No-Carrier-Added Iodine-131-MIBG: Evaluation of a Therapeutic Preparation

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Iodine-131-metaiodobenzylguanidine ([^{131}I]MIBG) is a radiopharmaceutical for imaging as well as targeted radiotherapy of neuroblastoma. It is predicted that the use of no-carrier-added [^{131}I]MIBG, rather than the conventional low specific activity preparation, will result in an enhanced therapeutic ratio because of different transport processes in neuroblastoma compared with most normal tissues. **Methods:** The main aims of the study were: (1) to determine whether [^{131}I]MIBG of substantially greater specific activity is transported into tumor cells by the same process as the existing compound; (2) to evaluate the effect of nonradiolabeled MIBG on the cytotoxicity of no-carrier-added [^{131}I]MIBG; and (3) to compare the biodistribution of both preparations of the radiochemical in neuroblastoma xenografts. **Results:** Active uptake of no-carrier-added [^{131}I]MIBG was temperature-, sodium- and oxygen-dependent; ouabain- and desmethylimipramine-inhibitable; and could be blocked competitively by monoamine inhibitors of the noradrenaline transport mechanism. The rank order of specific uptake capacity in a panel of neuroblastoma cell lines was the same for both low and high specific activity drug. Neuroblastoma spheroid regrowth was 85% inhibited by no-carrier-added [^{131}I]MIBG at 2 MBq · ml^{-1}. Inhibitory potency was reduced in a dose-dependent manner by nonradiolabeled MIBG. The accumulation of no-carrier-added [^{131}I]MIBG was significantly greater in tumor, adrenal, heart and skin of tumor-bearing mice than that of the conventional therapy preparation of [^{131}I]MIBG. **Conclusion:** These data indicate that there may be clinical advantages in the use of no-carrier-added [^{131}I]MIBG rather than conventional [^{131}I]MIBG.

Key Words: MIBG; neuroblastoma; no-carrier-added radiopharmaceuticals

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Metaiodobenzylguanidine (MIBG) is an analog of the adrenergic neuron blockers bretylium and guanethidine (*1*). It is actively transported by a process known as uptake-1 into cells of the adrenal medulla, adrenergic nerve cells and tumors such as pheochromocytoma and neuroblastoma, which form in the neural crest. When suitably radioiodinated, MIBG can be used for imaging (*2,3*) as well as for targeted radiotherapy (*4,5*). Although early studies of patients with recurrent or refractory neuroblastoma treated with [^{131}I]MIBG have been encouraging (*6*), it appears unlikely that [^{131}I]MIBG used alone will be curative (*7*).

Commercial preparation of [^{131}I]MIBG involves radioiodination by halogen exchange (*8*). The final product from this synthetic route incorporates a large excess of nonradiolabeled MIBG. Indeed, the ratio of radioiodine-conjugated to stable iodine-conjugated molecules is only about 1:2000. For a given radioactivity, no-carrier-added [^{131}I]MIBG preparations would contain only a maximum of 0.05% of the molar amount of drug found in [^{131}I]MIBG solutions prepared by iodide exchange (*9*). This has implications both for diagnostic and therapeutic applications of [^{131}I]MIBG (*10*).

Tumor uptake of MIBG occurs by two processes: specific, high affinity, active transport (uptake-1) and passive diffusion (uptake-2). Most normal tissues, with the exception of the adrenal medulla and sympathetically innervated organs such as the heart, absorb the drug by uptake-2. At low concentrations of MIBG, uptake-1 makes a greater contribution than uptake-2 to overall tumor accumulation of radiopharmaceutical (*11*). Therefore, there should be a greater difference in uptake between target and non-target tissue when a given activity of [^{131}I]MIBG is administered using no-carrier-added radiopharmaceutical rather than a conventional form. This is because the radioactivity of no-carrier-added [^{131}I]MIBG is carried by the minimum number of molecules of drug, whereas the standard preparation contains excess cold MIBG. This hypothesis (*10*) is supported by the observations that the cardiac uptake of [^{123}I]MIBG in rats is dependent on the specific activity (*12*), and that greater cytotoxicity is achieved in the cell line SK-N-SH with specific activities of about 1 GBq/mg^{-1} [^{131}I]MIBG than with the same total activity of [^{131}I]MIBG at a specific activity of 100-fold lower (*13*).

An indication of the clinical benefit that might accrue if no-carrier-added [^{131}I]MIBG were used instead of the con-

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ventional preparation comes from murine biodistribution experiments (*10*). Using the adrenals (which concentrate MIBG by uptake-1) as a target tissue and the liver as a typical non-target organ (which has no capacity for uptake-1), it is possible to calculate target to nontarget ratios. The adrenal-to-liver ratio of [^{131}I]MIBG 24 hr after injection was found to be 7.54 for the iodide exchange preparation, and 28.0 for no-carrier-added [^{131}I]MIBG. This fourfold improvement in target-to-nontarget ratio, although very encouraging, clearly needs to be confirmed in human neuroblastoma xenografts before the new preparation is tested clinically.

The aim of this study was to further evaluate the clinical potential of no-carrier-added [^{131}I]MIBG. The in vitro and in vivo pharmacokinetics of no-carrier-added [^{131}I]MIBG have been characterized in neuroblastoma cell lines and murine xenografts, and compared with those of [^{131}I]MIBG prepared by iodide exchange. The relative cytotoxicity of these two preparations has been determined in vitro.

MATERIALS AND METHODS

Synthesis of [^{131}I]MIBG. No-carrier-added [^{131}I]MIBG was synthesized by iododesilylation of meta-trimethylsilylbenzylguanidine according to the method of Vaidyanathan and Zalutsky (*10*) and purified using HPLC and solid-phase extraction (*9*). Iododesilylation provides a well-validated route for incorporation of radioiodine into organic compounds (*14–17*). The no-carrier-added [^{131}I]MIBG was reconstituted for use in phosphate-buffered saline after evaporation of its methanolic extracts in a stream of sterile nitrogen. The precursor for the radiolabeling procedure, metatrimethylsilylbenzylguanidine, was kindly supplied by Dr. Ganeshan Vaidyanathan (Department of Radiology, Duke University, Durham, NC). All other chemical reagents were obtained from Aldrich Chemical Co. (Dorset, UK). HPLC-grade solvents were purchased from Rathburn Chemicals Ltd. (Peebleshire, UK). Iodine-131-sodium iodide and commercial, exchange-labeled [^{131}I]MIBG were supplied by Amersham International (Buckinghamshire, UK). Nonradiolabeled MIBG was synthesized from MIBG according to the method of Wieland et al. (*1*).

Radiopharmaceutical Stability. An initial study of the stability of no-carrier-added [^{131}I]MIBG in phosphate-buffered saline was undertaken to ensure that the material used in the cell and animal experiments was predominantly (>95%) authentic [^{131}I]MIBG at time of use.

The effect of two different storage conditions, 20°C and –20°C, on the rate of deiodination of the [^{131}I]MIBG reconstituted in 200 μl of phosphate-buffered saline was determined by HPLC. This analysis was performed on a Waters Novapak RPC18 (Waterford, U.K.) column (3.9 × 150 mm) using a Waters 600 series pump with ultraviolet (Waters 490) and radiochemical detection.

Cell Culture. The human neuroblastoma cell lines used were SK-N-BE(2c) (*18*), SK-N-SH (*19*), NB1-G (*20*) and IMR-32 (*21*). One non-neuronal cell line was examined, A2780, a variant of NIH:OVCAR-3 (*22*). All cells were cultured in Eagle's minimal essential medium supplemented with 10% fetal calf serum, 2 mmole glutamine, penicillin-streptomycin (100 IU/ml^{-1}) and amphotericin B (2.5 μg/ml^{-1}). All media and supplements were purchased from Gibco (Paisley, UK).

Cell Uptake Experiments. Cells were seeded into six-well plates at an initial density of 5×10^5 per well and were cultured as monolayers on plastic for 2 to 4 days until semiconfluent. To determine their effect on uptake of no-carrier-added [^{131}I]MIBG, various inhibitory compounds were preincubated with the monolayers for 30 min. Medium was then aspirated and fresh medium containing both the potential inhibitory drug at the same final concentration and no-carrier-added [^{131}I]MIBG was added. Radiopharmaceutical accumulation was determined after 2 hr incubation. At the end of the incubation period, the monolayers were washed twice with cold phosphate-buffered saline and radioactivity was extracted with two aliquots of 10% (wt/vol) trichloroacetic acid. The activities of the combined extracts were measured in a sodium iodide crystal, gamma well detector (Canberra Packard, Berkshire, UK). In this way, using desmethylimipramine and ouabain, the percentage contribution of active uptake to total uptake was determined (*11*). Temperature dependence was assessed by measuring uptake at 4°C and 37°C. The sodium dependency of uptake was investigated by use of medium containing 125 mmole lithium chloride in place of sodium chloride. The effect of dissolved oxygen depletion was determined by the addition of sodium dithionite to the medium.

The potency of blockade of the uptake of no-carrier-added [^{131}I]MIBG by well established inhibitors of noradrenaline accumulation was monitored in neuroblastoma cells using a range of concentrations (10^{-9} to 10^{-3} mmole) of biogenic amines. Dopamine, imipramine, amitriptyline, desmethylimipramine, serotonin or the natural substrate, noradrenaline, were incubated with the radiopharmaceutical, and the 2-hr uptake was determined as described above.

To compare the radioactivity accumulation resulting from exposure of cells to no-carrier-added [^{131}I]MIBG or commercially available [^{131}I]MIBG, SK-N-BE(2c) neuroblastoma cells and A2780 ovarian carcinoma cells were incubated with a range of activities of the two preparations of the radiopharmaceutical. The former cell line has high capacity for specific uptake, while the latter cell line lacks the noradrenaline transporter and concentrates the drug only by passive diffusion (*23*). The percentage of specific binding was determined (*24*) by subtraction from total accumulated radioactivity of values observed in the presence of 1.5 μmole desmethylimipramine. This tricyclic antidepressant is a strong inhibitor of re-uptake of monoamine neurotransmitters by adrenergic neurons. A concentration of 1.5 μmole was used as this has previously been shown to be effective in blocking [^{131}I]MIBG uptake (*11*).

In order to determine the rank order of specific uptake in

various neuroblastoma cell lines, SK-N-BE(2c), SK-N-SH, NB1-G and IMR-32 were incubated with no-carrier-added [^{131}I]MIBG, with or without desmethylimipramine using the previously described procedures.

Cytotoxicity Assay. Cytotoxicity was determined by inhibition of regrowth of multicellular tumor spheroids (*25*). This model was chosen in preference to single cells because of the inefficient absorption of disintegration energy by tumors of dimensions less than the mean range of ^{131}I beta particles (*26,27*). Spheroids of SK-N-BE(2c) cells were prepared by placing one million cells in 50 ml of medium in a 100-ml Techne (Cambridge, UK) spinner vessel and stirring at 40 rpm. Spheroids of about 350 μm diameter were obtained after three to four days incubation at 37°C in 5% CO_2. These were incubated with no-carrier-added [^{131}I]MIBG (2 MBq/ml^{-1}) either alone or with a range of concentrations of nonradiolabeled MIBG for 2 hr to assess the effect of specific activity on cytotoxicity. The concentrations of nonradiolabeled MIBG used were 10 nmole, 20 nmole, 100 nmole, 200 nmole and 10 μmole. Spheroids were transferred into individual agar-coated wells of 24-well test plates with one plate being used for each treatment. Spheroids were measured immediately after treatment using image analysis (Analytical Instruments, Cambridge, U.K.) to determine cross-sectional area. This was converted to volume, on the assumption that the spheroids were true spheres. Cytotoxicity was defined as the proportion of spheroids that failed to achieve a tenfold volume increase within 28 days.

Experimental Animals. All animal work was carried out in accordance with the UK Coordinating Committee for Cancer Research guidelines on experimental neoplasia in animals under the authority of a project license granted by the UK Home Office under the Animals (Scientific Procedures) Act, 1986. Six-week-old male and female, congenitally athymic nude mice of strain MF1 nu/nu were obtained from Harlan Olac, Bicester, UK.

Neuroblastoma Xenografts. Xenografts were established in nude mice using the method described by Rutgers et al. (*28*). Briefly, a suspension containing 3 × 10^6 freshly harvested SK-N-BE(2c) cells was delivered by intrasplenic injection, and following a latent period of 3–12 weeks, palpable hepatic and splenic tumors developed. Tumor fragments of 2 mm to 3 mm in diameter were then implanted subcutaneously in the subcostal flank of 6-to-8-wk-old mice. Following passage of tumor fragments into fresh animals, subcutaneous xenografts developed in about 95%. Mice were used for pharmacokinetic and biodistribution experiments 3 to 4 weeks after implantation when the subcutaneous tumors had reached about 5 mm to 10 mm diameter.

MIBG Biodistribution Experiments. These experiments were performed on groups of seven mice. At least one hour before MIBG injection, tumor-bearing mice were weighed and injected intraperitoneally with 1 ml of a 0.1% (wt/vol) potassium iodide solution to diminish thyroid uptake of radioiodine. Then mice were injected intraperitoneally with about 5 MBq of either no-carrier-added [^{131}I]MIBG or commercial therapy preparation [^{131}I]MIBG, which was precisely quantified using a Curiemonitor-2 ionization chamber radionuclide meter (Radiation Components, Bracknell, UK).

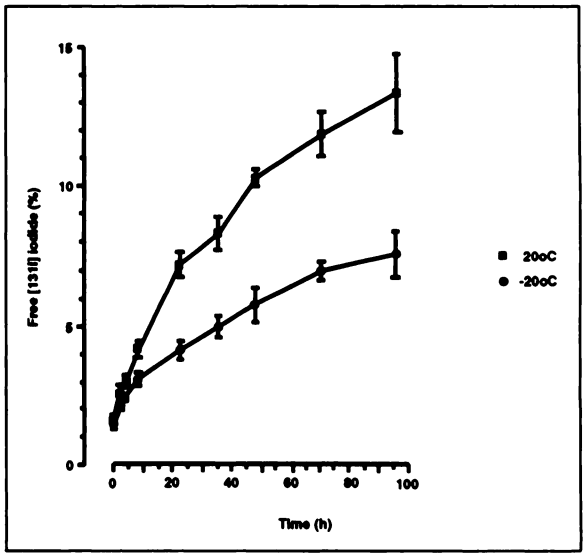

FIGURE 1. The effect of storage temperature on the stability of no-carrier-added [^{131}I]MIBG. No-carrier-added [^{131}I]MIBG was reconstituted in phosphate-buffered saline to a radioactivity concentration of 20.7 MBq · ml^{-1} before storage at 20°C, −20°C. Means and standard deviations of three determinations are presented.

At the appropriate time following [^{131}I]MIBG injection (1, 8, 16, 24, 48 or 72 hr later) the mice were killed. A blood sample was taken, and samples of the tumor, heart, lung, adrenal glands, kidney, spleen, skin, thyroid gland and skeletal muscle were excised and carefully dissected from any fatty or connective tissue. Tumor and tissue samples were placed in screw-capped, 1.5-ml Eppendorf tubes and weighed. Then the radioactivity in each tube was measured in an automated gamma counter.

The gamma counter measurement of each sample, in counts per minute, was converted to an absolute value, in MBq, by comparison with the measurements obtained from standards of known activity. From this value, and knowledge of the weight of the sample and activity of [^{131}I]MIBG initially administered to the mouse, the concentration of [^{131}I]MIBG in each organ and the tumor was expressed as the percent, in each gram of tissue, of the injected dose per mouse. Correction was made for the radioactive decay which had taken place since the time of injection.

RESULTS

Stability of No-Carrier-Added [^{131}I]MIBG. A comparison of the rates of deiodination of [^{131}I]MIBG stored in phosphate-buffered saline at 20°C and –20°C is presented in Figure 1. The increased stability of storage at –20°C was evident. After storage for 8 hr or longer, there was a statistically significant difference ($p < 0.05$) in the percentage

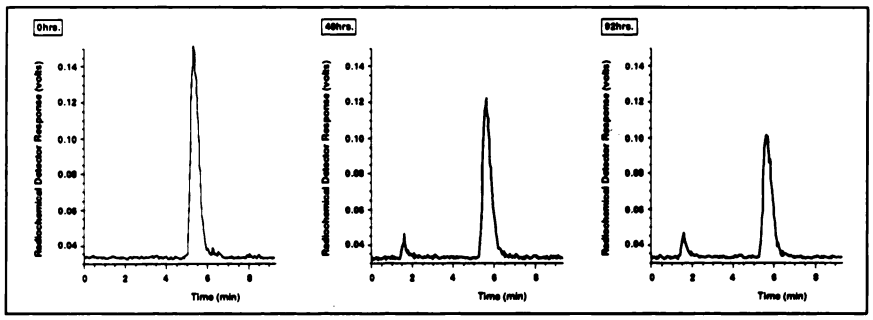

FIGURE 2. HPLC elution profiles of no-carrier-added [^{131}I]MIBG: 0, 48 and 92 hr after synthesis.

of free ^{131}I-iodide present in the sample maintained at room temperature compared with the frozen material. Thirty-six hours after synthesis, no more than 5% unbound iodide was present in the -20°C samples, whereas levels in excess of 8% were found in the material stored at ambient temperature. Due to its high specific activity, the physical amount of [^{131}I]MIBG in the analytical samples did not allow ultra-violet detection. Radiochemical detection showed only two active species at later times (Fig. 2). The major radioactive peak was shown to co-elute with an authentic sample of cold MIBG. The other radioactive peak eluted close to the void volume of the column, indicative of a highly polar species, presumably I^-. A sample of ^{131}I-sodium iodide had the same retention time.

The Effect of Inhibitors on No-Carrier-Added [^{131}I]MIBG Uptake. Uptake of drug at 4°C was reduced to 5.5% of the level at 37°C (control). The specific inhibitor, desmethylimipramine, at a concentration of 1.5 μmole decreased MIBG accumulation to 2.7% of the control values. Ouabain is a specific inhibitor of sodium-potassium-dependent adenosine triphosphatase transport mechanisms. Preincubation of SK-N-BE(2c) cells with 1 mmole ouabain reduced the uptake of radiopharmaceutical to 7.8% of control. The use of sodium-free medium diminished cellular concentration of the drug to 10.2%, and treatment with 1.5 mmole sodium dithionite reduced MIBG uptake to 6.8% of the control value.

Competition studies demonstrated that uptake of no-carrier-added [^{131}I]MIBG was reduced in a dose-dependent manner by noradrenaline and inhibitors of noradrenaline transport (Fig. 3). Blockade occurred with a rank order of potency identical to that observed for the inhibition of uptake of noradrenaline into SK-N-SH cells (*29*) and in HeLa cells transfected with noradrenaline transporter cDNA (*30*). The competitor concentrations required for 50% inhibition of uptake of no-carrier-added [^{131}I]MIBG were 1.5 nmole desmethylimipramine, 885 nmole imipramine, 135 nmole amitriptyline, 190 nmole dopamine, 304 nmole noradrenaline and 80 μmole serotonin. The rank order was also consistent with the results of low specific activity [^{131}I]MIBG uptake inhibition studies using a range of competitors of varying affinity for the noradrenaline transporter (*31*).

The assignment of ranking of capacity for active accumulation of no-carrier-added [^{131}I]MIBG by four neuroblastoma cell lines (SK-N-BE(2c), SK-N-SH, NB1-G and IMR-32) is given in Table 1. This is the same as the grading previously established for the uptake of low specific activity MIBG (*11,32*). To determine whether incubation with no-carrier-added [^{131}I]MIBG could result in a greater cellular concentration of radioactivity than is achievable with lower, specific-activity therapy preparations of the radiopharmaceutical, binding studies were performed using uptake-1 competent SK-N-BE(2c) cells and A2780 cells that concentrate MIBG passively. Over a wide range of activity, A2780 cells took up the same amount of radioactivity delivered in the form of no-carrier-added or low specific-activity [^{131}I]MIBG (Fig. 4). In contrast, as shown in Figure 5, radioactivity accumulation by SK-N-BE(2c) cells resulting from incubation with the commercial preparation of the drug tended to plateau beyond 100 kBq and the contribution of the specific uptake mechanism diminished from 96% at 1 kBq to 13% at 9 MBq. Over this same range, we observed only a 6% decrease in the proportion of accumulation by uptake-1 when radioactivity was in the form of no-carrier-added [^{131}I]MIBG.

The Relationship Between Specific Activity and Cytotoxicity of [^{131}I]MIBG. The effect of the addition of nonradiolabeled MIBG upon the toxicity of no-carrier-added [^{131}I]MIBG was assessed by means of spheroid regrowth monitoring (Fig. 6). In the absence of cold carrier MIBG, the no-carrier-added radiochemical at a radioactivity con-

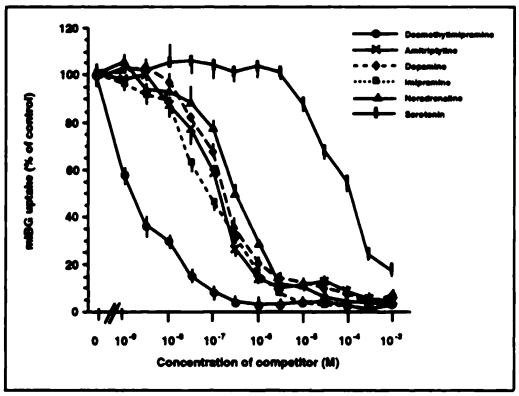

FIGURE 3. Inhibition of uptake of no-carrier-added [^{131}I]MIBG into SK-N-BE(2c) neuroblastoma cells by monoamine competitors. Cells were incubated with 7 kBq no-carrier-added [^{131}I]MIBG and competitor for 2 hr prior to measurement of cell-associated radioactivity. Means and standard deviations of three experiments in triplicate.

TABLE 1
MIBG Uptake by Neuroblastoma Cell Lines

Cell line	Desmethylimipramine-inhibitable MIBG uptake* (cpm × 10^3 per 10^6 cells)
SK-N-BE(2c)	114 ± 8
SK-N-SH	107 ± 10
NB1-G	26 ± 4
IMR-32	12 ± 2

*Uptake measurements were made after 2-hr incubation in the presence of 7 kBq no-carrier-added [^{131}I]MIBG with or without 1.5 μmole desmethylimipramine. Accumulated radioactivity is expressed as mean ± s.d. of three experiments in triplicate. Data from Mairs et al. (*23*).

centration of 2 MBq/ml permitted the regrowth of only 15% (3/19) of SK-N-BE(2c) spheroids. The addition of increasing concentrations of cold drug allowed the regrowth of a greater fraction of spheroids. Almost 100% (22/23) attained a tenfold increase in volume within the time period of the experiment when 10 μmole nonradiolabeled MIBG was coincubated with the radiopharmaceutical. No spheroid failed to regrow following incubation with iodide exchange [^{131}I]MIBG at a concentration of 2.4 MBq/ml^{-1} (*33*). The plasma drug concentrations observed during MIBG therapy are of the order of 10^{-7} mol (*34*). This value corresponds to that which occurs in the region of the point of inflection of the regrowth/concentration curve (Fig. 6).

Biodistribution Studies. The distribution in tumor-bearing nude mice of the two different MIBG preparations was compared by measurement of radioactivity in tissues ex-

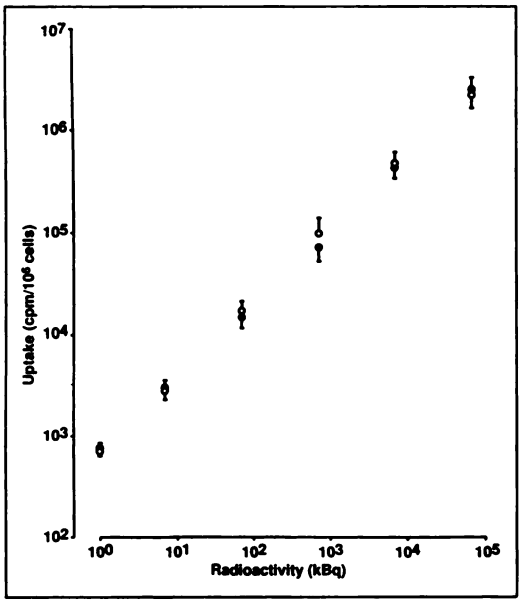

FIGURE 4. Uptake by A2780 ovarian carcinoma cells of no-carrier-added [^{131}I]MIBG (open circles) and commercial therapy preparation [^{131}I]MIBG (filled circles). Means and standard deviations of three experiments in triplicate.

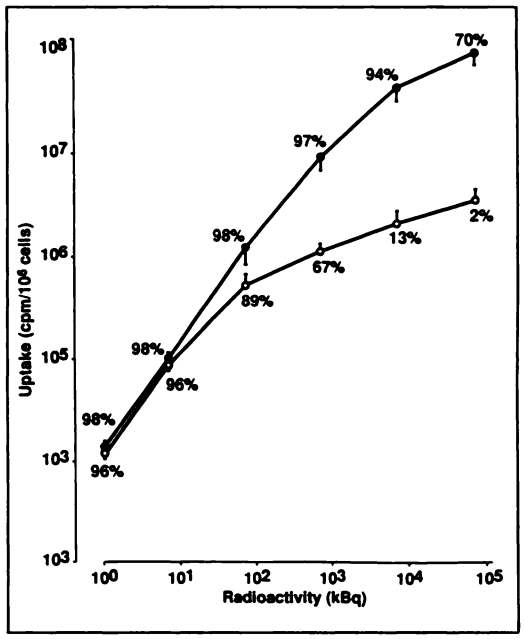

FIGURE 5. Uptake by SK-N-BE(2c) neuroblastoma cells of no-carrier-added [^{131}I]MIBG (filled circles) and commercial therapy preparation [^{131}I]MIBG (open circles). Mean and standard deviations of three experiments in triplicate. Percentages represent the proportion of uptake by the active process. Passive diffusion plus active uptake equals total uptake. Passive diffusion was determined by inhibition of active drug accumulation by incubation of cells with 1.5 μmole desmethylimipramine.

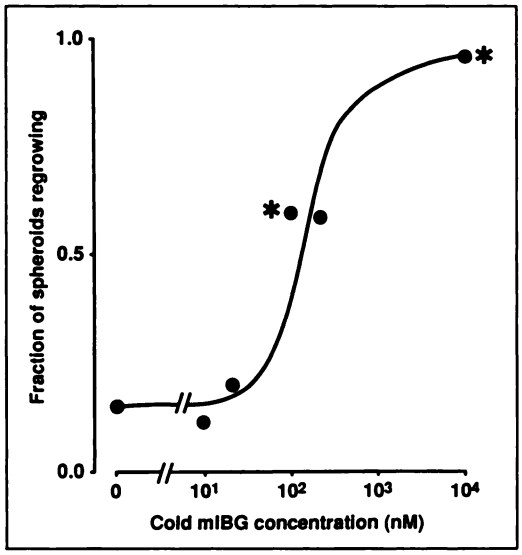

FIGURE 6. The effect of non-radiolabeled MIBG on the cytotoxicity of no-carrier-added [^{131}I]MIBG. SK-N-BE(2c) neuroblastoma spheroids were incubated with no-carrier-added [^{131}I]MIBG (2 MBq · ml^{-1}) and a range of concentrations of nonradiolabeled MIBG for 2 hr. Their growth was monitored over 28 days. Regrowing spheroids were defined as those which attained a ten-fold increase in volume within this time period. *Value significantly greater than that of preceding dose point ($p < 0.05$) calculated using 2 × 2 contingency tables.

TABLE 2
Biodistribution of Commercially Prepared [^{131}I]MIBG in Nude Mouse Xenografts as a Percentage of the Injected Dose per Gram*

	1 hr	8 hr	16 hr	24 hr	48 hr	72 hr
Tumor	0.74 (0.49)	0.68 (0.34)	0.80 (0.49)	1.08 (0.41)	0.39 (0.26)	0.25 (0.08)
Muscle	0.71 (0.15)	0.45 (0.30)	0.16 (0.09)	0.08 (0.02)	0.07 (0.03)	0.03 (0.02)
Liver	2.91 (1.09)	1.50 (0.60)	0.76 (0.31)	0.45 (0.22)	0.22 (0.08)	0.12 (0.07)
Spleen	2.26 (0.78)	1.36 (0.38)	0.59 (0.27)	0.68 (0.18)	0.29 (0.18)	0.09 (0.03)
Skin	1.78 (0.71)	1.22 (0.55)	0.60 (0.32)	0.43 (0.20)	0.22 (0.09)	0.08 (0.05)
Lung	1.63 (0.96)	1.02 (0.36)	0.77 (0.29)	0.30 (0.18)	0.11 (0.03)	0.06 (0.02)
Heart	3.08 (0.81)	0.94 (0.27)	0.35 (0.07)	0.39 (0.21)	0.19 (0.06)	0.14 (0.07)
Kidney	1.78 (0.61)	1.04 (0.25)	0.51 (0.29)	0.32 (0.13)	0.14 (0.05)	0.09 (0.04)
Thyroid	1.43 (0.52)	1.11 (0.38)	1.00 (0.32)	0.99 (0.36)	0.42 (0.29)	0.15 (0.07)
Blood	1.15 (0.38)	0.20 (0.25)	0.10 (0.05)	0.08 (0.05)	0.04 (0.02)	0.02 (0.02)
Adrenal	4.20 (1.09)	3.81 (0.88)	3.01 (0.74)	2.62 (0.70)	1.33 (0.56)	0.88 (0.32)

*Values are the mean (±s.d.).

cised from the animals, which were sacrificed at different times after intraperitoneal injection of the radiopharmaceutical (Tables 2 and 3). When [^{131}I]MIBG was administered in no-carrier-added form, significantly greater accumulation was observed at all times in tumor, adrenal glands and heart. In addition, significantly higher uptake values were obtained in skin at 16 hr and 24 hr, and in lungs at 48 hr. The concentration of MIBG in muscle was low compared to other nonsympathetically innervated tissues. There was a small but significant increase in muscle uptake evoked by the lower specific drug activity at 8 hr and 16 hr.

The means and standard deviations of tumor weight (mg) for the groups of mice treated with no-carrier-added or iodide-exchange MIBG were 212 ± 68 and 223 ± 77, respectively. Although there was wide variation in tumor size between animals, the difference between the two test groups was not significant.

The major determinant of the therapeutic index of targeted radiotherapy is the target-to-nontarget ratio of radionuclide accumulation. Figure 7 shows the ratio between tumor and liver, a representative normal tissue. At all times except 72 hr, enhanced tumor-to-liver uptake ratios were obtained using the higher specific activity radiopharmaceutical. Because the high initial MIBG levels in the liver declined rapidly (Tables 2 and 3), and the tumor MIBG levels did not peak until 24 hr, the tumor-to-liver ratios started at less than unity and gradually increased to achieve peak values at 24 hr followed by a gradual decline (Fig. 7). Comparison of tissue accumulation data at 24 hr (Tables 2 and 3) shows that no-carrier-added [^{131}I]MIBG elicited a threefold improvement in tumor uptake but with concomitant increases in uptake in adrenal glands, heart and skin.

DISCUSSION

This study was undertaken to determine whether no-carrier-added [^{131}I]MIBG offers any advantage over [^{131}I]MIBG produced by iodide exchange. We have shown that no-carrier-added [^{131}I]MIBG is accumulated by neuroblastoma cells in the same manner as the currently available drug preparation of lower specific activity. The rank order of MIBG uptake in the panel of neuroblastoma cell lines examined correlated with the cellular production of mRNA encoded by the noradrenaline transporter gene (*23*). It is

TABLE 3
Biodistribution of No-Carrier-Added [^{131}I]MIBG in Nude Mouse Xenografts as a Percentage of Injected Dose per Gram*

	1 hr	8 hr	16 hr	24 hr	48 hr	72 hr
Tumor	1.54 (0.50)‡	1.58 (0.68)§	2.12 (0.87)§	2.97 (0.93)§	0.90 (0.51)‡	0.33 (0.14)
Muscle	0.63 (0.17)	0.19 (0.12)†	0.08 (0.04)†	0.07 (0.04)	0.05 (0.03)	0.02 (0.01)
Liver	3.70 (0.88)	1.77 (0.83)	1.14 (0.59)	0.65 (0.28)	0.29 (0.11)	0.18 (0.08)
Spleen	2.05 (0.42)	1.19 (0.49)	0.60 (0.31)	0.64 (0.22)	0.18 (0.08)	0.07 (0.04)
Skin	2.27 (0.51)	1.67 (0.57)	0.96 (0.41)†	0.79 (0.40)†	0.27 (0.12)	0.12 (0.05)
Lung	2.33 (0.75)	1.39 (0.58)	0.59 (0.13)	0.47 (0.25)	0.20 (0.07)†	0.08 (0.03)
Heart	4.21 (1.08)†	3.07 (1.20)§	1.92 (0.83)§	0.95 (0.44)‡	0.30 (0.09)‡	0.19 (0.06)
Kidney	1.49 (0.48)	0.88 (0.30)	0.38 (0.11)	0.29 (0.16)	0.18 (0.05)	0.07 (0.02)
Thyroid	1.26 (0.46)	1.17 (0.28)	0.96 (0.40)	0.81 (0.31)	0.49 (0.27)	0.12 (0.09)
Blood	0.93 (0.12)	0.17 (0.10)	0.11 (0.04)	0.06 (0.02)	0.04 (0.02)	0.03 (0.01)
Adrenal	5.18 (1.44)†	7.04 (2.59)§	7.71 (3.15)§	9.06 (3.02)§	3.08 (0.90)§	1.16 (0.48)

*Values are the mean (±s.d.). Significance of difference between no-carrier-added [^{131}I]MIBG and commercial therapy preparation of [^{131}I]MIBG: †$p < 0.05$; ‡$p < 0.01$; §$p < 0.001$.

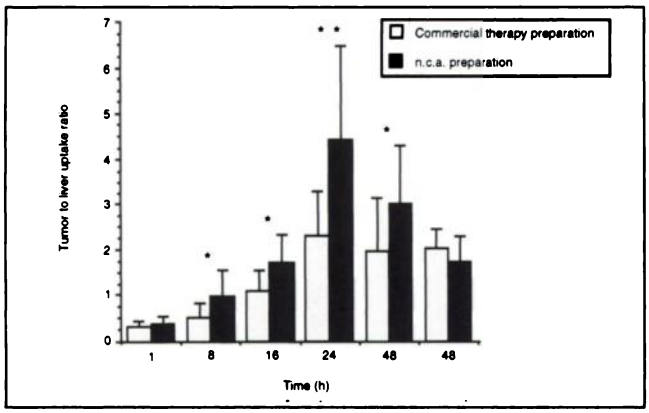

FIGURE 7. Tumor-to-liver radioactivity uptake ratios in nude mouse xenografts at various times after the administration of no-carrier-added [^{131}I]MIBG (filled bars) or commercial therapy preparation [^{131}I]MIBG (open bars) as means and standard deviations. Significance of difference between the two preparations of [^{131}I]MIBG: *$p < 0.05$; **$p < 0.01$.

important to establish whether MIBG treatment or chemotherapy have an effect on the synthesis of this transporter. This question is currently being addressed using in vitro models.

A previous study of in vitro MIBG uptake into SK-N-SH neuroblastoma cells (*10*) has shown that while the proportion of uptake due to specific binding was unaltered over a wide range of activities of no-carrier-added [^{131}I]MIBG, the binding of iodide-exchange-synthesized [^{131}I]MIBG declined over the same range. Our data on the accumulation of MIBG of low and high specific activity by SK-N-BE (2c) neuroblastoma cells confirm these findings and indicate that the presence of cold carrier MIBG molecules in the radiopharmaceutical preparation may adversely affect target cell uptake and hence the therapeutic efficacy of MIBG.

We have also shown that the cytotoxicity of no-carrier-added [^{131}I]MIBG in vitro is greater than that of the radiopharmaceutical contaminated with non-radiolabeled MIBG. The approximate plasma level of MIBG achieved during therapy is 10^{-7} mol (*34*). At this drug concentration in the experimental incubation medium, we observed a diminution of growth inhibitory potency, assessed using neuroblastoma spheroids. This suggests that therapeutic application of MIBG at the greatly reduced concentrations achievable with the carrier-free preparation may be more effective than iodide-exchange preparations of the drug.

The pattern of normal tissue uptake of the iodide exchange preparation of [^{131}I]MIBG was similar to that observed by other investigators (*10,28,35*), in that the time-dependent biodistribution profiles of carrier-free and carrier-added radiopharmaceutical in neuroblastoma xenografts showed an advantage for no-carrier-added [^{131}I]MIBG. A previous comparison of the biodistribution of intravenously injected no-carrier-added and iodide exchange [^{131}I]MIBG in tumor-free BALB/c mice (*10*) noted enhanced accumulation of the higher specific activity drug by heart and adrenals. Significant increases in uptake by these organs were also noted in the present study; however, at the comparable time (24 hr) the heart-to-liver and adrenal-to-liver ratios were approximately half of those previously observed (*10*). These discrepancies may be due to dissimilarities in the experimental model systems employed, such as a different mouse strain, presence or absence of tumor, different route of drug administration and different molar amount of radiopharmaceutical in the injected material.

Radiolabeled compounds are known to degrade due to self-irradiation. This autoradiolysis is a potential problem for a new radiopharmaceutical formulation. The loss of ^{131}I-iodide from [^{131}I]MIBG will decrease the specificity of radioactivity accumulation by tumor while increasing uptake in the thyroid. It is common clinical practice to discard [^{131}I]MIBG therapy solutions which contain more than 5% free ^{131}I-iodide (*36*). We found that at a radioactivity concentration of 20.7 MBq/ml^{-1}, the no-carrier-added radiopharmaceutical underwent less than 5% deiodination in 36 hr when stored at –20°C. This concentration of activity was used for pharmacokinetic studies in neuroblastoma xenografts.

In clinical practice, the dose of therapeutic [^{131}I]MIBG that can be given is limited by myelotoxicity to a whole-body radiation dose of about 2 Gy (*6*), unless the patient is rescued by bone marrow transplantation. The bulk of this whole-body dose comes from the uptake of [^{131}I]MIBG into normal tissues, not tumor. If a preparation of [^{131}I]MIBG that results in a more favorable tumor-to-normal-tissue ratio can be used, then a higher tumor dose, and hence an increased likelihood of benefit, will be achieved for a given whole-body radiation dose.

Our data show convincingly that no-carrier-added [^{131}I]MIBG results in a significantly higher tumor-to-normal-tissue ratio than does conventional [^{131}I]MIBG prepared by iodide exchange. Therefore, the use of no-carrier-added [^{131}I]MIBG offers the prospect of a real advance in the treatment of children with neuroblastoma.

The same difference in the ratio of tumor-to-nontumor uptake may also be of clinical significance in the diagnosis and staging of children with neuroblastoma. Iodine-131 or [^{123}I]MIBG scintigraphy is now in routine clinical use for the assessment of children with suspected neuroblastoma and for the re-evaluation of patients after treatment (*37*).

The greater tumor-to-background ratio seen with no-carrier-added [^{131}I]MIBG in this study should result in an improvement in the sensitivity of diagnostic scintigraphy. More accurate and reliable staging data should mean that patients with disseminated disease, which might previously have been unrecognized, may now be identified, enabling the most appropriate treatment to be used.

CONCLUSION

Normal tissue accumulation data show that no-carrier-added [^{131}I]MIBG results in significantly increased uptake in the skin, heart and adrenal glands in addition to improvement in tumor uptake. The relationship between targeting specificity and specific activity of the radiopharmaceutical there-

fore means that in general, the use of no-carrier-added [^{131}I]MIBG will lead to an increased tumor dose for the same normal tissue effects, although it is possible that there might be adverse cutaneous, cardiac or adrenal effects.

Following the demonstration of the benefits of no-carrier-added [^{131}I]MIBG in terms of biodistribution in experimental model systems, we propose to evaluate the comparative therapeutic efficacy of no-carrier-added [^{131}I]MIBG and [^{131}I]MIBG prepared by iodide exchange in neuroblastoma xenografts. Initial clinical studies with no-carrier-added [^{131}I]MIBG will investigate its place in diagnostic scintigraphy; we shall evaluate its role in therapy at a later time.

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