Liver and Kidney Imaging with Ga-68-Labeled Dihydroxyanthraquinones

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This paper describes the preparation of alizarin (1,2-dihydroxyanthraquinone) and alizarin red S (sodium 1,2-dihydroxyanthraquinone-3-sulfonate) labeled with Ga-68, which is obtained from a new high-yield Ge-68→Ga-68 generator. The uptake of Ga-68 alizarin by liver and spleen RES was studied in rats, dogs, and humans, and amounted to 80–85% of the administered dose within 5 min after i.v. injection. Gallium-68 alizarin red S was preferentially accumulated in the renal parenchyma to an extent of 70% within 2 hr after i.v. administration. Both substances combine simple and fast preparation with the potential advantages of positron scintigraphy. Complete labeling of 1 mCi Ga-68 was achieved by 100 μg of each compound, amounts that are without any known measurable harm to humans (LD_{50} alizarin red S for i.v. injected mice = 70 mg/kg (8); LD_{50} alizarin for i.p. injected mice >120 mg/kg (*18*)).

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Positron imaging in nuclear medicine—especially of deeply located organs—has several advantages over single-photon scintigraphy. The physical properties of positron emitters allow the selection of different focal planes, calculation of absorbed radiation, and quantitative activity determination; they also provide high sensitivity and spatial resolution. Because of intensified development of positron imaging devices during the last few years, the need for radiotracers labeled with positron emitters has increased. Besides the short-lived, positron-emitting nuclides C-11, N-13, O-15, and F-18—which are indispensable for basic research in biochemistry or drug metabolism but whose application requires the vicinity of a cyclotron—Ga-68 has become more interesting. The 68.3-min Ga-68, with β^+_{max} at 1.9 MeV, can be obtained as a generator product from its parent nuclide Ge-68 ($t_{1/2}$ 287 days) simply by elution, and exhibits excellent complex-forming properties. Radiopharmaceuticals based on Ga-68 and complex-forming agents have been reported for brain-tumor scanning (*1*), and for glomerular filtration of the kidneys (*2*), for bone scanning (*3*), and for blood-cell labeling (*4*).

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Among all the gallium-complexing substances we have investigated thus far, our most encouraging results have been with the hydroxylated anthraquinones. Whereas Ga-68-labeled 1,2-dihydroxyanthraquinone (alizarin) was rapidly accumulated in liver and spleen RES, Ga-68-labeled sodium 1,2-dihydroxyanthraquinone-3-sulfonate (alizarin red S) was preferentially taken up by the renal parenchyma.

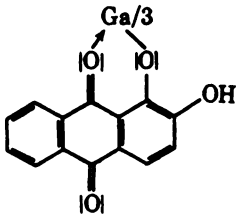
Hydroxylated anthraquinones are versatile compounds that have found applications in analytical chemistry (5–7) as well as in biology. Alizarin is known to form colored complexes with a variety of metal ions, but only zirconium and Group IIIa elements of the periodic table (Al, Ga, In) form complexes that are stable in neutral or slightly acid solutions.

In biology, alizarin red S has been used because of its accumulation in calcifying tissue, providing fluorescent red staining, especially of growth zones in bone and teeth (*8,9*).

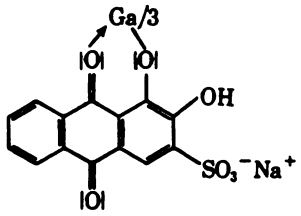
MATERIALS AND METHODS

All reagents used were of analytical grade. Alizarin and alizarin red S were checked additionally for their chemical purity by thin layer chromatography on silica gel with a solvent mixture of formic acid, n-butanol, and

water (6:25:9). Gallium-68 activity was obtained in 4 ml of 0.5 *N* HCl from a new type of Ge-68 → Ga-68 generator system, based on a pyrogallol ion exchanger coupled in series with a small anion exchange column* (*10*), with a concentration of 2.0 mCi Ga-68/ml. Preparation of Ga-68-labeled alizarin and alizarin red S (formulas I II) was similar. The 4-ml, Ga-68-containing eluate was made slightly alkaline (pH 9–12) with 1 *N* NaOH; after which 0.5 ml of either alizarin or alizarin red S in 0.1 *N* NaOH was added (1.5 mg/ml). During subsequent neutralization to pH 6.7–7.3 with 0.1 *N* HCl, the dark violet color of the alkaline solution of both reagents changed to brick red. The solution was stirred for 2–3 min.



I Ga-alizarin



II Ga-alizarin red S

In contrast to the clear red solution of Ga-68-labeled alizarin red S, which passed a 0.22 μm Millipore filter with no retention of Ga-68 activity, Ga-68 alizarin precipitated during neutralization as a red colloid and could be quantitatively retained on a similar Millipore filter. The particle size of the colloids was determined by activity retention on polycarbonate membrane filters of different pore sizes. Radiochemical purity of both Ga-68-labeled complexes was checked with paper chromatography; the radiochromatograms are shown in Fig. 1. The final pharmaceutical solutions, ready for injection, had a volume of about 7.0 ml, were 0.28 *M* in NaCl, contained 110 μg/ml of alizarin or alizarin red S, and had an activity concentration of 1 mCi Ga-68 per milliliter.

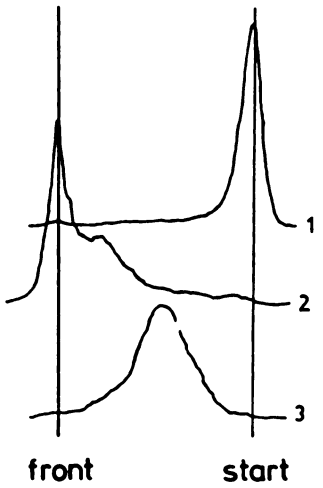


FIG. 1. Paper chromatography of $^{68}Ga(OH)_3$ (#1), Ga-68 alizarin red S (#2), and Ga-68 alizarin (#3). Ten μl of 0.25 *N* NaCl solution (pH 7.2) containing 0.3 μCi Ga-68 and 1 μg of each of indicated complexing agents were spotted. Support: Whatman No. 1 chromatographic paper. Solvent: ethylacetate:tetrahydrofurane:H_2O (6:35:47); ascending 1 hr.

Gallium-labeled alizarin and alizarin red S can be stored as long as desired in closed vials under light protection. Exposure to intense light slowly causes the photochemical reactions of anthraquinones in dilute HCl

TABLE 1. ORGAN DISTRIBUTIONS OF Ga-68 IN RATS* AFTER I.V. ADMINISTRATION OF Ga-68-LABELED ALIZARIN

	% dose, ± s.d., per gram organ†				
Organ	5 min	10 min	15 min	30 min	60 min
Blood	0.93 ± 0.36	0.46 ± 0.04	0.39 ± 0.04	0.28 ± 0.10	0.21 ± 0.03
Lung	0.53 ± 0.07	0.40 ± 0.05	0.35 ± 0.05	0.28 ± 0.03	0.20 ± 0.02
Spleen	1.45 ± 0.57	1.46 ± 0.36	1.61 ± 0.38	1.74 ± 0.51	1.41 ± 0.39
Duodenum‡	0.06 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.05 ± 0.01	0.04 ± 0.01
Bone‖	0.13 ± 0.03	0.12 ± 0.02	0.11 ± 0.02	0.14 ± 0.03	0.15 ± 0.03
Muscle	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.01
Liver	8.15 ± 0.72	7.86 ± 0.65	8.21 ± 0.36	8.31 ± 0.65	8.55 ± 0.83
Kidney	0.28 ± 0.05	0.23 ± 0.02	0.22 ± 0.03	0.22 ± 0.04	0.21 ± 0.02
	% dose, ± s.d., per whole organ§				
Kidneys	0.51 ± 0.09	0.42 ± 0.04	0.40 ± 0.05	0.40 ± 0.06	0.38 ± 0.04
Liver	82.3 ± 7.3	79.4 ± 6.6	82.9 ± 3.6	83.9 ± 6.5	86.4 ± 8.8
Spleen	0.93 ± 0.36	0.93 ± 0.23	1.03 ± 0.24	1.11 ± 0.33	0.90 ± 0.25

* Average weight 285.3 ± 36.8g (n = 35).
† n = 7.
‡ 10 cm of duodenum with contents, including the junction of gall ducts, were measured.
‖ One femur was measured.
§ Average weights of organs (n = 35): liver 10.1 ± 1.0 g; kidneys 1.82 ± 0.16 g; spleen 0.64 ± 0.11 g.

TABLE 2. ORGAN DISTRIBUTIONS OF Ga-68 IN RATS* AFTER I.V. ADMINISTRATION OF Ga-68-LABELED ALIZARIN RED S

Organ	% dose, ± s.d., per gram organ† 5 min	10 min	15 min	30 min	60 min
Blood	3.03 ± 0.27	2.14 ± 0.17	1.49 ± 0.20	0.93 ± 0.12	0.73 ± 0.09
Lung	1.05 ± 0.10	0.81 ± 0.07	0.69 ± 0.14	0.47 ± 0.06	0.37 ± 0.03
Spleen	0.55 ± 0.08	0.41 ± 0.05	0.34 ± 0.06	0.21 ± 0.03	0.20 ± 0.01
Duodenum‡	0.24 ± 0.04	0.23 ± 0.03	0.22 ± 0.04	0.13 ± 0.02	0.11 ± 0.01
Bone‖	0.26 ± 0.05	0.23 ± 0.03	0.19 ± 0.04	0.15 ± 0.02	0.14 ± 0.03
Muscle	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.06 ± 0.01
Liver	0.47 ± 0.06	0.40 ± 0.05	0.33 ± 0.05	0.26 ± 0.03	0.24 ± 0.01
Kidney	10.0 ± 2.12	18.19 ± 1.24	20.73 ± 4.01	24.75 ± 2.71	27.18 ± 0.90
	% dose, ± s.d., per whole organ§				
Kidneys	17.7 ± 3.75	32.2 ± 2.2	36.7 ± 7.1	43.8 ± 4.8	48.1 ± 1.6
Liver	4.89 ± 0.62	4.16 ± 0.52	3.43 ± 0.52	2.70 ± 0.26	2.50 ± 0.10
Spleen	0.35 ± 0.05	0.26 ± 0.03	0.22 ± 0.04	0.13 ± 0.02	0.13 ± 0.01

* Average weight: 295.6 ± 16.5 g (n = 35).
† n = 7.
‡ 10 cm of duodenum with content, including the junction of gall duct, was measured.
‖ One femur was measured.
§ Average weights of organs (n = 35): liver 10.4 ± 1.0 g; kidneys 1.77 ± 0.14 g; spleen 0.64 ± 0.10 g.

(*19*). Measurements of organ distribution of both substances were carried out in male Sprague-Dawley rats with an average weight of 300 g. Animals were injected i.v. with 0.4 ml of either Ga-68-labeled alizarin or alizarin red S solution, prepared as described above but subsequently diluted with water to an isotonic saline content (0.4 ml contained 35 μCi Ga-68 and 20 μg of the dye). At different times, as indicated, animals were anesthetized with ether and killed by heart puncture. Organs were removed and weighed, and 511-keV annihilation radiation was measured in a NaI(Tl) well detector coupled to a computerized multichannel analyzer. Care was taken to use identical geometry during measurement to avoid differences in sum-peak formation.

Scintigrams from dogs and humans were made with a positron scanner (*11*) constructed from 128 NaI(Tl) crystals and suitable for quantitative activity determination.

RESULTS

High chemical purity of commercially available alizarin and alizarin red S could be established by silica gel thin layer chromatography. Both substances migrated as single, narrow spots with R_f-values of 0.85 and 0.43, respectively. Filtration of Ga-68-labeled alizarin colloids through membrane filters showed the following size distribution: <0.2 μ ~ 0.2%, 0.2–1.0 μ ~ 1.0%, 1.0–3.0 μ ~ 90.2%, 3.0–5.0 μ ~ 6.1%, >5.0 μ ~ 2.5%. Radiochemical purity of the Ga-68 complexes was established by paper chromatography: There is no Ga-68 activity left at the origin, indicating full complexing of gallium. Whereas Ga-68 alazarin shows an R_f of about 0.5, Ga-68 alizarin red S, because of its higher water solubility, migrates with the front.

Organ distributions of Ga-68-labeled alizarin and alizarin red S in rats are presented in Tables 1 and 2. After i.v. administration, 80–85% of Ga-68 alizarin was taken up within 5 min by the liver and 1% by the spleen; neither moved further during the whole investigation time. Kidneys and bone also showed constant activities, whereas Ga-68 activity in blood, lungs, and duodenum decreased. Scintigrams of the liver and spleen of a healthy volunteer (Fig. 2) showed an 80–85% accumulation of Ga-68-labeled alizarin from 8 up to 70 min after

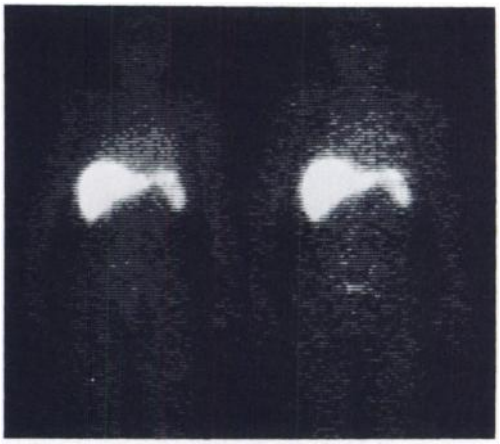


FIG. 2. Uptake of Ga-68 by liver of healthy volunteer at 8 min (left) and 70 min (right) after i.v. administration of 110 μg alizarin labeled with 615 μCi Ga-68.

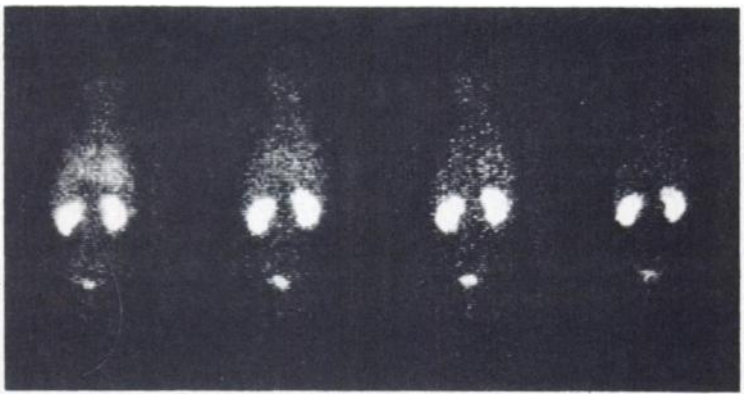


FIG. 3. Uptake of Ga-68 by renal parenchyma of normal human volunteer at 35, 70, 90, and 130 min (left to right) after i.v. administration of 90 μg alizarin red S labeled with 703 μCi Ga-68.

i.v. administration. Activity in the spleen of the volunteer amounted to 9%, as determined with the aforementioned positron scanner.

Accumulation of Ga-68 alizarin red S in the kidneys occurred much more slowly by comparison, and had not reached its full maximum at 60 min. This is also suggested by the constant decrease of Ga-68 activity in all other organs during the whole investigation period. This assumption was confirmed by means of scintigrams taken from a healthy volunteer at 35, 70, 90, and 130 min after i.v. administration of 0.7 mCi Ga-68-labeled alizarin red S (Fig. 3). Calculation of the activity in the kidneys showed a 70% uptake of Ga-68 at 130 min after administration of the compound. Activity in voided bladder urine was measured in a NaI(Tl) detector, and amounted to 3.5% of administered dose.

DISCUSSION

The above-described compounds represent the first attempt to broaden the spectrum of Ga-68-labeled radiopharmaceuticals by investigating the use of alizarin dyes and related complexing compounds. Due to the development of high-resolution, proportional-chamber positron cameras (*12*), Ga-68-labeled pharmaceuticals for static or slow functional imaging will achieve high interest because spatial resolution of several millimeters appears possible.

Gallium-68-labeled alizarin appears to be an efficient liver-RES imaging agent. In contrast to other Ga-68 colloids proposed for liver scanning (*13–15*) its preparation needs no inorganic carriers, such as $Fe(OH)_3$ or $CrPO_4$, no stabilizers like gelatin, and no heating; thus, preparation is quite simple. During more than 20 preparations, no problems with colloid size have occurred; thus the method is suitable for routine use. Regarding radiation exposure, a dose of 500 μCi Ga-68, which is sufficient for positron scanning of the liver, delivers 900 mrad of radiation to the liver. This is in the same range as the radiation dose from 2–3 mCi Tc-99m sulfur colloid, which is commonly used in liver scintigraphy. Likewise the biological behavior appears entirely comparable to this latter compound in routine clinical use.

Gallium-68-labeled alizarin red S is also distinguished by the same simplicity of preparation. Since it localizes in the renal parenchyma to the same extent as Tc-99m DMSA (dimercapto succinic acid) (*16,17*), it can be used for the determination of anatomical defects of the kidneys, as well as relative blood flow. In contrast to Tc-99m DMSA, there is a much smaller activity excretion by the kidneys (4–8% compared with 20–25% of the technetium compound) which shortens the waiting period for renal imaging to 1.5 hr after i.v. injection.

When one is comparing different patients, it is particularly helpful to be able to determine the absolute renal uptake, including suitable correction for photon scattering within the patient. Compared with the rapid renal uptake and excretion of Ga-68 EDTA, the slow accumulation of Ga-68 alizarin red S better meets the requirements of the currently available positron imaging devices, whose counting performance (with only several thousand coincident events per second) is too low for good spatial resolution and quantitative activity determinations of highly dynamic functions.

FOOTNOTE

*Bio Rad AG 1 × 8.

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