No-Carrier-Added Iodine-131-FIBG: Evaluation of an MIBG Analog

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The purpose of this study was to evaluate the properties of 4-fluoro-3-[^{131}I]iodobenzylguanidine ([^{131}I]FIBG), a potential neuroendocrine tumor and myocardial imaging radiopharmaceutical. **Methods:** The binding of [^{131}I]FIBG and [^{125}I]MIBG was compared in vitro using the SK-N-SH human neuroblastoma cell line. The role of the active uptake-1 mechanism was investigated by determining the effect on cell binding of desipramine (DMI), ouabain, norepinephrine (NE), unlabeled MIBG and FIBG and by incubation at 4°C. Finally, the tissue distributions of [^{131}I]FIBG and [^{125}I]MIBG were compared in normal mice. **Results:** The specific binding of [^{131}I]FIBG remained fairly constant (45%–60%) over a 2-3-log activity range and consistently was 11%–14% higher ($p < 0.05$) than that of [^{125}I]MIBG. The uptake of [^{131}I]FIBG was reduced to 13% of control values by 1.5 μM DMI, to 31% by 1 mM ouabain, to 8% by lower temperature, to 8% by 50 μM NE and to 6% and 5% by 10 μM each of unlabeled MIBG and FIBG, respectively. The amount of [^{131}I]FIBG retained by SK-N-SH cells was significantly higher than that of [^{125}I]MIBG with the maximum difference observed at 72 hr. In mice, the uptake of [^{131}I]FIBG was higher than that of [^{125}I]MIBG not only in target tissues (heart and adrenals) but also in many other normal tissues; conversely, thyroidal uptake of [^{131}I]FIBG was 2-3-fold lower than that of [^{125}I]MIBG. The uptake of [^{131}I]FIBG in the heart and adrenals was reduced by DMI. **Conclusion:** Iodine-131-FIBG is an analog of MIBG with prolonged binding to neuroblastoma cells in vitro and retention in the myocardium in vivo.

Key Words: iodine-125-MIBG; iodine-131-FIBG; neuroblastoma; neuroendocrine tumors

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When labeled with iodine radionuclides, the guanethedine analog MIBG is useful for scintigraphy and treatment of neural crest tumors such as pheochromocytoma and neuroblastoma (*1,2*), as well as myocardial imaging (*3,4*). Newer radiolabeling methods and MIBG analogs have been developed in an attempt to enhance the clinical usefulness of this approach. For example, the preparation of MIBG at a no-carrier-added level as a possible means to augment its uptake has been reported (*5,6*). Analogs of MIBG, labeled with radionuclides with physical properties more suitable for some potential imaging and therapy applications, also have been developed (*7–14*).

For imaging, [^{123}I]MIBG in combination with SPECT has advantages compared with planar scintigraphy with [^{131}I]MIBG (*15*). It may be possible to obtain better images by combining the distribution properties of MIBG with the superior imaging capabilities of PET. MIBG analogs labeled with the positron emitters ^{124}I and ^{76}Br have been reported; however, use of these agents for PET has been hindered by suboptimal physical properties and lack of routine availability of these radionuclides. The development of *meta-* and *para*-[^{18}F]fluorobenzylguanidines has been reported, but, compared with MIBG, their specific binding to SK-N-SH human neuroblastoma cells in vitro and their myocardial uptake in mice were significantly lower (*14*). We recently described (*9,10*) the preparation and validation of 4-[^{18}F]fluoro-3-iodobenzylguanidine ([^{18}F]FIBG; Figure 1). Preliminary results indicated that [^{18}F]FIBG is a suitable analog of MIBG and may find application in PET imaging of neuroendocrine tumors and the myocardium.

With regard to the oncologic strategy of using PET as a prelude to radionuclide therapy, utilization of radionuclides of the same element for both diagnosis and treatment would be advantageous, particularly if PET is to be used for dosimetry planning. For example, ^{124}I and ^{131}I, while not ideal, may be a useful pair of radionuclides for labeling MIBG or an MIBG analog with increased retention on neuroblastoma cells. Since our results with [^{18}F]FIBG suggest that [^{131}I]FIBG may offer higher binding to neuroblastoma cells than MIBG itself, we developed a no-carrier-added synthesis of [^{131}I]FIBG from a silicon precursor (*16*). In the current study, we evaluated [^{131}I]FIBG with regard to both its uptake and retention by SK-N-SH human neuroblastoma cells in vitro and its tissue distribution in normal mice.

MATERIALS AND METHODS

All chemicals were purchased from Aldrich Chemical Company except as noted. Desipramine (DMI) and norepinephrine (arterenol; NE) were obtained from Sigma. A previously reported method (*9*) was used to prepare unlabeled 4-fluoro-3-iodobenzylguanidine. Sodium [$^{131/125}$I]iodide in 0.1 N NaOH was supplied by DuPont-New England Nuclear (North Billerica, MA). Iodine-131-FIBG and [^{125}I]MIBG were prepared following previously reported procedures (*5,16*).

Cells and Culture Conditions

The human neuroblastoma cell lines SK-N-SH (uptake-1 positive) and SK-N-MC (uptake-1 negative) (*17*) were purchased from the American Type Culture Collection (Rockville, MD). The incubation medium (JRH Biosciences, Lenexa, KS) was made by mixing 440 ml RPMI 1640, 50 ml of Serum Plus, 5 ml of penicillin-G/streptomycin (5000 U penicillin and 5000 μg streptomycin in 1 ml) and 5 ml glutamine (200 mM in saline).

Paired-Label Binding of Iodine-131-FIBG and Iodine-125-MIBG to SK-N-SH Human Neuroblastoma Cells

The cells were seeded into 24-well plates (4×10^5 cells per well in 500 μl medium) and incubated for 24 hr at 37°C in a 5% CO_2 humidified atmosphere. After removing the medium, fresh medium containing 100 nCi each of [^{131}I]FIBG and [^{125}I]MIBG was added to each well and incubated at 37°C. After 0.5, 1 and 2 hr, the medium was removed and the cells were washed twice with phosphate-buffered saline (PBS). The cells were solubilized with 500 μl of 0.5 N NaOH for 30 min at room temperature and then removed with cotton swabs. The cell-bound activity was counted along with input standards using an LKB 1282 (Wallac, Finland) automated gamma counter with crossover correction for ^{131}I in the ^{125}I counting window. Nonspecific binding was determined either by preincubating cells with desipramine (50 μM) for 30 min before adding the tracers or by measuring binding to the negative cell line SK-N-MC; both methods gave equivalent results. Each

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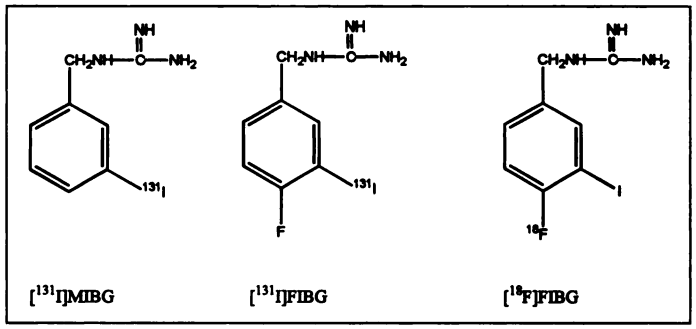

FIGURE 1. Chemical structures of [^{131}I]MIBG, [^{131}I]FIBG and [^{18}F]FIBG.

measurement was performed in quadruplicate. A 2-hr incubation was selected to determine whether tracer uptake was saturable at higher activity levels. Cells were incubated with 7.5–750 nCi each of [^{131}I]FIBG and [^{125}I]MIBG and cell-bound activity was determined as described above.

Specificity of Uptake of Iodine-131-FIBG in SK-N-SH Cells

The cells were seeded into 24-well plates (4×10^5 cells per well in 500 μl medium) and incubated for 24 hr. Before addition of [^{131}I]FIBG (75 nCi), cells were incubated for 30 min at 37°C with 500 μl of medium containing either 1.5 μM DMI or 1 mM ouabain. The potential blocking effects of NE (50 μM), MIBG (10 μM) and FIBG (10 μM) were determined by co-incubating the cells with these agents. All incubations were performed for 2 hr at 37°C. To determine the effect of temperature, cells were equilibrated with 450 μl of medium at 4°C for at least 1 hr. Subsequently, 75 nCi [^{131}I]FIBG were added and incubated for 2 hr at 4°C. In all experiments, the cell-associated activity was determined as described before. The percent binding to the cells was normalized to simultaneously performed incubations without interventional agents (control). Each measurement was performed in quadruplicate.

Paired-Label Binding and Retention of Iodine-131-FIBG and Iodine-125-MIBG in SK-N-SH Cells

SK-N-SH cells (5×10^5 cells per well in 500 μl of medium) in 24-well plates were incubated at 37°C for 24 hr. The medium was replaced with fresh medium containing 150 nCi of each tracer in a total volume of 500 μl per well. The cells were incubated for 2 hr at 37°C, the medium was removed and then supplemented with 500 μl of fresh medium. Cell-bound activity was determined in quadruplicate at 0, 2, 4, 8, 24, 48, 72 and 96 hr. The specificity of cell-associated activity was determined in parallel wells to which 1.5 μM DMI had been added after the initial tracer incubation period.

Biodistribution in Normal Mice

Three experiments were performed. Groups of five BALB/c mice were used for each time point. The mice were killed by an overdose of halothane, tissues of interest were isolated, washed, blot-dried and counted for ^{131}I, and ^{125}I, using a single-label or a dual-label program on the automated gamma counter (LKB 1282). A 5% or 10% aliquot of the injected dose was also counted and the percentage of injected dose per organ or per gram of tissue was computed.

In an initial experiment, 3 μCi each [^{131}I]FIBG and [^{125}I]MIBG were administered and the tissue distribution was determined at 1, 4 and 24 hr. In a subsequent study, 6 μCi of each tracer was injected and the tissue distribution was measured at 1, 2, 3, 6 and 7 days. Finally, the specificity of heart and adrenal uptake of [^{131}I]FIBG was determined at 5 min and 1 hr by pretreatment of the animals with DMI (10 mg/kg in PBS) or PBS i.p. 30 min before tracer injection.

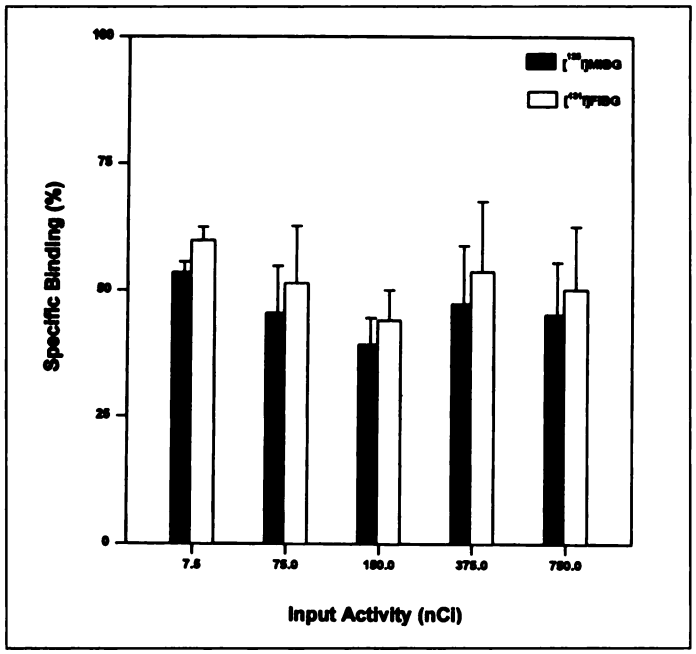

FIGURE 2. Paired-label binding of [^{131}I]FIBG and [^{125}I]MIBG to SK-N-SH human neuroblastoma cells in vitro as a function of activity concentration.

RESULTS

Iodine-131-FIBG Binding to SK-N-SH Neuroblastoma Cells

To determine the optimum time required for maximal binding, an assay was performed in a paired-label format using [^{131}I]FIBG and [^{125}I]MIBG. The uptake was similar for both tracers at all time points and the percent binding appeared to level off at 2 hr. For example, with [^{131}I]FIBG, the percentage activity bound to the cells was 46.5% ± 3.2%, 68.4% ± 1.6% and 81.1% ± 1.0%, at 0.5, 1 and 2 hr, respectively.

The percent uptake of [^{131}I]FIBG and [^{125}I]MIBG by SK-N-SH cells as a function of activity concentration is shown in Figure 2. The binding of [^{131}I]FIBG (45%–60%) was fairly constant over a 2-3-log activity range and was 11%–14% higher than that of [^{125}I]MIBG ($p < 0.05$) at all activity concentrations tested. Nonspecific binding was less than 2% in all cases.

Specificity of Iodine-131-FIBG Uptake by SK-N-SH Cells

The uptake of [^{131}I]FIBG was blocked to varying degrees by several interventional agents and by performing the incubation at 4°C. The uptake-1 inhibitor DMI (1.5 μM) reduced the binding of [^{131}I]FIBG to 13% of the control value. Ouabain (1 mM) and incubation at 4°C reduced the uptake to 31% and 8% of the control value, respectively, suggesting that [^{131}I]FIBG uptake in this cell line is energy-dependent. The specificity of [^{131}I]FIBG uptake is further demonstrated by the reduction of its binding to 8%, 6% and 5% of control value by 50 μM norepinephrine, 10 μM MIBG and 10 μM FIBG, respectively.

Retention of Iodine-131-FIBG and Iodine-125-MIBG by SK-N-SH Cells

As shown in Figure 3, 76% of the originally bound [^{131}I]FIBG activity was retained in SK-N-SH cells after 3 days compared with 30% for [^{125}I]MIBG. Using these binding data, time-activity curves were constructed assuming that both tracers were labeled with ^{131}I. The area under the FIBG time-activity curve extrapolated to infinity was about twice that for MIBG, suggesting a significant advantage in radiation absorbed dose to this cell line might be achievable with [^{131}I]FIBG. Further, it was demonstrated that DMI enhanced the washout of initially bound [^{125}I]MIBG and [^{131}I]FIBG, indicating that tracer retention is mediated by the re-uptake of released activity.

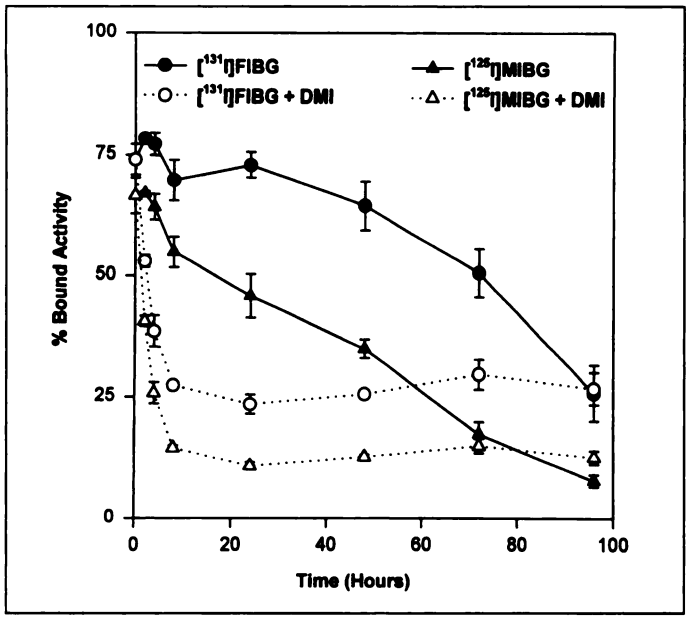

FIGURE 3. Retention of [^{131}I]FIBG and [^{125}I]MIBG by SK-N-SH cells in the absence and presence of DMI, after removal of unbound activity.

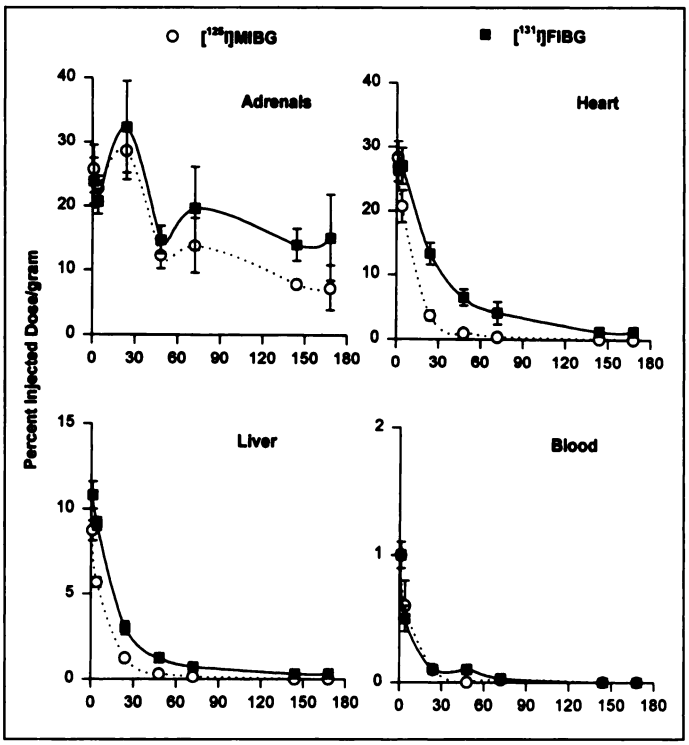

FIGURE 4. Paired-label uptake of [^{131}I]FIBG and [^{125}I]MIBG in mouse heart, adrenals, liver and blood.

Biodistribution Studies

The results of tissue distribution of [^{131}I]FIBG and [^{125}I]MIBG in normal mice are summarized in Table 1 and in Figure 4. High uptake of [^{131}I]FIBG was seen in both heart and adrenals. The [^{131}I]FIBG/[^{125}I]MIBG myocardial uptake ratio increased from slightly less than 1 at 1 hr to 1.3, 3.8 and 12.2 at 4 hr, 1 day and 3 days, respectively. In the adrenals, the uptake of both tracers was similar up to 2 days; however, a 1.4-2-fold higher retention of [^{131}I]FIBG was noted from 3 to 7 days postinjection. Several other tissues also retained [^{131}I]FIBG to a significantly higher degree than [^{125}I]MIBG. A notable exception was the thyroid. At 24 hr, thyroidal uptake of ^{131}I (1.9 ± 0.4% ID/g) was half that of ^{125}I (3.7 ± 1.7% ID/g; $p < 0.05$). On Days 6 and 7, thyroidal retention of ^{131}I was only one-third that of ^{125}I, suggesting that [^{131}I]FIBG is less susceptible to deiodination than [^{125}I]MIBG.

Inhibition of [^{131}I]FIBG accumulation in the heart and adrenals by DMI pretreatment confirmed the specificity of uptake in these tissues. Five minutes after injection, the heart and adrenal uptake of DMI-treated mice was reduced to 80% and 73% of control values, respectively. One hour after tracer injection, DMI reduced the heart and adrenal uptake of [^{131}I]FIBG to 48% and 60% of the control values, respectively. The reductions in heart and adrenal accumulation by DMI were statistically significant ($p < 0.05$).

DISCUSSION

FIBG is an MIBG analog that can be labeled either with ^{18}F or a variety of iodine radionuclides. When labeled with ^{18}F, FIBG exhibited 10%–15% higher specific binding to SK-N-SH

TABLE 1
Paired-Label Tissue Distribution of Radioactivity in Normal Mice after Intravenous Injection of Iodine-131 FIBG and Iodine-125-MIBG

	Percent injected dose per gram of tissue*							
	1 hr		4 hr		1 d		3 d	
Tissue	[^{125}I]MIBG	[^{131}I]FIBG	[^{125}I]MIBG	[^{131}I]FIBG	[^{125}I]MIBG	[^{131}I]FIBG	[^{125}I]MIBG	[^{131}I]FIBG
Liver	8.72 ± 0.56	10.80 ± 0.84	5.68 ± 0.25	9.13 ± 0.39	1.14 ± 0.14	2.98 ± 0.38	0.13 ± 0.03	0.69 ± 0.18
Spleen	5.13 ± 0.60	4.72 ± 0.50	4.72 ± 0.54	4.81 ± 0.48	1.78 ± 0.35	3.54 ± 0.74	0.30 ± 0.14	1.82 ± 0.41
Lungs	13.04 ± 1.76	16.88 ± 2.17	4.40 ± 1.19	6.25 ± 1.68	1.10 ± 0.54	2.50 ± 1.04	0.12 ± 0.05	0.67 ± 0.32
Heart	28.26 ± 2.56	26.67 ± 2.12	20.65 ± 2.52	27.00 ± 2.76	3.54 ± 0.75	13.29 ± 1.77	0.33 ± 0.12	4.03 ± 1.78
Kidney	3.04 ± 0.45	3.09 ± 0.50	2.37 ± 0.14	2.81 ± 0.16	0.80 ± 0.14	1.63 ± 0.30	0.19 ± 0.10	0.64 ± 0.19
Stomach	5.57 ± 1.29	4.64 ± 1.18	3.24 ± 0.63	2.79 ± 0.52	1.72 ± 0.36	2.33 ± 0.63	0.50 ± 0.07	1.09 ± 0.15
Small intestine	7.28 ± 1.00	6.11 ± 1.04	5.07 ± 0.19	4.23 ± 0.20	1.43 ± 1.19	2.22 ± 0.33	0.30 ± 0.34	1.03 ± 1.20
Large intestine	4.08 ± 0.52	3.53 ± 0.45	6.60 ± 0.27	4.96 ± 0.38	1.84 ± 0.19	2.17 ± 0.33	0.20 ± 0.08	0.62 ± 0.15
Thyroid	3.55 ± 0.59	3.18 ± 0.52	3.76 ± 0.69	3.12 ± 0.52	3.70 ± 1.72	1.87 ± 0.44	3.62 ± 1.72	1.38 ± 0.47
Muscle	2.47 ± 0.17	2.17 ± 0.15	1.87 ± 0.20	1.87 ± 0.19†	0.42 ± 0.06	0.70 ± 0.09	0.05 ± 0.01	0.20 ± 0.04
Bone	1.68 ± 0.14	1.66 ± 0.14†	1.23 ± 0.15	1.33 ± 0.12	0.23 ± 0.04	0.34 ± 0.09	0.04 ± 0.04	0.11 ± 0.08
Blood	1.03 ± 0.10	0.98 ± 0.11	0.58 ± 0.23	0.52 ± 0.07†	0.10 ± 0.02	0.12 ± 0.09	0.02 ± 0.01	0.03 ± 0.01
Brain	0.15 ± 0.01	0.15 ± 0.01†	0.11 ± 0.03	0.12 ± 0.02†	0.06 ± 0.06	0.11 ± 0.11	0.01 ± 0.00	0.02 ± 0.01
Adrenals	25.76 ± 3.71	23.87 ± 3.59	22.77 ± 1.98	20.67 ± 1.86	28.61 ± 4.39	32.39 ± 7.13†	13.91 ± 4.27	19.74 ± 6.51

*Mean ± s.d.; n = 5–10 animals per group.
†Differences not statistically significant by paired t-test ($p > 0.05$).

human neuroblastoma cells than MIBG itself (*10*). While this property might be advantageous for imaging, it might be even more useful for therapeutic application, particularly if the higher retention of [^{131}I]FIBG in tumor cells could be maintained for several days. Retention of radionuclide in tumor for a period compatible with their physical half-life is desirable to maximize therapeutic efficacy. With [^{131}I]MIBG, this is not the case, since considerable amounts of [^{131}I]MIBG generally are released from SK-N-SH cells within 24 hr (*8,18–21*). A similar observation has been made with other human neuroblastoma cell lines (*22,23*).

In the current study, FIBG was labeled with ^{131}I and its binding properties compared with MIBG. As observed previously with [^{18}F]FIBG, specific binding of [^{131}I]FIBG to SK-N-SH cells was higher than that of [^{125}I]MIBG. The nonspecific binding of [^{131}I]FIBG, like [^{125}I]MIBG, was only 1%–2%, suggesting that the higher uptake of [^{131}I]FIBG in SK-N-SH cells was not due to its slightly higher lipophilicity (*9*). Perhaps the most significant observation in the current study was the fact about 75% of the [^{131}I]FIBG taken up by SK-N-SH cells remained cell-associated after 3 days compared with about 30% for [^{125}I]MIBG. These results suggest that a higher tumor dose might be achieved by using [^{131}I]FIBG instead of [^{131}I]MIBG. The presence of DMI in the incubation medium enhanced the release of [^{131}I]FIBG, albeit to a lesser degree than observed with [^{125}I]MIBG, suggesting some difference in the mechanisms for cellular retention of the two agents.

It is well established that MIBG is taken up in SK-N-SH cells by an active uptake-1 mechanism (*18,19*). This mechanism is characterized by sodium- and temperature-dependency, high affinity and low capacity, saturability and ouabain- and DMI-sensitivity (*24*). Our results indicate that [^{131}I]FIBG is similar to MIBG in this respect. The tricyclic antidepressant drug DMI, an inhibitor of the uptake-1 mechanism, reduced the uptake of [^{131}I]FIBG to 13% of the control values, a reduction similar to that reported for MIBG (*18*). The specificity of [^{131}I]FIBG uptake was further demonstrated by its ability to be blocked by NE as well as unlabeled MIBG and FIBG. Again, the extent of blocking observed with NE and MIBG was similar to that reported for [^{125}I]MIBG (*18*) and [^{18}F]FIBG (*10*). The energy dependence of [^{131}I]FIBG uptake was demonstrated by the fact that pretreatment with ouabain and incubation at 4°C resulted in substantially lower accumulation in SK-N-SH cells. These data demonstrate that [^{131}I]FIBG, like MIBG, is taken up in SK-N-SH cells through uptake-1 mechanism.

Although not universally accepted, it has been proposed that the polar basic guanidino group of benzylguanidines is responsible for their high affinity interaction with the norepinephrine transporter (*24–26*). If this is the case, then it is not clear why substitution of an aromatic hydrogen by fluorine would result in such significant difference in halobenzylguanidine re-uptake by neuroblastoma cell lines. Recently, it has been proposed that cellular release of MIBG is mediated by a specific carrier, most probably an uptake carrier working in reverse mode (*27*). It is tempting to speculate that FIBG may be a good substrate for the uptake carrier and not for the reverse one. Another reasonable explanation may be differential retention of label on the two tracers in the intracellular environment (vide infra). Regardless of the mechanism of retention, the ability to retain the intact tracers may be similar; however, MIBG and FIBG may be deiodinated at different rates. Experiments are under way to identify the catabolic products of MIBG and FIBG that are retained and released by SK-N-SH cells over time.

Paired-label tissue distribution studies were performed in normal mice to compare the tissue distribution of [^{131}I]FIBG and [^{125}I]MIBG. The initial uptake of both tracers was similar; however, in most tissues, ^{131}I levels were higher than ^{125}I with this difference increasing with time. Differences were most striking in the heart, an organ with an active uptake-1 mechanism, with an FIBG:MIBG uptake ratio of 12 observed at 3 days. Conversely, in the adrenals, another tissue which can accumulate MIBG by an uptake-1 process, only modest differences in ^{125}I and ^{131}I levels were seen. While the reason for the different uptake ratios seen in heart and adrenals is unclear, it should be noted that the clearance of MIBG is relatively fast from the heart and relatively slow in the adrenals, suggesting that different factors influence halobenzylguanidine retention in these tissues.

Although MIBG is considered to be metabolically stable (*28*), our results indicate that FIBG is more inert towards deiodination in vivo than MIBG. This is reflected by the fact that [^{131}I]FIBG uptake was considerably lower than that of [^{125}I]MIBG in the thyroid. Thyroid uptake of radioiodine is frequently used as an indicator of the susceptibility to deiodination of radioiodinated compounds (*29*). The fact that FIBG is less susceptible to deiodination than [^{125}I]MIBG is surprising since the substitution of an aromatic hydrogen by a fluorine should enhance the susceptibility to deiodination if in vivo deiodination goes through the expected nucleophilic displacement pathway.

Future studies will endeavor to determine the labeled catabolic products resultant from the exposure of [^{131}I]FIBG and [^{125}I]MIBG to tissues in vivo and in vitro. Although the increased retention of [^{131}I]FIBG in neuroblastoma cells in vitro is encouraging, the higher normal tissue retention of this agent could be problematic. Further studies in human neuroblastoma xenograft models will be needed to determine whether the integrated dose advantage observed in vitro can be extended to in vivo situations and whether tumor-to-normal tissue radiation absorbed dose ratios for FIBG will be more favorable than those for MIBG.

CONCLUSION

Various in vitro and in vivo assays demonstrated that [^{131}I]FIBG possesses characteristics similar but not identical to MIBG. In vitro retention studies showed a two-fold advantage in the integrated tumor dose that might be achievable with [^{131}I]FIBG compared with [^{131}I]MIBG. However, the practical significance of this difference must be confirmed in human tumor xenograft models because [^{131}I]FIBG is also retained to a greater extent in many normal tissues. Prolonged retention in normal target tissues such as heart and adrenals may be of potential utility in the imaging of these organs at extended time points. This may be of particular relevance for serial PET imaging with [^{124}I]FIBG.

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REFERENCES

1. McEwan AJ, Shapiro B, Sisson J, Beierwaltes WH, Ackery DM. Radioiodobenzylguanidine for the scintigraphic location and therapy of adrenergic tumors. *Semin Nucl Med* 1985;15:132–153.
2. McEwan AJ, Wyeth P, Ackery D. Radioiodinated iodobenzylguanidines for diagnosis and therapy. *Appl Radiat Isot* 1986;37:765–775.
3. Wakasugi S, Wada A, Hasegawa Y, Nakano S, Shibata N. Detection of abnormal cardiac adrenergic neuron activity in adriamycin-induced cardiomyopathy with iodine-125-metaiodobenzylguanidine. *J Nucl Med* 1992;33:208–214.
4. Stanton MS, Tuli MM, Radtke NL, et al. Regional sympathetic denervation after myocardial infarction in humans detected noninvasively using ^{123}I-metaiodobenzylguanidine. *J Am Coll Cardiol* 1988;14:1519–1529.

5. Vaidyanathan G, Zalutsky MR. No-carrier-added synthesis of *meta*-[^{131}I]iodobenzylguanidine. *Appl Radiat Isot* 1993;44:621–628.
6. Vaidyanathan G, Zalutsky MR. No-carrier-added [^{123}I]MIBG: synthesis and preliminary evaluation. *Nucl Med Biol* 1995;22:61–64.
7. Vaidyanathan G, Zalutsky MR. 1-(*m*-[^{211}At]Astatobenzyl)guanidine: synthesis via astato demetalation and preliminary in vitro and in vivo evaluation. *Bioconj Chem* 1992;3:499–503.
8. Vaidyanathan G, Strickland DK, Zalutsky MR. *Meta*-[^{211}At]astatobenzylguanidine: further evaluation of a potential therapeutic agent. *Int J Cancer* 1994;57:908–913.
9. Vaidyanathan G, Affleck DJ, Zalutsky MR. 4-[^{18}F]Fluoro-3-iodobenzylguanidine: a potential MIBG analog for positron emission tomography. *J Med Chem* 1994;37:3655–3662.
10. Vaidyanathan G, Affleck DJ, Zalutsky MR. Validation of 4-[^{18}F]fluoro-3-iodobenzylguanidine as a potential PET analog of MIBG. *J Nucl Med* 1995;36:644–650.
11. Ott RJ, Tait D, Flower MA, Babich JW, Lambrecht RM. Treatment planning for ^{131}I-MIBG radiotherapy of neural crest tumours using ^{124}I-MIBG positron emission. *Br J Radiol* 1992;65:787–791.
12. Loc'h C, Mardon K, Valette H, Brutesco C, Merlet P, Syrota A, Maziere B. Preparation and pharmacological characterization of [^{76}Br]-*meta*-bromobenzylguanidine ([^{76}Br]MBBG). *Nucl Med Biol* 1994;21:49–55.
13. Valette H, Loc'h C, Mardon K, et al. Bromine-76-metabromobenzylguanidine: a PET radiotracer for mapping sympathetic nerves of the heart. *J Nucl Med* 1993;34:1739–1744.
14. Garg PK, Garg S, Zalutsky MR. Synthesis and preliminary evaluation of *para*- and *meta*-[^{18}F]fluorobenzylguanidine. *Nucl Med Biol* 1994;21:97–103.
15. Shulkin BL, Shapiro B, Francis I, Door R, Shew S-W, Sisson JC. Primary extra-adrenal pheochromocytoma positive ^{123}I MIBG imaging with negative ^{131}I MIBG imaging. *Clin Nucl Med* 1986;11:851–854.
16. Vaidyanathan G, Affleck DJ, Zalutsky MR. No-carrier-added 4-fluoro-3-[^{131}I]iodobenzylguanidine and 3-[^{211}At]astato-4-fluorobenzylguanidine. *Bioconj Chem* 1996;7:102–107.
17. Biedler JL, Helson L, Spengler BA. Morphology and growth, tumorigenicity and cytogenetics of human neuroblastoma cells in continuous culture. *Cancer Res* 1973;33:2643–2652.
18. Smets LA, Loesberg C, Janssen M, Metwally EA, Huiskamp R. Active uptake and extravesicular storage of *m*-iodobenzylguanidine in human neuroblastoma SK-N-SH cells. *Cancer Res* 1989;49:2941–2944.
19. Buck J, Bruchelt G, Girgert R, Treuner J, Niethammer D. Specific uptake of m-[^{125}I]iodobenzylguanidine in the human neuroblastoma cell line SK-N-SH. *Cancer Res* 1985;45:6366–6370.
20. Smets LA, Janssen M, Rutgers M, Ritzen K, Buitenhuis C. Pharmacokinetics and intracellular distribution of the tumor-targeted radiopharmaceutical *m*-iodo-benzylguanidine in SK-N-SH neuroblastoma and PC-12 pheochromocytoma cells. *Int J Cancer* 1991;48:609–615.
21. Guerreau D, Thedrez P, Fritsch P, et al. In vitro therapeutic targeting of neuroblastoma using ^{125}I-labeled meta-iodobenzylguanidine. *Int J Cancer* 1990;45:1164–1168.
22. Mairs RJ, Gaze MN, Barret A. The uptake and retention of metaiodobenzylguanidine by the neuroblastoma cell line NB1-G. *Br J Cancer* 1991;64:293–295.
23. Lashford LS, Hancock JP, Kemshead JT. *Meta*-iodobenzylguanidine (MIBG) uptake and storage in the human neuroblastoma cell line SK-N-BE(2C). *Int J Cancer* 1991;47:105–109.
24. Jaques S, Tobes MC, Sisson JC. Sodium dependency of uptake of norepinephrine and m-iodobenzylguanidine into cultured human pheochromocytoma cells: evidence for uptake-one. *Cancer Res* 1987;47:3920–3928.
25. Smets LA, Janssen M, Metwally E, Loesberg C. Extragranular storage of the neuron blocking agent meta-iodobenzylguanidine (MIBG) in human neuroblastoma cells. *Biochem Pharmacol* 1990;39:1959–1964.
26. Smets LA, Bout B, Wisse J. Cytotoxic and antitumor effects of the norepinephrine analog meta-iodo-benzylguanidine (MIBG). *Cancer Chemother Pharmacol* 1988;21:9–13.
27. Servidei T, Iavarone A, Lasorella A, Mastrangelo S, Riccardi R. Release mechanisms of [^{125}I]metaiodobenzylguanidine in neuroblastoma cells: evidence of a carrier-mediated efflux. *Eur J Cancer* 1995;31A:591–595.
28. Mangner TJ, Tobes MC, Wieland DW, Sisson JC, Shapiro B. Metabolism of iodine-131-metaiodobenzylguanidine in patients with metastatic pheochromocytoma. *J Nucl Med* 1986;27:37–44.
29. Zalutsky MR, Narula AS. A method for the radiohalogenation of proteins resulting in decreased thyroid uptake of radioiodine. *Appl Radiat Isot* 1987;38:1051–1055.

Weighted Summation of Oxygen-15-Water PET Data to Increase Signal-to-Noise Ratio for Activation Studies

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Data with the highest possible signal-to-noise (S/N) ratios are desirable when performing nonquantitative perturbation studies with PET and ^{15}O-water. To achieve this, protocols have been suggested in which the stimulus is switched off before the washout phase. An alternative strategy is suggested for cases in which the stimulus is not easily discontinued. **Methods:** For a given subject, a theoretical signal curve is created by simulating tissue time-activity curves for baseline and activated states and their subtraction. The curve is created from a typical arterial curve, and values for delay and flow are estimated for that subject. When summing the activity data before image reconstruction, the values from the signal curve are used as weights. Thus, data with high information content regarding changes in blood flow are given a large weight, and data with less information are given a smaller weight. The method is examined by simulations, and the results are validated by application to data from 10 individuals from an activation study. **Results:** Simulations show that the S/N ratio peaks for a given summation time and then decline for longer times when performing a straight summation of data. This time is not constant and varies both with the whole brain flow level and the magnitude of the activation. When using weighted summation on the other hand, the S/N ratio approaches asymptotically its optimal value. The optimal S/N value for weighted summation is 5%–10% higher than the peak value obtained with straight summation. The results are confirmed by the experimental data, indicating a shift in optimal summation time from 60–100 sec and an increase by 6% in the S/N ratio for weighted compared to straight summation. **Conclusion:** The method presented in this paper offers a way to significantly increase the S/N ratio in ^{15}O-water perturbation studies without increasing invasiveness or complicating the experimental protocol.

Key Words: PET; activation study; signal-to-noise ratio

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PET studies with ^{15}O-water are a powerful tool to study altered cerebral blood flow as a consequence of physiological activation (*1*), pharmacological perturbation (*2*) or physiological or pathophysiological state (*3*). The methodology is based on the detection of regional differences between two or more scans in which the subject is in different states. Absolute quantification of the magnitude of change requires arterial sampling (*4,5*) and is therefore often sacrificed to enable simpler and less invasive procedures (*6*). There has been, however, some controversy about which integration time to use for optimal S/N ratios with respect to changes in blood flow. Time suggestions range from 60 to 100 sec after the arrival of the bolus to the brain (*7,8*). While the noise level tends to decrease with increased integration time, the signal will peak

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