In Vivo Stability and Distribution of [^{131}I]Iodomethyl Trimethylammonium Chloride: Concise Communication

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[^{131}I]Iodomethyl trimethylammonium chloride (I-131 TMA) was prepared by the isotope-exchange reaction of diiodomethane with Na^{131}I, followed by reaction with trimethylamine. A specific activity of 20 μCi/mg was obtained.

Tissue distributions following intravenous injection of I-131 TMA in mice showed that between 10 min and 2 hr, the highest accumulations of radioactivity were in the urine, kideny, and heart. The uptake of this compound in the heart was very rapid, and the levels of radioactivity remained fairly high up to 2 hr. At 10, 30, 60, and 120 min the heart-to-blood ratios were, respectively, 10.2, 7.7, 8.3, and 9.6.

Thyroid uptake of this compound in the rats and analysis of urine samples of the mouse indicated no extensive deiodination of the compound in vivo.

These results show that this compound does have potential for use in myocardial imaging. In addition, its stability in vivo makes it very useful for the radiolabeling of compounds containing quaternary ammonium moieties.

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One of the major problems associated with radioiodinated compounds is the susceptibility of compounds such as 19-iodocholesterol (*1*) and 16-iodo-9-hexadecenoic acid (*2*) to undergo in vivo deiodination. The stability of the iodinated compounds can be greatly increased by attaching iodine to aromatic rings, e.g., *o*-iodohippurate (*3*) and iodinated bretylium analogs (*4*). However, not all the compounds can be labeled with radioiodine attached to aromatic rings. To introduce an aromatic ring that is not an original entity of a molecule could in many instances lead to a complete change of biological activity of the compound. Recently a radioiodinated heterocyclic compound, ortho-iodosophenylphosphoric acid (*5*) was found to be relatively stable in vivo. Nevertheless, the application of this class of heterocyclics as radiopharmaceuticals is quite limited.

Several monocationic ions, such as Cs, Rb, K, and Tl, seem able to accumulate intracellularly in the myocardium by an ion-exchange mechanism. Recently [^{13}N]-ammonia has been used in myocardial imaging (*6, 7*). It was also shown that high myocardium-to-liver ratios were obtained with radioiodinated bretylium analogs in several species (*4*). As a result, we reasoned that the combination of the above structural features would produce an ion such as [^{131}I]iodomethyl trimethylammonium (*8*) that would have potential for myocardial imaging.

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MATERIALS AND METHODS

Chemistry. Iodomethyl trimethylammonium iodide was converted to the chloride form by elution with water through an anion-exchange column (AG1 × 8, Cl^- form, 100–200 mesh, 20 × 5 mm). The chloride had a mp of 177–178°C and gave the following elemental analysis (%): Calculated for $C_4H_{11}ClN$: C, 20.40; H, 4.71; N, 5.95. Found: C, 20.64; H, 4.86; N, 6.12.

[^{131}I]iodomethyl trimethylammonium chloride (I-131 TMA, Fig. 1) was prepared by the isotope-exchange reaction of diiodomethane (15 mg) with Na^{131}I (1 mCi in ~0.1 *M* NaOH solution) in methyl ethyl ketone (1 ml)

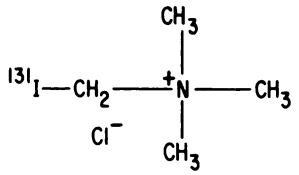

FIG. 1. Structure of [^{131}I]iodomethyl trimethylammonium chloride.

at 110°C for 4 hr, followed by reaction of the mixture with 25% trimethylamine in methanol (0.6 ml) at 60°C for 2 hr. To prevent any escape of elemental radioiodine, an activated charcoal filter was attached to the top of the condenser and the reaction was run in a well-ventilated hood. After removal of the solvent under a stream of nitrogen, the iodide anion was converted to chloride by elution with water through an anion-exchange column. The final volume of the solution was made up to 3 ml with water. The total radiochemical yield was 30%, and a specific activity of 20 μCi/mg (100 μCi/ml) was obtained. Higher specific activity could be obtained by using more concentrated Na^{131}I. For example, a specific activity of 160 μCi/mg was achieved with the same conditions except that 10 mCi of Na^{131}I was used. The purity of the labeled compound was ascertained by thin layer chromatography (TLC) on plastic sheets coated with cellulose, using two different solvent systems. A single peak was obtained on the radiochromatogram and was coincident with the single spot obtained with the authentic unlabeled compound. The R_f of TMA was 0.65 in methanol, and 0.88 in water:methanol (1:1).

Animal studies. Tissue distributions with I-131 TMA were determined on ICR white female mice (20–25 g) or Sprague-Dawley male rats (140 g). After filtration of the compound through a Millipore filter (0.22 μm), it was administered through the tail vein of the mouse (2 μCi/mouse) or the rat (6 μCi/rat). Pentobarbital (2 mg) was injected intraperitoneally 5 min before the sacrifice of the mice, and the rats were killed under ether anesthesia. Animals were killed at different time periods, and the major tissues were removed and counted in vials in a well counter. The results were expressed as percentage of the administered dose per gram of wet tissue × grams of body weight. Urine samples were developed on TLC plates in the systems described above, and the radiochromatograms were compared with the original sample. The remaining urine samples were eluted with water through an anion-exchange column (AG1 × 8). The column and the solution collected from the elution were counted in a well counter.

Whole-body clearance studies in the mouse were performed by placing the animal (in a 50-ml polyethylene tube fitted with a perforated cap) on top of the NaI detector of the well counter. The mouse was counted for radioactivity at different periods and then was returned to the mouse cage.

RESULTS

Tissue distributions in mice with I-131 TMA (Table 1) showed that between 10 min and 2 hr the highest ac-

TABLE 1. TISSUE DISTRIBUTION IN MICE* WITH I-131 TMA (BODY WT IN g × % DOSE/g TISSUE—MEAN AND RANGE)

Tissue	10 min	30 min	60 min	120 min
Blood	29.36 (25.99–34.94)	24.01 (19.84–26.70)	23.11 (21.21–24.09)	15.77 (12.65–18.77)
Liver	200.45 (161.03–234.69)	144.90 (127.78–178.5)	138.60 (104.72–176.90)	103.41 (79.25–120.61)
Lung	116.71 (103.09–139.91)	77.35 (65.58–86.1)	74.17 (68.38–77.49)	74.14 (44.18–93.56)
Kidney	775.31 (655.61–861.08)	779.45 (683.41–888.3)	728.57 (496.23–1117.33)	676.48 (436.01–939.23)
Heart	299.93 (295.01–308.92)	184.53 (149.80–204.6)	191.29 (165.76–216.58)	152.00 (92.35–208.66)
Spleen	27.81 (67.52–82.50)	77.41 (64.86–87.00)	73.01 (69.51–78.3)	57.49 (39.29–73.97)
Stomach	38.14 (25.99–48.64)	20.53 (7.26–32.7)	22.82 (3.11–39.51)	28.84 (12.65–38.09)
Brain	26.08 (21.91–34.18)	20.51 (16.21–23.7)	18.21 (16.84–25.83)	13.30 (7.99–16.28)
Urine†		1095.03 (1022.45–1167.6)		1230.75 (662.4–1799.09)

* Three mice per group.
† Two mice per group.

cumulations of radioactivity were in the kidney, urine, and heart. The uptake of this compound in the heart was very rapid, and the levels of radioactivity remained fairly high up to 2 hr. However, the radioactivity in the blood was relatively low and showed little change during the periods studied. At 10, 30, 60, and 120 min, the levels in the heart were, respectively, 299.93, 184.53, 191.29, 152.00, as percentages of dose per gram of tissue × grams of body weight. These corresponded to heart-to-blood ratios of 10.2, 7.7, 8.3, and 9.6. The very high accumulation of this compound in the kidney and urine was expected, since most ammonium compounds are known to be excreted through the kidney by the urine.

There appeared to be no loading dose effect, since injection of only 1/20 of the original dose used for the distribution studies did not show any significant change in the distribution profile at 10 min after injection.

Whole-body clearance studies with a mouse showed that less than 1% of the injected activity was retained 24 hr after injection.

Iodine-131 TMA in water did not show any significant decomposition after dark storage in the refrigerator for a month. Table 2 shows the uptake of radioactivity in the thyroid of rats. A thyroid-to-blood ratio of 6.1 at 30 min and 23.7 at 2 hr indicated that no extensive in vivo deiodination occurred in the rats. TLC and radiochromatograms of mouse urine samples taken at 0.5 and 2 hr after injection showed no detectable iodide or other metabolites. Chromatography of the urine samples through anion-exchange columns also confirmed the above results. Only 1.3% (30-min samples) and 1.5% (2-hr samples) of the total activity in the urine was retained in the columns, presumably as $^{131}I^-$. The majority of the radioactivity was eluted in the solutions and was identified as the original compound.

The mice showed no acute toxicity with nonradioactive TMA at the dose level (0.1 mg) used for the distribution studies. The LD_{50} was determined to be approximately 33 mg/kg. Since it was shown earlier that much higher specific activity than that of the injected dose could be obtained, the toxicity effect would be reduced to minimum.

TABLE 2. DISTRIBUTION IN THE THYROID AND BLOOD OF RATS* WITH I-131 TMA (BODY WT IN g × % DOSE /g TISSUE— MEAN AND RANGE)

Tissue	30 min	2 hr
Blood	30.36 (26.46–33.60)	12.59 (11.16–13.3)
Thyroid	185.95 (160.74–224.91)	297.95 (255.44–343.14)

* Three rats per group.

DISCUSSION

The tissue distributions with I-131 TMA confirmed our postulate that this compound did have the propensity to accumulate in the myocardium, possibly by the same ion-exchange mechanism as the other monocationic species. The very little in vivo deiodination of this compound indicates that its in vivo stability is comparable with most of the stable radioiodinated compounds. The significance of our research with this tracer is that the compound not only showed good uptake in the myocardium and had potential for myocardial imaging, but is also promising for radiopharmaceutical synthesis. For example, iodomethyl ammonium can be introduced into many drugs or biologically important compounds containing quaternary ammonium moieties (acetylcholine, hexamethonium, bretylium, etc.) and it may be used for studying the metabolism of these related drugs. Furthermore, the substitution of other radiohalogens for I-131 is also possible. We hope that this type of labeling will involve minimal alterations of chemical structures and biological activities.

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