Gallium-68 Labeled Red Cells and Platelets

New Agents for Positron Tomography

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Red cells and platelets are labeled with Ga-68 by preparing the Ga-68-hydroxy-quinoline (oxine) complex and mixing the complex with the separated cellular components. The complex is prepared by first breaking the Ga-68 EDTA chelate with concentrated hydrochloric acid, adsorption of the gallium on a strong anionic ion-exchange column, and elution of the gallium activity with distilled water. Oxine is added, the oxine complex extracted into chloroform, and—following evaporation and dissolution—the solution is added to either red cells or platelets that have been centrifuged from whole blood, washed, and suspended in normal saline. After 15 min of incubation, labeling efficiencies of >90% and yields of ~7 mCi are obtained from 18 mCi of Ga-68 EDTA eluted from the generator. After injection of the gallium-labeled red cells in dogs, excellent blood pool images were obtained by positron tomography. The distribution corresponded to that of ^{11}CO-labeled red cells. When labeled platelets were injected into dogs with experimentally induced intimal injury of on carotid artery, the area of injury was clearly visualized 40 min after tracer injection.

J Nucl Med 18: 558–562, 1977

For the past decade the major advances in clinical nuclear medicine have been strongly dominated by the use of the scintillation camera and ^{99m}Tc-labeled radiopharmaceuticals. This approach suffers from three limitations (*1*).

1. The scintillation camera compresses into a two-dimensional image the distribution of activity within a three-dimensional object. Further, activity contained in tissue overlying and underlying the region of interest is superimposed on the image. This may seriously interfere with the identification of the structures of interest.

2. The gamma radiation detected by the camera is attenuated, in general unaccountably, by the tissues interposed between the region of interest and the crystal of the camera. In most cases this makes quantitation difficult if not impossible.

3. The field of view, resolution, and sensitivity change with distance.

These disadvantages can be eliminated by the utilization of emission tomography (*2–4*) with positron-emitting radiopharmaceuticals, which permits accurate quantification of the radiopharmaceutical distribution.

One major limitation to the general applicability of positron tomography is the scarcity of suitable radiopharmaceuticals. To date, the applications of such devices discussed in the literature (*5–8*) mainly involve radiopharmaceuticals labeled with the very-short-lived nuclides ^{11}C, ^{13}N, ^{15}O. Although compounds labeled with these emitters have great potential for the study of regional physiology, their

Received Oct. 8, 1976; revision accepted Dec. 28, 1976.

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use is limited to institutions with an "in-house" biomedical cyclotron.

As a result, more widespread application of positron tomography will probably require the use of positron-emitting radionuclides that can be produced without an in-house cyclotron. A leading candidate is Ga-68 ($T_{1/2}$ 68 min), which is formed by the decay of 275-day ^{68}Ge. The commercially available generator system allows the elution of the gallium in the form of Ga-68 EDTA (*9*). In spite of the complexity of procedures where other than Ga-68 EDTA is used, several radiopharmaceuticals have been labeled with Ga-68 after the gallium-EDTA complex has been decomposed (*9–19*). In the present work we prepared two new agents, Ga-68-labeled red blood cells and platelets, and have studied their in vivo behavior in dogs utilizing the positron-emission transaxial tomographic (PETT) scanner.

METHODS

Gallium-68 is obtained as Ga-EDTA by elution of a 25-mCi Ga-68 generator* with 10 ml of 0.05 *M* EDTA solution (pH 7.0). Ten milliliters of 12 *M* hydrochloric acid is added and the solution is passed through a small column (2 cm long by 0.3-cm diam) containing a strong anion-exchange resin† (*9*). This resin adsorbs the gallium as $GaCl_4^-$ and the EDTA is washed off the column. After a rinse with 20 ml of 6 *M* hydrochloric acid, the gallium is eluted off the ion-exchange column with 2 ml of distilled water. This solution is still acidic; it is boiled to dryness in a 30-ml beaker, the residue dissolved in 2 ml of 0.02 *M* hydrochloric acid, and the pH adjusted by the addition of 200 μl of acetate buffer (0.5 *M*, pH 5.5). During this preparation of Ga^{3+}, care must be taken to remove all traces of metals from the solutions involved. All glassware must be thoroughly cleaned and all the hydrochloric acid solution used must be purified by passage through a large column containing the strong anion-exchange resin.

8-hydroxyquinoline (150 μg in 150 μl of ethanol) is added to the gallium solution and mixed thoroughly. The resulting complex is then extracted in two equal volumes (1.5 ml each) of chloroform. Typically, from 50 to 80% of the activity is extracted. After evaporation of the chloroform to dryness in a boiling-water bath, the residue is dissolved in 0.5 ml of 20% (v/v) ethanol.

Cell separation and labeling. Red cells are separated from 20 ml of heparinized whole blood by centrifugation at 180g for 15 min. The separated cells are then washed twice with equal volumes of isotonic saline solution. Following the final wash, the gallium-oxine complex is added to the cells and the mixture is incubated at room temperature for 15 min.

For the platelet separation, 43 ml of whole blood is collected in a 50-ml syringe containing 7 ml of ACD solution. The blood is carefully transferred to a 50-ml sterile plastic centrifuge tube and centrifuged at 180g for 15 min. With siliconized Pasteur pipets, the platelet-rich plasma is separated into two equal volumes in sterile conical tubes, and is further centrifuged at 1500g for 15 min. The platelet-poor plasma is removed and the platelets gently resuspended in 10 ml isotonic saline solution. The platelets are again centrifuged at 1500g for 15 min, resuspended, and the centrifugation repeated. Finally, the washed platelets are suspended in ~7 ml of isotonic saline and the solution containing the ^{68}Ga activity added. This platelet suspension is allowed to incubate for 15 min at room temperature. The labeling yield in each study was determined by centrifugation of an aliquot of the sample that was injected, the cellular component being separated from the supernatant and each fraction counted.

Animal studies. Dogs anesthetized with sodium pentobarbital (30 mg/kg) were positioned in the positron-emission transaxial tomograph (PETT) scanner (*2,3*) on their backs so that the tomographic slice would be through the region of interest. In all cases transmission scans (*2,3*) were performed before injection of the radiopharmaceutical, using a ring filled Cu-64 in order to obtain data for the attenuation correction of the emission image.

The labeled blood components (~7 mCi) were injected intravenously. For comparison studies, in some cases ^{11}C-labeled carbon monoxide was administered by inhalation to form [^{11}C]-carboxyhemoglobin. Before administration, the ^{11}CO is collected in an ambu bag and diluted with air. The ^{11}CO was produced by the ^{10}B(d,n)^{11}C reaction using the deuteron beam University Medical School cyclotron, with boric oxide as the target material. The resulting mixture of ^{11}CO and $^{11}CO_2$ is swept out of the target by helium gas and passed over zinc at 700°C to reduce $^{11}CO_2$ to ^{11}CO.

The accumulation of platelets in areas of endothelial injury was studied in four dogs by damaging the endothelium of the carotid artery (*19*). The femoral artery was exposed and, under fluoroscopic control, a 4F Fogarty arterial catheter was inserted into the carotid artery. The labeled platelets were administered, the catheter balloon inflated to 700 mm Hg pressure and pulled back into the aortic arch. In one of the dogs the catheter was simply inserted and left in place, uninflated, for approximately 15 min. The catheter was then removed, the femoral artery repaired, and the surgical incision closed.

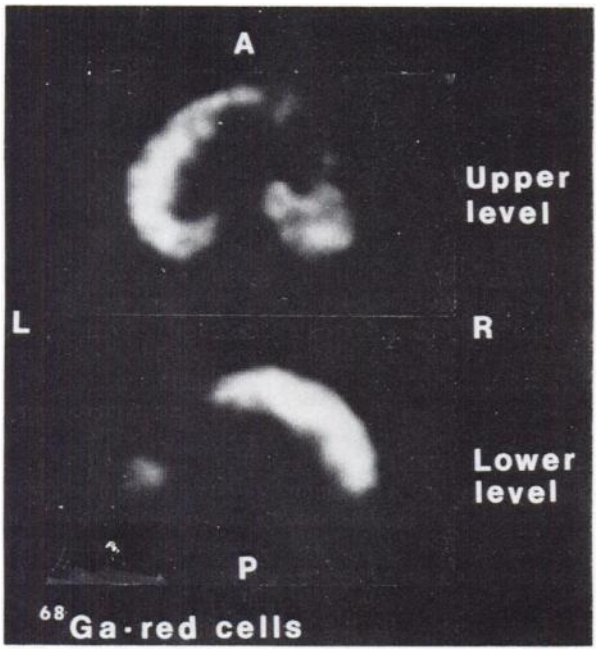

FIG. 1. Upper-level tomographic image of Ga-68-labeled red cells injected intravenously in dog, showing accumulation in liver and spleen. Lower-level tomogram reveals mainly spleen and only small amount of activity in tip of liver. Images were taken within 30 min of injection, and time of study (including transmission images) was approximately 1 hr.

RESULTS AND DISCUSSION

Cell labeling. High yields of labeled red cells were obtained in all cases. In five studies, yields of 93 ± 5% were obtained. The generator was eluted when the cells were separated and washing was almost complete, so that there was minimal delay between the procedures involving the short-lived gallium. Typically the cells were ready for injection ~35 min following generator elution; ~17 mCi of ^{68}Ga was eluted from the generator, ~10 mCi of the gallium chloride was formed, and ~7 mCi of cells was injected into the dogs. Analysis of the injectate by centrifugation showed that >90% of the activity was associated with the cells. Attempts to label the blood components with Ga-68 produced by thermal decomposition of the Ga-68 EDTA (*9*) proved unsuccessful.

Labeling yields with platelets tended to be more variable, and maximum yields were only obtained when all materials used in labeling were purified as described in the section on methods. Low yields can be caused by incomplete removal of transferrin from the cells or platelets before labeling. Like indium-transferrin (*20*), gallium-transferrin has a high stability constant and will form preferentially if all the transferrin is not removed. The overall labeling procedure takes ~70 min. The Ga-68 was eluted from the generator before the last platelet wash, and the 8-hydroxyquinoline complex was synthesized during the last wash. Owing to the lower concentration of platelets, this exchange is a greater problem with the labeling of platelets than with the labeling of red cells. The similarity of indium and gallium is also evident in the fact that the stability and the methods of preparation of these gallium-labeled blood components parallel those previously observed for In-111-labeled blood components (*21–25*). The labeling is thought to occur (*26*) by the diffusion of the gallium-8-hydroxyquinoline through the cell membrane and the transfer of the gallium to intracellular components.

In vivo studies. As with indium-labeled blood components (*21–26*) the Ga-68 remained bound to the components for several hours after injection, and the tracer thus remained in the blood pool. In the abdomen (Fig. 1) excellent tomographic sections of the liver (upper level) and spleen (lower level) were obtained. When C-11 was administered, sections taken, and gallium-labeled red cells administered after the decay of the ^{11}C, very similar images were obtained with the two blood-pool agents. Figure 2 shows typical images of the two tracers at the levels of the heart and abdomen. The slight difference observed between the two images of the heart is probably due to the difficulty in repositioning the animals for cardiac tomography, where a difference of 2 mm will produce a different image (unpublished data). The images obtained show the potential of Ga-68-labeled red cells. It seems probable that they can be used as a substitute for C-11-labeled car-

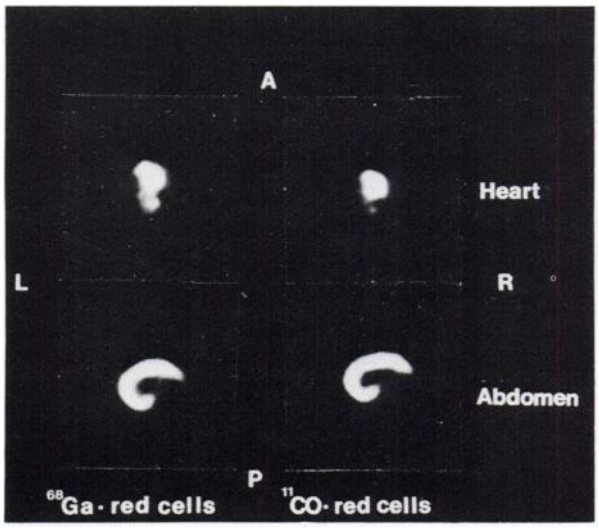

FIG. 2. Tomographic images of dog, showing similarity of images obtained with Ga-68 red cells and [^{11}CO] carboxyhemoglobin. Red cells were injected intravenously, whereas ^{11}CO was administered by inhalation.

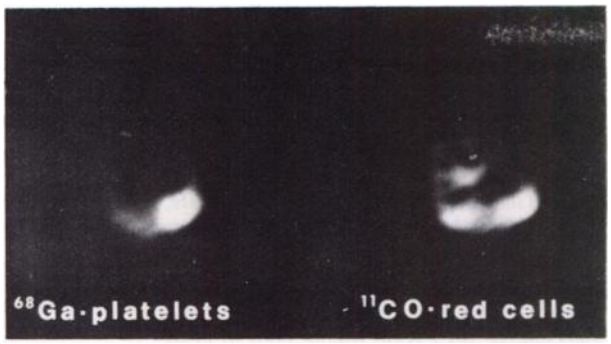

FIG. 3. Tomograms of neck of dog viewed from below with induced endothelial damage of one carotid, showing marked accumulation of Ga-68-labeled platelets in abnormal carotid. Red-cell image with ^{11}CO reveals only slight asymmetry of carotids, which is related to residual Ga-68 activity in damaged carotid. Ga-68-labeled platelets were administered intravenously, ^{11}CO by inhalation.

boxyhemoglobin in institutions having no cyclotron.

Figure 3 shows a typical image obtained following the administration of Ga-68-labeled platelets to a dog with intimal injury in one carotid artery. The image was taken 30 min after injection of the radiopharmaceutical. The digital printout of the actual activity present in the tomogram showed that in this image the ratio of activity in the damaged carotid to that in the undamaged carotid was ~15:1. In the four dogs studied the ratios were all greater than 12:1. In one dog the ratio could not be calculated because the normal carotid was not observable over the background in the neck. We have previously shown histologically (*23*) that intimal damage is produced in this model, and that there is platelet accumulation in the damaged area. In the dog where the catheter was not inflated, the ratio of activity in "damaged" carotid to the activity in "normal carotid" was ~2:1. The simple insertion of the catheter appears to damage the artery sufficiently for platelets to accumulate in amounts detectable by positron tomography.

Figure 3 also shows a tomogram taken following administration of ^{11}CO to the animal by inhalation, with visualization of both carotids. The right carotid appears to contain the greater activity, but there is still residual gallium activity in the left carotid. When the digital printouts of the activity of the gallium-platelet scan and the ^{11}CO scan were compared, and the residual gallium activity was subtracted from the [^{11}CO]-carboxyhemoglobin picture, the ^{11}C activity was equal in both arteries. In the study where the catheter was not inflated, the carboxyhemoglobin activity was the same in both arteries.

CONCLUSION

The combination of positron tomography and the Ga-68-labeled blood components appears to have great potential for imaging blood pools and assessing areas of platelet accumulation. These two new radiopharmaceuticals add to the Ga-68 radiopharmaceuticals available for use with positron tomography.

ACKNOWLEDGMENTS

This work was supported by NIH SCOR in Thrombosis HL14147 and NIH 5 P01 HL13851. The authors thank Robert Feldhaus, Joan Primeau, Barbara Asberry, Mickey Clarke, and Carol Higgins for their assistance.

Mohan Patel is a World Health Organization Fellow.

FOOTNOTES

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