jnm/ RADIOCHEMISTRY AND RADIOPHARMACY

IODINE-123-ROSE BENGAL: AN IMPROVED HEPATOBILIARY IMAGING AGENT

A. N. Serafini, W. M. Smoak, H. B. Hupf, J. E. Beaver, J. Holder, and A. J. Gilson

University of Miami School of Medicine, Mount Sinai Medical Center, Miami Beach, Florida

A practical method for preparing ^{123}I-rose bengal that allows for its rapid and efficient incorporation into the molecule is reported. Administration of ^{123}I-rose bengal to normal healthy patients showing the normal uptake and excretory pattern visualized with this radiopharmaceutical is also presented. The overall reduction in imaging time and radiation exposure together with the improved images possible should greatly improve our diagnostic capabilities in evaluating the jaundiced patient.

Iodine-131-rose bengal was first developed for use as a test of liver function by Taplin, et al in 1955 (*1*). Since then the ^{131}I-rose bengal test in conjunction with other liver function tests has been shown to be useful in evaluating the jaundiced patient by a number of investigators (*2–4*). The technique currently utilizes dynamic scintigraphy with the Anger scintillation camera as described by Burke and Halko (*5*) in which the visual delineation of both hepatic uptake and excretion of the radiolabeled dye and the time sequence of their occurrence can be assessed. This is usually combined with a blood pool clearance study performed by placing a scintillation detector against the side of the head so that a 20-min retention can be measured. Analysis of these data has been found to correlate with the clearance of the radiopharmaceutical from the blood (*6*).

Because of the relatively high absorbed radiation dose produced by the beta decay and the 8-day half-life of ^{131}I-rose bengal, we are limited in the amount of activity that can be administered to the patient (usually 200–300 μCi). The low photon flux with low count statistics results in a relatively prolonged imaging time or poor resolution image. The problems are often aggravated by the poor functional capacity of the hepatobiliary system in the presence of disease. With the availability of large quantities of cyclotron-produced ^{123}I at our institution, the feasibility of developing a new formulation of rose bengal was investigated.

The preparation of the ^{123}I-labeled rose bengal and its distribution in animals was recently reported (*7*). This has since led us to investigate further and evaluate this radiopharmaceutical in humans. This study outlines some of our preliminary findings.

MATERIALS AND METHODS

The ^{123}I used in this study was produced on the Mount Sinai Medical Center cyclotron using the ^{124}Te(p,2n)^{123}I reaction. Details regarding the production, chemical separation, and radionuclidic purity have been published elsewhere (*8,9*).

Chemically, rose bengal is a halogenated fluorescein dye, principally tetrachlorotetraiodofluorescein. Commercially available nonradioactive rose bengal may contain a number of related hologenated fluoresceins that may or may not be handled similarly by the liver (*10,11*). In order to reduce variations possibly resulting from the starting material, we tested the rose bengal of three commercial suppliers (Eastman, K&K, and Fisher) for chemical homogeneity. Ten milligrams of rose bengal from each supplier were dissolved in isotonic sodium chloride solution and applied separately to a 37- $\times$ 2-cm column of Bio-Gel P-2. The rose bengal was slowly eluted with saline causing a separation of various components of the dye identifiable by visible and ultraviolet light spectroscopy. The rose bengal from two of the suppliers contained numerous components

Received Dec. 26, 1974; original accepted Jan. 23, 1975.

For reprints contact: Aldo N. Serafini, Div. of Nuclear Medicine, University of Miami School of Medicine, Mount Sinai Medical Center, Miami Beach, Fla. 33140.

while that from the third (Fisher) contained three, two of which were capable of being tagged by our iodination procedure. The major component, having about 95% of the tagged radioactivity, has an absorption maxima at 550 mμ corresponding to 4,5,6,7 tetrachloro-2′4′5′7′ tetraiodofluorescein (*12*). The rose bengal from Fisher Scientific was used for our studies.

Iodination of the rose bengal is performed by refluxing a mixture of 10–50 mg rose bengal, 10–50 mCi of ^{123}I, and 0.15 ml hydrogen peroxide contained in a maximum of 10 ml acetate buffer at pH 5.2 for 1 hr. Just prior to the addition to the reaction vessel of the ^{123}I contained in a maximum volume of 4 ml, the pH is adjusted to 2 followed by the addition of 0.25 mg of sodium iodide per milliliter of ^{123}I solution. Following reflux, the unbound radioactivity is converted to iodide by the addition of 50 mg sodium thiosulfate and is separated by precipitating the rose bengal with 2 ml of 6 *M* HCl and filtering the supernate. The precipitate is washed with 100 ml of 0.01 *M* HCl followed by sterile water until the rose bengal begins to dissolve as evidenced by a slight pink tint in the wash water. The iodinated rose bengal is dissolved in phosphate buffer, pH 7.2, filtered and adjusted with sterile diluent to contain 1.8–2.2 mg rose bengal/ml, 0.9–1.1 mCi ^{123}I/ml and pH 7–7.5. The yields of the iodination procedure range between 65 and 75%.

Radiochemical purity is determined by ascending chromotography in a 50:50 mixture of 5.8% ammonium hydroxide and ethanol using Whatman No. 1 paper. In this system, the R_f values for rose bengal and iodide are 0.43 and 0.85, respectively. The final product is autoclaved at 121°C for 30 min and each batch is tested for sterility and pyrogens according to USP 18 procedures. The total time in preparing the radiopharmaceutical by this method is about 3 hr.

Seven normal healthy volunteers with no clinical or biochemical evidence of hepatobiliary disease were studied. Patients were prepared by having them fast from midnight the night before the exam. Each patient was injected intravenously with 2 mCi of ^{123}I-rose bengal. Blood clearance studies were performed in addition to measuring 24-hr urinary excretion. Simultaneously, sequential scintiphotos of the cardiac pool, liver, biliary system, and intestines were taken at various intervals throughout the study using the Anger scintillation camera with a high-energy parallel-hole collimator. Polaroid scintiphotos with 300,000 counts per view were taken. Utilizing an on-line computer, the data were also recorded and stored for subsequent playback analysis. Time–activity curves could thus be generated from various selected regions of interest that included the activity in the cardiac pool, liver, gallbladder, common bile duct, where visible, and in the gastrointestinal tract.

The estimated absorbed dose from our ^{123}I-rose bengal in rads per millicurie is as follows: liver, 0.2; gallbladder, 3.8; ovaries, 0.3; testes, 0.03; and total body, 0.08 (*13*).

RESULTS

The scintiphoto sequence of events recorded in two typical normal patients is shown in Figs. 1 and 2. Significant findings are that, within the first 30 min, sufficient localization of the radiopharmaceutical within the liver parenchymal cells is seen so that good anatomic delineation of the liver can be obtained.

Soon thereafter, two different patterns seem to emerge. First, there may be subsequent localization of the activity within the region of the gallbladder that increases with time. At a certain point, usually before maximal concentration in the gallbladder is reached, evacuation of the rose bengal into the gastrointestinal tract is visualized. This emptying is manifested by visible activity in the region of the common duct and subsequently the duodenum (Fig. 1). Pooling of activity within the region of the jejunal loop is then noted. In some instances, especially if the rate of emptying occurs early, the duct and duodenum are not well visualized and only activity within the proximal portion of the jejunem is seen initially. This may be visualized sometimes even before gallbladder filling occurs.

Subsequent progressive decline in activity within the liver with diminishing activity in the gallbladder is seen during the next 4–6 hr. At 24 hr only activity throughout the bowel is seen. The marked improvement in the quality of the images is apparent. Liver images approaching the quality obtained with ^{99m}Tc-sulfur colloid were observed in most instances. Marked improvement in the anatomic detail of the biliary tree was evident with excretion of the ^{123}I-rose bengal into the gastrointestinal tract being readily visible. The overall imaging time to obtain the 300,000 counts per scintiphoto varied between 90–360 sec depending on the time after injection the scintiphoto was obtained. This is a considerable reduction in time when compared with ^{131}I-rose bengal.

To ascertain that the ^{123}I-rose bengal was stable in vivo, sequential whole-body scans to encompass the thyroid and stomach were performed. The whole-body scans showed that after injection, the majority of the radioactivity was concentrated in the liver and the amount then decreased sharply with a concomitant increase in the intestinal tract. After 24 hr some thyroid uptake was observed suggesting that some iodine is slowly released from the rose bengal

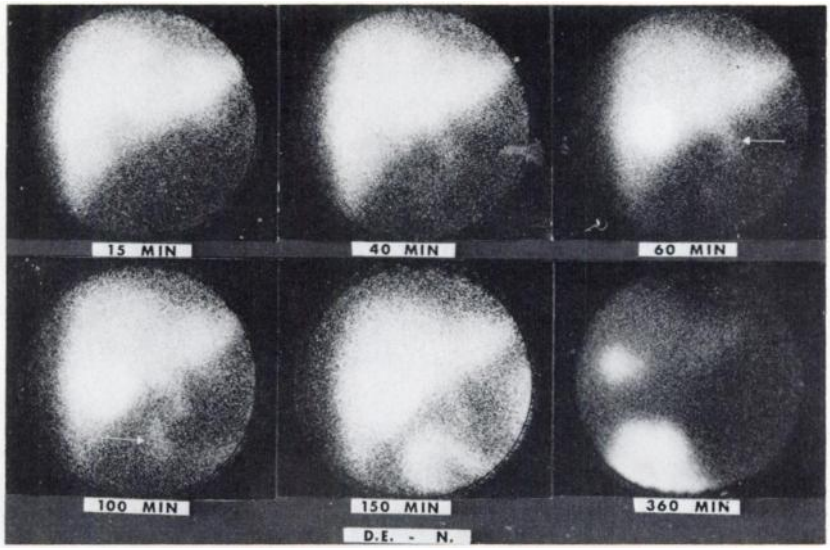

FIG. 1. Sequence of events demonstrating initial uptake of ^{123}I-rose bengal into parenchymal cells with subsequent visualization of common duct at 60 min and excretion into second portion of duodenum at 100 min. Note that excretion is occurring prior to complete filling of gallbladder.

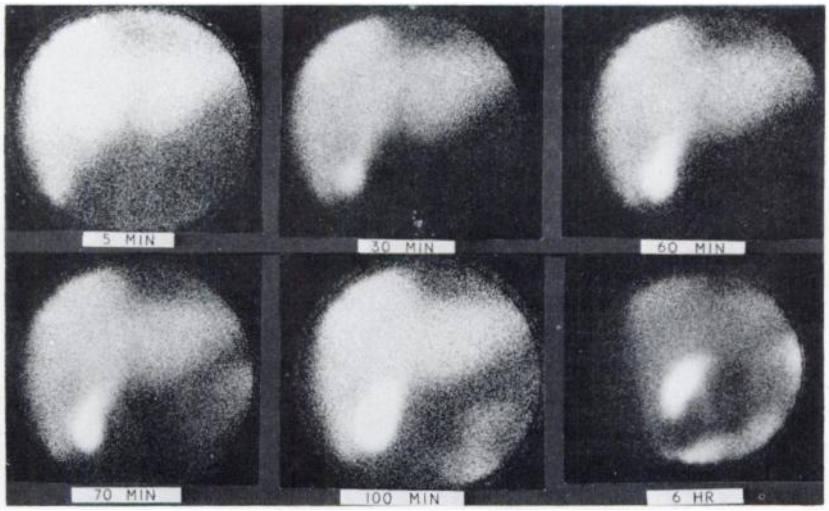

FIG. 2. Sequence of events demonstrating initial uptake of ^{123}I-rose bengal into parenchymal cells with subsequent visualization of gallbladder. Activity in common duct or duodenum is not seen; however, excretion is appreciated by activity present in left upper quadrant in proximal portion of duodenum.

molecule in vivo probably due to metabolism. The blood clearance during the first 30 min is demonstrated in Fig. 3 and is comparable to the results reported with ^{131}I-rose bengal (*6*). The 24-hr urinary excretion in this group averaged 2% of the administered activity.

DISCUSSION

Iodine-123 when compared with ^{131}I has several desirable physical properties that have encouraged us to substitute ^{123}I as a label for radiopharmaceuticals (*14,15*). The major deterrent to the use of ^{123}I has been lack of availability in sufficient quantities for routine clinical use. Hundred-millicurie amounts of ^{123}I have been produced at the Mount Sinai Medical Center cyclotron from an enriched tellurium target with a radionuclidic purity of 99.1% at the end of bombardment. With the availability of these high yields, millicurie quantities of high specific activity radiopharmaceuticals become possible. The

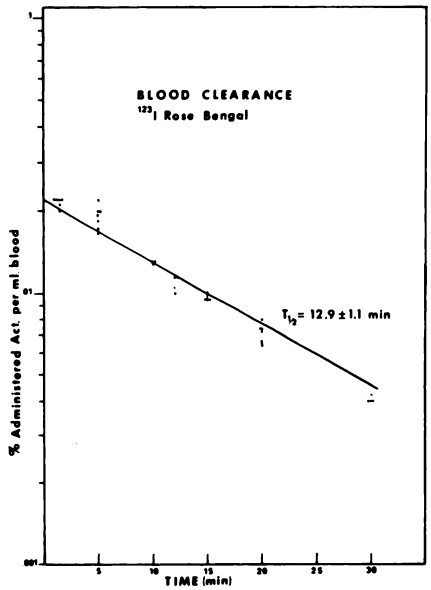

FIG. 3. Blood clearance of ^{123}I-rose bengal in normal patients during first 30 min.

relatively short 13-hr half-life of ^{123}I decreases patient exposure, and administration of millicurie quantities for diagnostic studies becomes feasible. However, rapid and efficient incorporation of ^{123}I in the molecule becomes more important than with 8-day half-life ^{131}I analogs. We have developed an expeditious method of routinely preparing high specific activity ^{123}I-rose bengal. Administration of ^{123}I-rose bengal to normal healthy volunteers vividly demonstrates the functioning anatomy of the liver, gallbladder, and biliary tree. A practical method for preparing large quantities of ^{123}I, considerations in selection of a rose bengal dye, the technique used for formulating the radiopharmaceutical, and distribution studies are reported in this study.

Based on these results, we feel that ^{123}I-rose bengal will prove advantageous in the evaluation of hepatobiliary disorders especially in the jaundiced patient in whom the differentiation between hepatocellular and extrahepatic obstructive jaundice may not be apparent from the biochemical and clinical presentation.

ACKNOWLEDGMENT

The authors wish to express their gratitude to Betsy Christy King for her valuable assistance in this study.

REFERENCES

1. TAPLIN GV, MEREDITH OM, KADE H: The radioactive (I-131 tagged) Rosebengal uptake excretion test for liver function using external gamma ray scintillation counting techniques. *J Lab Clin Med* 45: 665–678, 1955

2. ROSENTHALL LM: *The Application of Radioiodinated Rosebengal and Colloidal Gold in the Detection of Hepatobiliary Disease.* St. Louis, Warren H Green, 1969, pp 65–86

3. EYLER WR, SCHUMAN BM, DUSAULT LA, et al: The Radioiodinated Rose Bengal line scan as an aid in the differential diagnosis of Jaundice. *Am J Roentgenol Radium Ther Nucl Med* 94: 469–476, 1965

4. SILVERBERG M, ROSENTHALL L, FREEMAN LM: Rose Bengal excretion studies as an aid in the differential diagnosis of neonatal jaundice. *Semin Nucl Med* 3: 69–80, 1973

5. BURKE G, HALKO A: Dynamic clinical studies with radioisotopes and the scintillation camera. *JAMA* 198: 140–150, 1966

6. NORDYKE RA, BLAHD WH: Blood disappearance of radioactive Rose Bengal—rapid simple test of line function. *JAMA* 170: 1159, 1959

7. CHRISTY B, KING G, SMOAK WM: Preparation of ^{123}I-labeled rose bengal and its distribution in animals. *J Nucl Med* 15: 484, 1974

8. ASHKAR FS, BEAVER JE, HUPF HB: Dynamic studies of thyroid function using parenteral NaI-123: In *Proceedings of the IAEA Symposium on Dynamic Studies with Radioisotopes in Clinical Medicine and Research*, Knoxville, Tenn, IAEA-SM-185/79, 1974

9. BEAVER JE: I-123 production and availability. In *Proceedings of Thyroid and Endocrine System Investigations with Radionuclides.* Medcom: (in press) 1974

10. RABAN P, GREGORA V, BROUSIL J: Control of Rose Bengal quality with a view to its use for liver function tests. *Acta Univ Carol [Med] (Praha)* 17:5/6, 291–319, 1971

11. BROUSIL J, RABAN P, GREGORA V: Investigation of possible reasons for the variation of results in the liver function test with labeled Rose Bengal. *Acta Univ Carol [Med] (Praha)* 17:5/6, 321–338, 1971

12. U.S. Pharmacopeia XVIII. Easton, Pa, Mack Publishing Co, 1970, p 661

13. SMITH EM: Personal communication

14. MYERS WG: Radioiodine-123 for scanning. *J Nucl Med* 7: 390–391, 1966

15. NELSON JP, WATSON DD, KING AG, et al: Clinical and physical aspects of the use of I-123-Rose Bengal. *South Med J* 66: 1331, 1973