Bone Accumulation of the Tc-99m Complex of Carbamyl Phosphate and Its Analogs

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Carbamyl phosphate, an organic molecule containing a single phosphate group, has been used in the therapy of sickle-cell disease. Carbamyl phosphate bound Tc-99m and achieved bone uptake in mice, rabbits, and a human volunteer. By examination of the structural formula, a working hypothesis was developed that predicted that the Tc-99m complexes of the analogous compounds acetyl phosphate, propionyl phosphate, and butyryl phosphate, each carrying single phosphate and carbonyl groups, would also show bone specificity. This was confirmed experimentally. Phosphonoacetic acid is a structural analog of these compounds. The structural analysis also predicted that aminomethylphosphonic acid and phosphoenolpyruvate would not have as avid bone affinity, and this was also confirmed. These compounds represent a new class of bone-seeking agents that have the common properties of a lone phosphate and a carbonyl function. Such agents may permit the synthesis of additional analogs in an effort to obtain optimal affinity in the Tc-99m complexes.

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In sickle-cell disease, red-cell deformation can occur at low oxygen tensions. The mechanism appears related to intramolecular bonding within hemoglobin S. One approach to the prevention of this bonding has been the attempted chemical modification of hemoglobin by use of agents such as carbamyl phosphate (*1,2*). In 1976 we therefore began a program to investigate the binding of Tc-99m by carbamyl phosphate. The resultant complex turned out to be a bone-seeking agent. Carbamyl phosphate [$H_2N \cdot CO \cdot OPO \cdot (OH)_2$] contains but a single phosphate group as contrasted with the two phosphates or phosphonates in currently used bone-imaging agents such as pyrophosphate, imidodiphosphate and diphosphonate. We therefore constructed a working hypothesis to indicate which groupings on the carbamyl phosphate molecule were necessary for the bone affinity of the Tc-99m complex. This hypothesis was tested by studying a series of analogs.

MATERIALS AND METHODS

The disodium salt of carbamyl phosphate, aminomethylphosphonic acid, and phosphoenolpyruvate were used as obtained commercially. Acetyl phosphate was purchased as the mixed lithium-potassium salt; the latter ions were exchanged for hydrogen before use by means of a 50W-X2 resin*. Propionyl phosphate and butyryl phosphate were synthesized from silver phosphate and the corresponding acid chloride by the method of Lipmann and Tuttle (*3*). The compounds were used directly as the silver salts.

The chemicals were labeled with Tc-99m in the presence of stannous chloride. A solution of the compound of interest (0.1% by weight in isotonic saline) was mixed with stannous chloride solution (0.1% by weight in 0.1*N* HCl) in the ratio of 10:1. The pH of the product was adjusted between 5 and 6. It was then purged with nitrogen gas and finally passed through a 0.22-μm filter. To 1 ml of the resultant solution was added 1 ml of the eluate of a pertechnetate generator that contained 1–30 mCi of radio-

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TABLE 1. STRUCTURAL FORMULAE OF THE TEST COMPOUNDS AS RELATED TO BONE UPTAKE OF THE Tc-99m COMPLEXES*

Formula	Name	Bone uptake of Tc-99m complex?
$H_2N-CH-PO(OH)_2$ (H)	Aminomethyl-phosphonic acid	No
$HO-C(=O)-C(=CH_2)-O-PO(OH)_2$	Phosphoenol-pyruvate	No
$H_2N-C(=O)-O-PO(OH)_2$	Carbamyl phosphate	Yes
$H_3C-C(=O)-O-PO(OH)_2$	Acetyl phosphate	Yes
$H_3C-H_2C-C(=O)-O-PO(OH)_2$	Propionyl phosphate	Yes
$H_3C-H_2C-H_2C-C(=O)-O-PO(OH)_2$	Butyryl phosphate	Yes
$HO-C(=O)-CH-PO(OH)_2$ (H)	Phosphono-acetic acid	Yes†

* The structure of phosphonoacetic acid is shown for comparison.
† Reported by Kung and associates (4).

activity. As a reference material in the biologic studies, we used methylene diphosphonate, prepared exactly as the other agents were. Each radiopharmaceutical was then tested by chromatography for free pertechnetate. Two systems were used. The first was Whatman No. 1 paper in the ascending direction, using 85% methanol. The second was a silical-gel plate†. This was chromatogrammed first in acetone and then, after air drying, in 0.9% saline. [^{99m}Tc] pertechnetate was used as a reference.

Biologic evaluations were carried out in mice for each of the compounds. Studies with carbamyl phosphate were extended to three 2-kg albino rabbits and a male human volunteer. Swiss-Webster mice (5 per compound) were injected intravenously with 0.2 ml of the tracer solution. Mice were killed 2 hr after i.v. administration of the radiolabeled materials. Blood, the femurs, and various organs were obtained. After weighing, each sample was counted for radioactivity and compared with an aliquot of the injected dose diluted in a known volume of saline. The percentage of injected dose per organ was calculated. In the rabbits and the human volunteer, multiple blood samples were also obtained. Imaging was done with a gamma camera and a rectilinear scanner.

RESULTS

Methylene diphosphonate and the test compounds each bound Tc-99m, as shown chromatographically. That is, there was no evidence of free pertechnetate in either system. The structural formulae are shown in Table 1. The distribution of radioactivity for each test compound, in mice, is shown in Table 2. Aminomethyl phosphonate and phosphoenol pyruvate had bone uptake less than half that of the other complexes. In rabbits, there was also bone uptake of radioactivity after injection of the Tc-99m-carbamyl phosphate complex. This was shown by imaging (Fig. 1) as well as by autopsy data (Table 3). In the three rabbits, the mean blood levels of radioactivity were 14.3% of the injected dose at 10 min, 5.8% at 30 min, and 4.2% at 60 min. In the human

TABLE 2. DISTRIBUTION OF RADIOACTIVITY IN MICE 2 HR AFTER I.V. ADMINISTRATION OF THE Tc-99m COMPLEX OF THE INDICATED COMPOUND (FIVE MICE EACH)*

	Femurs	Blood	Liver	Kidneys	G.I.	Carcass	Lung
Methylene diphosphonate	1.85 ± 0.15	0.36 ± 0.13	0.29 ± 0.08	0.38 ± 0.03	1.58 ± 1.22	22.87 ± 5.22	0.14 ± 0.02
Aminomethyl phosphonate	0.79 ± 0.05	2.63 ± 1.27	3.42 ± 2.42	2.27 ± 1.94	4.12 ± 2.21	17.73 ± 1.41	0.15 ± 0.10
Phosphoenolpyruvate	0.73 ± 0.04	7.57 ± 3.67	2.62 ± 3.40	4.55 ± 0.97	2.36 ± 1.19	21.88 ± 2.96	0.39 ± 0.12
Carbamyl phosphate	2.07 ± 0.17	2.19 ± 0.35	1.24 ± 0.23	1.92 ± 0.59	1.65 ± 0.57	26.36 ± 7.85	0.13 ± 0.04
Acetyl phosphate	1.86 ± 1.10	3.38 ± 1.95	9.37 ± 5.55	2.26 ± 0.80	3.51 ± 1.47	31.0 ± 7.17	0.24 ± 0.09
Propionyl phosphate	1.72 ± 0.44	1.83 ± 0.38	0.68 ± 0.16	1.13 ± 0.23	1.83 ± 0.62	20.42 ± 3.94	0.12 ± 0.03
Butyryl phosphate	2.22 ± 0.29	1.81 ± 0.24	2.00 ± 0.22	1.17 ± 0.24	1.58 ± 0.56	21.95 ± 0.71	0.14 ± 0.01

* Mean ± s.d.

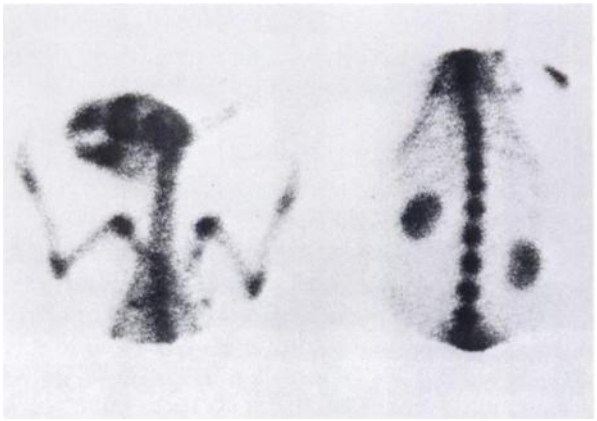

FIG. 1. Gamma camera images of a rabbit, obtained 2 hr after i.v. administration of Tc-99m carbamyl phosphate. Left hand view, of skull and thorax, was taken anteriorly. On right is posterior view of thorax and abdomen.

TABLE 3. MEAN VALUES FOR RADIOACTIVITY IN RABBIT TISSUES (THREE ANIMALS), 2 HR AFTER I.V. ADMINISTRATION OF Tc-99m CARBAMYL PHOSPHATE

	Percentage of administered dose per organ (mean)
Femurs	5.56
Blood	2.67
Liver	1.08
Kidneys	1.94
Lung	0.12
Heart	0.06

volunteer, blood levels of radioactivity were: 2 min, 50.8%; 5 min, 34.4%; 10 min, 27.5%; 30 min, 19.8%; 1 hr, 14.0%; 2 hr, 11.7%; and 3 hr, 10.9%. A posterior rectilinear scan that was started at one hour (Fig. 2) revealed good bone delineation with activity still present in the cardiac blood pool.

DISCUSSION

We had not anticipated the finding of bone accumulation of radioactivity after i.v. administration of the Tc-99m complex of carbamyl phosphate, since the compound has only one phosphate group. When the observation was made, a working hypothesis was developed, based on the structural formula, to indicate which compounds of this type would show similar behavior. The prediction noted two groupings on the molecule:

1. A single phosphate (perhaps replaceable by a phosphonate attached to a small grouping such as CH_2).
2. The carbonyl moiety (=C=O).

On the basis of this analysis, Tc-99m complexes of the carbamyl phosphate analogs (acetyl phosphate, propionyl phosphate, and butyryl phosphate) were predicted to have bone-seeking properties. This was found experimentally (Table 2). During these studies, the Tc-99m complex with phosphonoacetic acid was reported, by Kung and coworkers (*4*), to localize in bone. Phosphonoacetate resembles these compounds but, as shown in Table 1, the –O– grouping between the P and carbonyl is replaced by a CH_2 moiety. Binding of Sn-reduced pertechnetate by the carbonyl phosphates might involve an interaction between the =C=O and –PO $(OH)_2$ groupings, or the cooperative interplay between two or more of the molecules. Loss of the carbonyl grouping (e.g. in phosphoenolpyruvate and aminomethylphosphonic acid) might be expected to result in the loss of bone specificity. This was found experimentally. Some activity is noted in the bones with almost any Tc-99m-based compound due to nonspecific uptake and to reticuloendothelial trapping of any radiocolloid that is formed. Limited studies with aminomethyl phosphonic acid and phosphoenolpyruvate at 1 and 3 hr postinjection in mice, and with the compounds prepared at slightly higher or lower pH values, have failed to show increased uptake in bone.

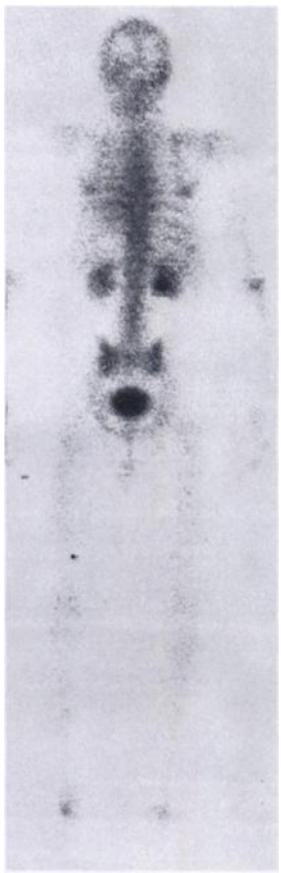

FIG. 2. Posterior rectilinear scan of adult male. Study was begun 1 hr after i.v. administration of Tc-99m carbamyl phosphate. Skeletal system can be identified, as well as activity in cardiac blood pool.

The present formulations of these new Tc-99m complexes appear to have higher blood retention than methylene diphosphonate. However, the study in a male volunteer did not reveal any major red-cell binding of radioactivity (radioactivity in plasma and whole blood fell in parallel). These compounds, of course, suggest further analogs that might be tried in an effort to obtain optimal bone accumulation. The smallest analog would be formyl phosphate $H-\overset{O}{\overset{\|}{C}}-O-PO(OH)_2$. Although this compound has been referred to in the literature, an isolated stable preparation does not seem to have been made.

FOOTNOTES

* Bio-Rad AG, Richmond, Calif.
† Baker-flex®.

ACKNOWLEDGMENT

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