosen as the particulate matter for our present study. The isotope was currently available, it is easy to measure quantitatively,
Prevention of Hepatic Metastases by i.v. $^{198}$Au

and much has been known about its chemical preparation to small particles of approximately known size (22). A uniform small particle size (20 to 40 nm) is essential to avoid capillary aggregation and blockage and to allow effective reticuloendothelial phagocytosis. Unlike chromic [32$^p$]phosphate, cumbersome mixing procedures are not necessary for its preparation. Radioactive gold has a half-life of 2.73 days, emits negative $\beta$ particles with an average energy of 0.315 MeV, and an $\alpha$-ray with an energy of 0.411 MeV. Its tissue distribution and radiation effects have been extensively studied in rats, dogs, guinea pigs, and humans. The greatest part of its i.v. administered dose (80 to 90%) is concentrated by the liver. Only 5 to 15% is taken up by the spleen, which is relatively radioresistant, and very small quantities reach other tissues of the body. The compound is cleared from the blood within minutes inasmuch as most of the gold given becomes firmly lodged within Kupffer cells and distribution is in a fairly homogeneous pattern throughout the liver parenchyma (16, 18, 19, 21). The administration of $^{198}$Au in larger i.v. doses has not been associated with untoward effects in humans (16). There is also virtually no problem of bone marrow depression following injection of the material. No immediate or long-term hepatic damage has been described following radiation of the organ with 2500 R. After massive experimental doses in rats, liver necrosis had occurred but the doses were considerably higher (50 times) than were those used in our present study. The smallest amount of $^{198}$Au reported in the literature that has caused liver damage is 12,000 equivalent R, this suggests that the threshold for necroses in rat livers is between 25,000 and 12,000 R (11). Four hundred $\mu$Ci of $^{198}$Au colloid with $t_{1/2}$ physical assumed equal to $t_{1/2}$ effective were calculated to deliver 2944 rads to the liver (2840 from $\beta$-radiation, 104 from $\alpha$-radiation) and in our study appeared to be the minimal effective dose in preventing liver "metastases." The human threshold for hepatic damage that is secondary to total fractionated hepatic radiation is approximately 3000 rads (9, 20), although dramatic recovery of liver morphology and function has been noted in humans who have received 3000 to 5900 rads after fractionated hepatic radiotherapy (15). Dosage increments of $^{198}$Au colloid from 400 to 2000 $\mu$Ci did not cause significant changes in several parameters of rat liver function that were monitored at 7 and 21 days: serum bilirubin, alkaline phosophate, SGOT and SGPT. Bile acid secretion rates in the isolated hepatocyte after exposure to 400 $\mu$Ci were unaltered. Morphological liver damage was produced only at significantly higher doses (3000 $\mu$Ci) at 6 months. The radiocolloid tissue distribution also remained similar to previously described patterns (16, 18, 19).

In the main experiment $^{198}$Au was injected either into a mesenteric or peripheral vein. Although the phenomenon of "streamlining" of portal flow that caused uneven hepatic distribution following mesenteric vein injection is a clinical consideration (8), it was accepted as part of the experimental structure. Streamlining effects were of course obviated by the femoral vein injection route, which not only proved equally effective but may also be more practical and adaptable to the clinical situation.

The resulting reduction in the incidence of liver "takes" to 19 and 8% after mesenteric or femoral vein $^{198}$Au treatment, respectively, is significant ($p < 10^{-9}$). These results have paralleled other studies with respect to optimal time of isotope administration, which was within 48 hr after tumor injection (1, 5). Treatment results were poorer when $^{198}$Au administration was delayed for 2 weeks but significantly different from control values. This observation is correlated with the fact that clinically obvious liver tumor growths were usually established after this time interval (2 weeks) in our experimental rat livers. Root (16) has similarly achieved poor therapeutic results in the treatment of established liver tumors after i.v. administered colloidal radiogold. In the areas of discrete "takes" where reticuloendothelial tissues have been replaced, the radionuclide is not concentrated in the hepatic tissue immediately adjacent to the tumor nodules. This is in striking contrast to the very high levels of radioactivity in the remaining grossly normal liver parenchyma.

While showers of viable malignant cells have been isolated from the portal venous effluent area of the primary tumor after surgical manipulations, the cause of liver metastases may be in the presence of already disseminated occult tumor microfoci at the time of initial resection. Current knowledge of tumor population growth kinetics suggests that micrometastases are rendered cytotoxically more sensitive after removal of all gross disease as the fraction of remaining actively dividing viable tumor cells increases in an inverse relationship to total tumor population size (17). It is therefore logical to treat the disease in its earlier stages rather than wait until a point at which, to date, only palliative results may be expected at best. Unfortunately, external irradiation and infusion or systemic chemotherapy leave much to be desired in an adjuvant setting because of inherent accompanying systemic and local toxicity.

It is apparent that this radionuclide offers a means of achieving localized radiation distribution to the liver following i.v. administration with minimal systemic and local untoward effects. The chief therapeutic use reported for $^{198}$Au has been in the treatment of leukemia and lymphoma (16). While therapeutic possibilities of i.v. administered radiogold colloid in the treatment of established hepatic metastases has appeared exceedingly limited, the unique characteristics of colloidal radionuclides and their preferential concentration in the reticuloendothelial tissues of the liver suggest that they may prove advantageous in the treatment of disseminated micrometastatic hepatic tumor foci.

REFERENCES

Prevention of Hepatic Metastases by Intravenous Radioactive Gold


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