PRELIMINARY INVESTIGATION OF ^{68}Ga -PYROPHOSPHATE
AS A POTENTIAL SCANNING AGENT

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INFORMAL REPORT

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DISCONTINUED

Most radiopharmaceuticals which have been used or contemplated for the assessment of myocardial perfusion have been potassium isotopes or analogs. More recently it has become possible to positively identify infarcted regions of the myocardium by "hot spot" imaging with ^{99m}Tc -labeled pharmaceuticals such as tetracycline (1) and bone agents (2,3,4), principally ^{99m}Tc -pyrophosphate.

Since it is our belief that positron imaging will be a major modality for nuclear cardiology in the future, we undertook a preliminary study of the organ distribution of gallium-pyrophosphate on the basis of the known affinity of both gallium and pyrophosphate for bone. The use of radiogallium in ionic form for bone imaging is restricted by the need to saturate blood binding sites with relatively large amounts of stable gallium before sufficient radio-gallium concentration in bone occurs (5). For myocardial imaging low blood levels of radioactivity is also mandatory. The organ distribution of gallium-pyrophosphate (^{68}Ga -PYP and ^{67}Ga -PYP) was studied with and without added carrier and compared to ^{99m}Tc -pyrophosphate in order to determine the optimum conditions for bone uptake of gallium-pyrophosphate. In the study of different levels of added carrier, ^{67}Ga -PYP was used because of the greater ease in counting samples containing an isotope with a relatively long physical half-life.

Materials and Methods

The ^{68}Ga activity was obtained as an EDTA complex by eluting a ^{68}Ge - ^{68}Ga generator (New England Nuclear Corporation); ^{67}Ga as $^{67}\text{GaCl}_3$, carrier-free, in 0.05N HCl (Diagnostic Isotopes); ^{99m}Tc from a ^{99}Mo - ^{99m}Tc generator (Squibb). The ^{68}Ga -EDTA complex was dissociated with concentrated HCl and the resulting GaCl_4^- complex separated from EDTA by adsorption on a Dowex anion exchange column. The anion exchange column was washed with 1.5 ml pyrogen-free water to remove the $^{68}\text{GaCl}_4^-$, 2-3 drops 10N NaOH were added followed by 0.5 ml sodium pyrophosphate solution (2mg/ml $\text{Na}_4\text{P}_2\text{O}_7 \cdot 10\text{H}_2\text{O}$). The final pH was

adjusted to 7 with dilute NaOH. The solution was further diluted with water to yield a final concentration of $50 \mu\text{g Na}_4\text{P}_2\text{O}_7 \cdot 10 \text{ H}_2\text{O}$ per 0.2 ml.

To $5\text{--}10 \mu\text{l } ^{67}\text{GaCl}_3$ was added $17.5 \text{ mg Na}_4\text{P}_2\text{O}_7 \cdot 10 \text{ H}_2\text{O}$ and 4 ml saline, stirred until dissolved and pH adjusted to 7 with 0.1 N HCl. The final preparation for injection contained about $1 \mu\text{Ci}$ of ^{67}Ga activity and $500 \mu\text{g Na}_4\text{P}_2\text{O}_7 \cdot 10 \text{ H}_2\text{O}$ per 0.2 ml. Gallium chloride was added to an appropriate carrier level. For 5 and 10 mg/kg carrier levels, freshly made gallium citrate was used for preinjections.

$^{99\text{m}}\text{Tc}$ -pyrophosphate was prepared using a commercially available kit (Phosphotec, Squibb), containing a final concentration of about $500 \mu\text{g Na}_4\text{P}_2\text{O}_7 \cdot 10 \text{ H}_2\text{O}$ per 0.2 ml.

The quality of the gallium pyrophosphate preparations was checked by gel permeation chromatography using $0.9 \times 35 \text{ cm Biogel P-30}$ columns, precalibrated with appropriate standards.

A total of 8 groups of 12 BNL white mice each weighing 25-30 grams were injected into a tail vein with a 0.2 ml volume of the pyrophosphate complex and the added carrier. At 5 and 10 mg/kg levels of added gallium carrier; however, the mice were pre-injected 30 minutes before the administration of the radioactivity with the carrier in a 0.2 ml volume. The animals were sacrificed by ether overdose 1 and 2 hours after administration of radioactivity.

Appropriate standards, organs of interest, and all samples were counted in a NaI automatic well counter, making corrections for decay. Complete organs in the case of the liver, kidneys, stomach, intestine, spleen, gonads, heart, lungs and tail were removed and counted, while only samples of muscle, bone and blood were taken for counting. Muscle, bone and blood were assumed to constitute 43, 10 and 7 percent respectively of the animal's total weight.

Results were expressed both as percent injected dose per gram and as percent injected dose per organ.

The ^{68}Ga -PYP compound was also evaluated in rabbits with (5 mg) and without added carrier gallium citrate and also following administration of 3 mg iron dextran 20 minutes before the ^{68}Ga -PYP. Distribution of radioactivity was studied using a gamma-camera equipped with a pinhole collimator and the distribution of radioactivity followed up to 90 minutes post-injection.

Results

Gel chromatography of the gallium-PYP preparations revealed that only one species was present, presumably that of the desired complex. Recovery of activity from the column bed was always complete.

Tables 1-4 list the results obtained at 1 and 2 hours post injection with ^{67}Ga -PYP with and without different levels of added carrier, ^{68}Ga -PYP and $^{99\text{m}}\text{Tc}$ -PYP. Each value represents the mean $\pm$ 1 S.D. of six mice. The results are expressed both as percent of injected dose per organ and as percent of injected dose per gram of tissue.

In going from no added carrier to high levels of gallium, the percentage of activity in the bone increased, reaching values similar to $^{99\text{m}}\text{Tc}$ -PYP at the 5 and 10 mg/kg levels. With the exception of the kidneys, the percentages in the other tissues decreased, but even at a level of 10 mg/kg added carrier gallium the concentration in the blood was higher than with $^{99\text{m}}\text{Tc}$ -PYP. Similarly the bone to blood ratios increased from 0.57 to 6.29 and from 1.37 to 9.84 at 1 and 2 hours respectively with increasing levels of added carrier compared to 22.40 and 15.55 for $^{99\text{m}}\text{Tc}$ -PYP.

At added levels of 10 mg/kg of gallium a 30% mortality within a few minutes after injection was found. No mortality or other obvious deleterious effects were observed during the period of study at lower levels of added carrier.

In rabbits clearer delineation of bone structure was also seen following carrier administration. Pretreatment with iron-dextran, however, led to very slight uptake of radioactivity in bone and resulted in clear delineation of liver activity.

Discussion

Gallium-68 ($T_{1/2}$ 68 min.) is a convenient label for radiopharmaceuticals designed for use with positron detection devices; the radionuclide is a generator product obtained from its parent ^{68}Ge ($T_{1/2}$ 287 days). Although the bone seeking properties of gallium have been investigated previously, the lack of early specific localization in bone at low levels of gallium has been shown to be due to the binding of gallium to serum proteins, specifically transferrin (5). The additions of carrier gallium to a ^{68}Ga citrate preparation enhanced the bone uptake of the ^{68}Ga due to saturation of the plasma protein binding sites. Although a similar type of carrier effect was noted with ^{68}Ga -tripolyphosphate (6), a recent publication claims good bone uptake without added carrier for ^{68}Ga -EDTMP (7)*.

Although uptake of ^{68}Ga -PYP in bone occurred without any added carrier, levels close to that obtainable with $^{99\text{m}}\text{Tc}$ -PYP could only be reached with high (5-10 mg/kg) levels of added carrier. Carrier gallium at a dose of 4 mg gallium per kg has been reported to result in no apparent toxicity to patients during a clinical study with ^{68}Ga -citrate with added carrier gallium (5). Although the LD_{50} and LD_{10} for $\text{Ga}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ has been reported to be 80 mg/kg and 63 mg/kg respectively in male mice (10), we found about a 30% mortality at a level of 10 mg/kg gallium.

* However, availability of EDTMP sufficiently pure for human use (devoid of toxic nitrogenous contaminants), at present is questionable (8). Also, the nature and the stability of metal-EDTMP chelates have recently been reported to be dependent upon the purity of this ligand (9).

It was not found practical to saturate the gallium binding capacity of the serum proteins by pre-injection of iron-dextran, possibly due to the coprecipitation of gallium activity with insolubilized iron in-vivo and subsequent deposition of the colloid in the liver and RES.

Our study seems to indicate that it would be possible to obtain the ideal low levels of blood radioactivity, required for heart imaging, with ^{68}Ga -PYP, only with the addition of relatively high (5-10 mg/kg) levels of carrier gallium. Since the toxicity of such levels of gallium could be outside of the safety margin, the usefulness of the method for clinical studies may be limited.

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TABLE 1

Pyrophosphate (Carrier effect)1 Hr. Post-injection% Injected dose/organ

	⁶⁷ Ga-PYP No added carrier	0.1mg/kg	⁶⁷ Ga-PYP and Added Carrier 0.5mg/kg	1mg/kg	5mg/kg	10mg/kg	⁶⁸ Ga-PYP No carrier	^{99m} Tc PYP
Bone	14.48 ±1.38	19.31 ±4.90	19.66 ±2.32	22.38 ±2.08	28.95 ±2.63	33.62 ±3.31	18.39 ±2.29	31.00 ±8.81
Blood	17.68 ±3.38	16.09 ±3.26	6.57 ±1.87	6.16 ±2.52	6.86 ±0.89	3.76 ±1.14	22.32 ±4.16	1.54 ±0.54
Muscle	19.84 ±2.59	24.27 ±1.15	11.52 ±2.15	9.55 ±3.17	9.16 ±1.54	5.93 ±1.64	26.01 ±1.88	2.81 ±1.38
Kidneys	1.80 ±0.50	1.95 ±0.30	1.79 ±0.30	2.01 ±0.29	2.24 ±0.18	2.14 ±0.48	2.33 ±0.54	1.25 ±0.20
Liver	7.12 ±2.46	7.53 ±1.02	4.69 ±0.51	5.41 ±0.95	5.03 ±0.67	4.28 ±0.58	8.21 ±2.06	6.41 ±0.37
Gut & Feces	10.69 ±1.79	10.46 ±0.62	5.19 ±0.22	4.04 ±1.02	4.27 ±0.69	2.82 ±0.63	11.60 ±0.91	1.59 ±0.36
Stomach	0.66 ±0.06	0.87 ±0.08	0.32 ±0.03	0.28 ±0.08	0.26 ±0.02	0.19 ±0.05	0.86 ±0.13	0.15 ±0.04
Spleen	0.27 ±0.12	0.30 ±0.04	0.14 ±0.02	0.15 ±0.05	0.16 ±0.03	0.10 ±0.03	0.39 ±0.15	0.12 ±0.04
Gonads	0.22 ±0.07	0.25 ±0.04	0.11 ±0.02	0.11 ±0.05	0.14 ±0.03	0.09 ±0.03	0.31 ±0.07	0.02 ±0.01
Heart	0.39 ±0.10	0.36 ±0.08	0.22 ±0.07	0.15 ±0.05	0.16 ±0.03	0.10 ±0.03	0.52 ±0.15	0.04 ±0.01
Lungs	1.38 ±0.50	1.25 ±0.71	0.53 ±0.17	0.66 ±0.20	0.37 ±0.03	0.27 ±0.07	1.77 ±0.59	0.15 ±0.04
Tail	4.24 ±0.81	3.80 ±0.55	2.78 ±1.41	2.62 ±0.79	3.21 ±0.79	2.21 ±0.43	3.70 ±0.39	1.83 ±0.34
Total	78.75	86.44	53.53	53.50	60.80	55.52	96.40	46.91

6 mice in each group. Mean ± 1 S.D.

TABLE 2

Pyrophosphate (Carrier effect)1 Hr. Post-injection% Injected dose/gm.

	⁶⁷ Ga PYP No added carrier	0.1mg/kg	0.5mg/kg	1mg/kg	5mg/kg	10mg/kg	⁶⁸ Ga PYP No carrier	^{99m} Tc PYP
Bone	5.38 ±0.69	7.72 ±2.25	7.54 ±0.99	8.02 ±0.73	10.50 ±1.08	13.01 ±1.50	6.61 ±0.79	11.31 ±2.87
Blood	9.47 ±1.50	9.12 ±1.69	3.59 ±1.01	3.17 ±1.35	3.54 ±0.51	2.07 ±0.63	11.43 ±1.46	0.81 ±0.27
Muscle	1.71 ±0.22	2.25 ±0.19	1.02 ±0.17	0.81 ±0.31	0.77 ±0.15	0.53 ±0.16	2.18 ±0.18	0.24 ±0.11
Kidneys	3.88 ±1.23	4.23 ±0.46	3.61 ±0.91	4.28 ±1.46	4.32 ±0.29	4.38 ±0.87	4.80 ±0.99	2.60 ±0.45
Liver	4.11 ±1.41	4.68 ±0.41	2.89 ±0.30	3.02 ±0.52	2.98 ±0.43	2.67 ±0.42	4.94 ±1.09	3.58 ±0.23
Gut & Feces	3.05 ±0.65	2.90 ±0.35	1.55 ±0.11	1.14 ±0.30	1.18 ±0.16	0.82 ±0.20	2.97 ±0.23	0.45 ±0.08
Stomach	1.36 ±0.18	1.84 ±0.36	0.82 ±0.13	0.59 ±0.18	0.57 ±0.07	0.57 ±0.14	2.08 ±0.29	0.42 ±0.20
Spleen	1.92 ±0.74	2.58 ±0.39	1.02 ±0.17	0.97 ±0.27	1.03 ±0.13	0.84 ±0.24	2.72 ±0.82	1.01 ±0.38
Gonads	1.95 ±1.01	2.63 ±1.80	0.75 ±0.13	0.75 ±0.31	0.89 ±0.19	0.65 ±0.20	2.00 ±0.41	0.17 ±0.10
Heart	2.85 ±0.95	3.38 ±0.32	1.45 ±0.42	1.12 ±0.41	1.18 ±0.23	0.78 ±0.23	3.90 ±1.00	0.30 ±0.06
Lungs	5.93 ±2.01	5.61 ±2.14	2.36 ±0.36	2.39 ±0.77	1.98 ±0.23	1.45 ±0.43	8.56 ±2.72	0.81 ±0.22

6 mice in each group. Mean ± 1 S.D.

TABLE 3

Pyrophosphate (Carrier effect)2 Hr. Post-injection% Injected dose/organ

	⁶⁷ Ga-PYP No added carrier	0.1mg/kg	0.5mg/kg	1mg/kg	5mg/kg	10mg/kg	⁶⁸ Ga-PYP No carrier	^{99m} Tc PYP
Bone	18.33 ±2.49	22.01 ±6.21	23.80 ±2.25	24.63 ±4.52	38.83 ±3.53	22.98 ±3.91	23.31 ±1.39	27.77 ±6.86
Blood	9.25 ±2.74	10.21 ±4.07	6.27 ±2.13	4.51 ±1.19	3.76 ±0.88	3.33 ±1.16	15.97 ±3.59	1.24 ±0.34
Muscle	17.87 ±2.08	20.46 ±2.79	11.21 ±1.77	8.46 ±0.58	8.89 ±2.38	4.92 ±1.83	22.77 ±2.30	2.23 ±0.74
Kidneys	1.66 ±0.46	1.65 ±0.36	1.67 ±0.31	1.88 ±0.42	1.85 ±0.26	2.13 ±0.43	2.06 ±0.36	1.21 ±0.31
Liver	6.24 ±1.03	6.43 ±1.19	5.34 ±1.00	5.44 ±0.83	4.56 ±0.26	4.24 ±0.86	7.04 ±1.88	5.39 ±0.69
Gut & Feces	11.63 ±2.22	11.25 ±1.22	6.37 ±0.82	4.50 ±0.71	5.04 ±1.22	2.81 ±0.81	12.41 ±0.82	1.59 ±0.16
Stomach	0.79 ±0.12	0.88 ±0.16	0.36 ±0.05	0.25 ±0.03	0.24 ±0.04	0.16 ±0.06	0.86 ±0.13	0.15 ±0.02
Spleen	0.18 ±0.05	0.23 ±0.07	0.16 ±0.05	0.13 ±0.03	0.12 ±0.04	0.10 ±0.02	0.28 ±0.08	0.10 ±0.03
Gonads	0.18 ±0.07	0.23 ±0.05	0.12 ±0.05	0.12 ±0.02	0.14 ±0.07	0.07 ±0.02	0.28 ±0.07	0.02 ±0.01
Heart	0.25 ±0.06	0.28 ±0.08	0.18 ±0.05	0.10 ±0.02	0.09 ±0.02	0.07 ±0.02	0.30 ±0.07	0.03 ±0.01
Lungs	0.87 ±0.40	0.68 ±0.19	0.43 ±0.11	0.37 ±0.10	0.35 ±0.08	0.24 ±0.09	1.10 ±0.28	0.10 ±0.03
Tail	3.39 ±1.36	2.96 ±1.47	2.33 ±0.58	1.94 ±0.85	2.80 ±0.52	2.03 ±0.28	3.83 ±1.11	1.47 ±0.43
Total	70.64	77.26	58.22	52.33	66.67	43.05	90.20	41.30

6 mice in each group. Mean ± 1 S.D.

TABLE 4

Pyrophosphate (Carrier effect)2 Hr. Post-injection% Injected dose/gm.

	⁶⁷ Ga-PYP No added carrier	0.1mg/kg	0.5mg/kg	1mg/kg	5mg/kg	10mg/kg	⁶⁸ Ga-PYP No carrier	^{99m} Tc PYP
Bone	7.50 ±1.80	9.29 ±2.80	8.87 ±1.00	9.27 ±2.03	14.70 ±1.38	12.10 ±1.70	8.43 ±0.75	9.95 ±2.26
Blood	5.46 ±1.95	6.04 ±1.96	3.31 ±1.10	2.41 ±0.64	2.03 ±0.45	1.23 ±0.37	8.14 ±1.98	0.64 ±0.20
Muscle	1.68 ±0.19	2.02 ±0.37	0.97 ±0.18	0.74 ±0.06	0.78 ±0.22	0.42 ±0.15	1.90 ±0.21	0.19 ±0.07
Kidneys	3.57 ±1.11	3.82 ±0.55	3.27 ±0.37	3.59 ±0.48	4.00 ±0.24	4.29 ±0.73	4.26 ±0.73	2.36 ±0.64
Liver	3.94 ±1.18	4.27 ±0.50	3.20 ±0.54	3.14 ±0.30	2.86 ±0.22	2.40 ±0.21	4.62 ±1.26	3.11 ±0.48
Gut & Feces	3.62 ±1.01	3.32 ±0.31	1.71 ±0.25	1.28 ±0.19	1.48 ±0.34	0.77 ±0.18	3.39 ±0.30	0.46 ±0.07
Stomach	1.83 ±0.33	1.79 ±0.30	0.78 ±0.16	0.65 ±0.09	0.58 ±0.07	0.46 ±0.14	2.03 ±0.24	0.34 ±0.10
Spleen	1.46 ±0.55	1.90 ±0.51	0.98 ±0.34	0.91 ±0.13	1.08 ±0.14	0.69 ±0.08	2.19 ±0.73	0.88 ±0.31
Gonads	1.14 ±0.31	1.55 ±0.22	0.81 ±0.24	0.89 ±0.36	1.11 ±0.64	0.49 ±0.19	1.76 ±0.51	0.13 ±0.04
Heart	1.87 ±0.31	2.37 ±0.53	1.29 ±0.33	0.83 ±0.12	0.73 ±0.15	0.51 ±0.13	2.49 ±0.63	0.23 ±0.07
Lungs	3.90 ±0.82	3.71 ±0.52	2.08 ±0.50	1.76 ±0.34	1.68 ±0.38	1.00 ±0.27	5.68 ±1.02	0.53 ±0.10

6 mice in each group. Mean ± 1 S.D.