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Enhanced Tumour Uptake and *In Vitro* Radiotoxicity of No-carrier-added [^{131}I] Meta-iodobenzylguanidine: Implications for the Targeted Radiotherapy of Neuroblastoma

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***In vitro* and *in vivo* neuroblastoma models were used to determine whether improvements in tumour targeting *in vivo* and therapeutic efficacy *in vitro* could result from the use of no-carrier-added (n.c.a.) [^{131}I]MIBG. Results were compared with use of the conventional therapy MIBG preparation (ex. [^{131}I]MIBG) of lower specific activity which is produced by iodide exchange reaction. The efficacy of n.c.a. [^{131}I]MIBG was compared with that of [^{131}I]MIBG over a range of specific activities by the assessment of neuroblastoma spheroid growth delay. Whereas n.c.a. [^{131}I]MIBG at a radioactivity concentration of 2 MBq/ml prevented the regrowth of 84% of spheroids, toxicity was significantly reduced by the addition of non-radiolabelled MIBG to the incubation medium. The time-dependent biodistribution of n.c.a. [^{131}I]MIBG in nude mice bearing human neuroblastoma xenografts was compared with that of the conventional therapy radiopharmaceutical. The n.c.a. agent gave improved tumour uptake but also significantly greater accumulation in normal tissues known to accumulate MIBG such as heart, adrenal and skin. However, uptake and retention in the blood was unaltered. For all tissues examined, the 3-day cumulative tumour to normal tissue radiation dose ratio was greater for n.c.a. [^{131}I]MIBG. Theoretical calculations were undertaken to predict organ to tumour dose ratios which would result in human neuroblastoma patients with each of the [^{131}I]MIBG preparations. These results suggest that significant therapeutic gain may be achieved by the use of n.c.a. [^{131}I]MIBG as a treatment agent in neuroblastoma.**

Key words: no-carrier-added [^{131}I]MIBG, pharmacokinetics, spheroid toxicity, xenografts

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INTRODUCTION

META-IODOBENZYLGUANIDINE (MIBG) was developed for the scintigraphic visualisation of the adrenal medulla [1]. This compound emulates noradrenaline uptake and storage [2] in adrenomedullary cells and sympathetic neurones. Radio-iodinated MIBG is an established radiopharmaceutical for the imaging [3, 4] and targeted radiotherapy [5, 6] of neural crest-derived tumours, such as pheochromocytoma and neuroblastoma.

The production of commercial therapy [^{131}I]MIBG involves an iodide exchange reaction, whereby a radioiodine is substituted for stable iodine on a "cold" MIBG molecule [7]. This method results in a preparation with a considerable amount of cold MIBG, such that less than 0.05% of the molecules are in the form of [^{131}I]MIBG. Since these cold molecules can compete with radiolabelled MIBG for tumour uptake, the presence of a saturable mechanism for tumour accumulation would make it highly unlikely that MIBG produced by the iodide exchange method would be optimal for achieving selective targeting in neuroblastoma.

Indeed, the basis for discrimination by MIBG between tumour and non-target tissues is the expression of neuroblastoma cell membrane-associated, high affinity, noradrenaline transporter. In contrast, most normal tissues accumulate MIBG inefficiently by passive diffusion [8]. In neuroblastoma cells in culture, active uptake (Uptake-1) of MIBG by the noradrenaline transporter makes a greater contribution to total drug accumulation than passive uptake when MIBG is present at low concentrations [9–12]. This suggests that tumour-specific uptake should be enhanced by the administration of lower molar amounts through the use of MIBG of high specific activity [13].

Indications that improved selectivity may be achieved by the use of high specific activity radioiodinated MIBG have been provided by *in vivo* and *in vitro* investigations. For example, [^{123}I]MIBG uptake into rat heart (a sympathetically innervated organ) is specific activity dependent [14], and the ability to kill

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cultured SK-N-SH neuroblastoma cells is greater in higher specific activity preparations of [^{131}I]MIBG [15]. Moreover, it has recently been demonstrated by tissue uptake studies in normal mice that the Uptake-1-rich organs, heart and adrenals, acquired significantly more radioactivity when animals were injected with no-carrier-added (n.c.a.) [^{131}I]MIBG than when the exchange preparation of MIBG was used [13].

The purposes of the present study were to evaluate the influence of carrier MIBG on the cytotoxicity of [^{131}I]MIBG for neuroblastoma spheroids, and to compare the pharmacokinetics and dosimetry in tumour-bearing mice of n.c.a. [^{131}I]MIBG with the current, commercially available, therapy preparation (ex.[^{131}I]MIBG).

MATERIALS AND METHODS

Radiochemical synthesis

Preparation of n.c.a. [^{131}I]MIBG was accomplished by iododesilylation of meta-trimethylsilylbenzylguanidine [13]. The product was purified by HPLC and solid phase extraction [16], dried in a stream of nitrogen and dissolved in phosphate buffered saline. Non-radiolabelled MIBG was synthesised from meta-iodobenzylamine hydrochloride according to the method of Wieland and associates [1].

Determination of cytotoxicity

The human neuroblastoma cell line, SK-N-BE(2c) [17], was cultured in Eagle's minimal essential medium supplemented with 10% fetal calf serum, 2 mM glutamine, penicillin-streptomycin (100 IU/ml) and amphotericin B (2.5 μg/ml). It has previously been demonstrated that SK-N-BE(2c) cells have the capacity for high affinity uptake of MIBG [12, 18, 19]. The determination of [^{131}I]MIBG toxicity was by inhibition of the regrowth of multicellular spheroids [20]. The spheroid model was chosen in preference to cells growing in monolayer because targeted radiotherapy using ^{131}I-labelled compounds should result in the sterilisation of small tumour deposits or clumps of cells, while being relatively non-toxic to single cells [21]. This is due to the inefficient absorption of decay energy by tumours of dimensions less than the mean range of ^{131}I β-particles [22, 23].

Spheroids of SK-N-BE(2c) cells were prepared by placing one million cells in 50 ml medium in a 100 ml Techne (Cambridge, U.K.) spinner vessel and stirring at 40 rpm. Spheroids of approximately 350 μm diameter were obtained after 3–4 days incubation at 37°C in 5% CO_2. These were incubated with n.c.a. [^{131}I]MIBG (2 MBq/ml) either alone or with a range of concentrations of non-radiolabelled MIBG for 2 h to assess the effect of specific activity on cytotoxicity. The concentrations of non-radiolabelled MIBG used were 10, 20, 100 and 200 nM and 10 μM. 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. For the puproses of this assay, spheroids which failed to achieve a 10-fold increase in volume within 28 days of treatment where considered to be cured. Cytotoxicity was expressed as a function of cold MIBG in the incubation medium.

Neuroblastoma xenografts

All animal work was carried out in accordance with the U.K. Coordinating Committee for Cancer Research guidelines on experimental neoplasia in animals under the authority of a project licence granted by the U.K. Home Office under the Animals (Scientific Procedures) Act, 1986. 6-week-old male and female congenitally athymic nude mice of strain MF1 nu/nu were obtained from Harlan Olac, Bicester, U.K.

Xenografts were established in nude mice using the method described by Rutgers and associates [24]. 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 tumours developed. Tumour fragments 2–3 mm in diameter were then implanted subcutaneously in the subcostal flank of 6–8 week old mice. Following passage of tumour fragments into other animals, subcutaneous xenografts developed in approximately 95% of recipients. Mice were used for pharmacokinetic and biodistribution experiments 3–4 weeks after implantation, when the subcutaneous tumours had reached approximately 5–10 mm diameter.

MIBG biodistribution experiments

These experiments were performed in groups of 7 mice. At least 1 h before MIBG injection, tumour-bearing mice were weighed and injected intraperitoneally with 1 ml of a 0.1 % (w/v) potassium iodide solution to diminish thyroid uptake of radioiodine. Then mice were injected intraperitoneally with 5 MBq of either n.c.a. [^{131}I]MIBG or the commercial therapy [^{131}I]MIBG preparation. Groups of mice were killed at 1, 8, 16, 24, 48 and 72 h following [^{131}I]MIBG injection. A blood sample was taken, and samples of the tumour, heart, lung, adrenal glands, kidney, spleen, skin, thyroid gland and skeletal muscle were excised and carefully dissected free of any fat or connective tissue. Tissue samples were placed in screw-capped 1.5 ml Eppendorff tubes, weighed, and ^{131}I activity levels were measured in an automated gamma counter. By comparison with injection standards of appropriate count rate, tissue distribution results were expressed as percentage injected dose per gram of tissue.

RESULTS

The effect of non-radiolabelled MIBG on the toxicity of n.c.a. [^{131}I]MIBG

Spheroid regrowth assay was used to evaluate the effect of carrier MIBG on the cytotoxicity of n.c.a. [^{131}I]MIBG (Figure 1). In the absence of carrier, n.c.a. [^{131}I]MIBG at a radioactivity concentration of 2 MBq/ml inhibited the regrowth of 84%($^{16}/_{19}$) of SK-N-BE(2c) spheroids. At an identical constant concentration of the n.c.a. radiopharmaceutical, addition of cold MIBG to the incubation medium resulted in a dose-related decrease in inhibitory potency. Spheroid cure was reduced to approximately 50% by 100 nM cold MIBG. This concentration corresponds to plasma levels of the drug observed during therapy of stage IV neuroblastoma patients [25]. When the concentration of unlabelled MIBG was 10 μM, only 4% ($^1/_{23}$) of the spheroids failed to grow to ten times original volume within 28 days. For comparison, previous studies have shown that ex.[^{131}I]MIBG at a radioactivity concentration of 2.4 MBq/ml had no effect on the regrowth of spheroids derived from SK-N-BE(2c) cells [23].

Biodistribution

The time-dependent distribution of radioiodine activity in tumour and normal tissues following the intraperitoneal injection of n.c.a.[^{131}I]MIBG or ex.[^{131}I]MIBG is shown in Figure 2. Significantly higher tumour uptake was observed for the n.c.a. radiopharmaceutical in tumour at most time points ($P < 0.01$, 1 and 48 h; $P < 0.001$, 8, 16 and 24 h). In addition, significantly higher accumulation of n.c.a. [^{131}I]MIBG was seen in both heart

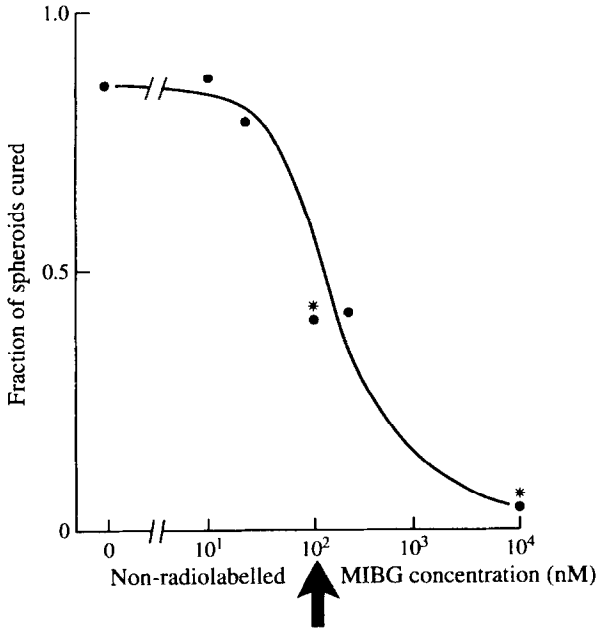

Figure 1. The effect of non-radiolabelled MIBG on the cytotoxicity of n.c.a. [^{131}I]MIBG in SK-N-BE(2c) neuroblastoma spheroids. * Value significantly greater than that of preceding dose point ($P < 0.05$), calculated using 2 × 2 contingency tables. The arrow indicates the approximate plasma concentration resulting from the administration of commercial therapy preparation [^{131}I]MIBG [25].

($P < 0.05$, 1 h; $P < 0.01$, 24 and 48 h; $P < 0.001$, 8 and 16 h) and in adrenal glands ($P < 0.05$, 1 h; $P < 0.001$; 8, 16, 24 and 48 h). Other tissues transiently exhibiting significantly higher concentrations of n.c.a. [^{131}I]MIBG were skin at 16 and 24 h ($P < 0.05$) and lung at 48 h ($P < 0.05$). The concentration of [^{131}I]MIBG was lowest in muscle, and there was a small but significant increase in muscle uptake for the ex.[^{131}I]MIBG preparation at 8 and 16 h ($P < 0.05$). Maximal tumour uptake of MIBG occurred 24 h post-injection (Figure 3). At this time, n.c.a.[^{131}I]MIBG produced a 3-fold improvement in accumulation of radiopharmaceutical by tumour.

The areas under the decay corrected uptake curves were estimated using a trapezoidal approximation. These were used to derive values of absorbed dose per unit injected activity (Gy/MBq) assuming electron equilibrium conditions (Figure 4). Using these data, cumulative tumour to organ absorbed dose ratios were calculated for both n.c.a. [^{131}I]MIBG and ex.[^{131}I]MIBG (Figure 5). As anticipated, these were most favourable for both preparations in muscle and blood, and least satisfactory with normal tissues known to specifically accumulate MIBG, such as adrenals and the heart. Comparison of cumulative radiation absorbed dose ratios revealed an advantage for n.c.a. [^{131}I]MIBG in all tissues examined. Although heart exhibited higher uptake of n.c.a. [^{131}I]MIBG than of ex. [^{131}I]MIBG at all time points (Figure 2), the enhanced tumour accumulation of n.c.a. [^{131}I]MIBG resulted in a tumour to heart ratio which was marginally greater than that produced by the low specific activity preparation of [^{131}I]MIBG.

Predicted therapeutic advantage in human neuroblastoma patients

It is possible to use these murine data to estimate the therapeutic advantage resulting from the use of n.c.a. [^{131}I]MIBG instead of the commercial radiopharmaceutical in human neuroblastoma patients. Figure 6 shows the predicted ratios of absorbed dose per unit injected activity for tumour and all organs between n.c.a. and exchange preparations. For the same injected activity, the predicted tumour absorbed dose is higher by a factor of approximately 2.3 for n.c.a. [^{131}I]MIBG. The predicted absorbed doses in the adrenals and heart are also higher by a similar factor. Doses to the liver, skin and lung would be anticipated to be higher by between 30–45%, while those to the remaining organs would be less ranging from 95% for thyroid to 65% for muscle.

DISCUSSION

In some cases, the commercially available preparation of [^{131}I]MIBG has been shown to be efficacious in the treatment of patients with neuroblastoma [6], although improvements in this therapeutic approach are clearly needed. A number of strategies are under investigation in our institution including combining MIBG treatment with high dose chemotherapy and total body irradiation [26]. Herein, we report our efforts in another area—improving the properties of the radiopharmaceutical itself through the use of a n.c.a. preparation. The results of experiments performed both *in vitro* and *in vivo* suggest that this strategy may be beneficial.

In the cytotoxicity experiments, n.c.a. [^{131}I]MIBG regrowth inhibition of SK-N-BE(2c) neuroblastoma spheroids was reduced in a dose-dependent manner by carrier MIBG. This observation is consistent with reports of decreased cellular uptake of the radiopharmaceutical, via ATPase-mediated transport, due to the presence of cold competitor [13, 27]. A similar relationship between MIBG potency and specific activity has been noted by others in neuroblastoma monolayer cell cultures [15].

A critical issue is the relevance of these results to the clinical situation. In that regard, the plasma concentration of cold MIBG following the administration of therapeutic doses of the conventional iodide exchange preparation is of the order of 100 nM. The inclusion of this concentration of carrier MIBG in the spheroid incubation medium decreased the cytotoxic effect, suggesting that improved tumour toxicity may be achievable in patients through the use of n.c.a. [^{131}I]MIBG instead of ex. [^{131}I]MIBG.

The tissue distribution of ex.[^{131}I]MIBG was similar to that previously observed [13, 24, 28]. A comparison of the pharmacokinetics of n.c.a. [^{131}I]MIBG and ex.[^{131}I]MIBG showed significant advantages for the former. Although n.c.a. [^{131}I]MIBG was more highly concentrated in sympathetically innervated tissues such as heart, adrenals and skin, this was accompanied by an even more pronounced increased uptake in tumour from 1 to 48 h post-injection. As a result, calculations based on these biodistribution data indicate that n.c.a. [^{131}I]MIBG increases cumulative tumour to normal tissue radiation absorbed dose ratios, and suggest the possibility of improved targeted radiation treatment due to the enhanced therapeutic index associated with n.c.a. [^{131}I]MIBG.

A previous comparison of murine tissue uptake of ex. [^{131}I]MIBG and n.c.a. [^{131}I]MIBG also found greater concentrations of the higher specific activity radiopharmaceutical in heart and adrenals [13]. However, the uptake of these organs, relative to that of non-sympathetically innervated liver, was approximately twice that observed in the present biodistribution study. This may be due to differences in strain of mouse, route of injection of radiopharmaceutical, administered dose, or presence or absence of tumour.

A recent investigation of the pharmacokinetics of [^{131}I]MIBG

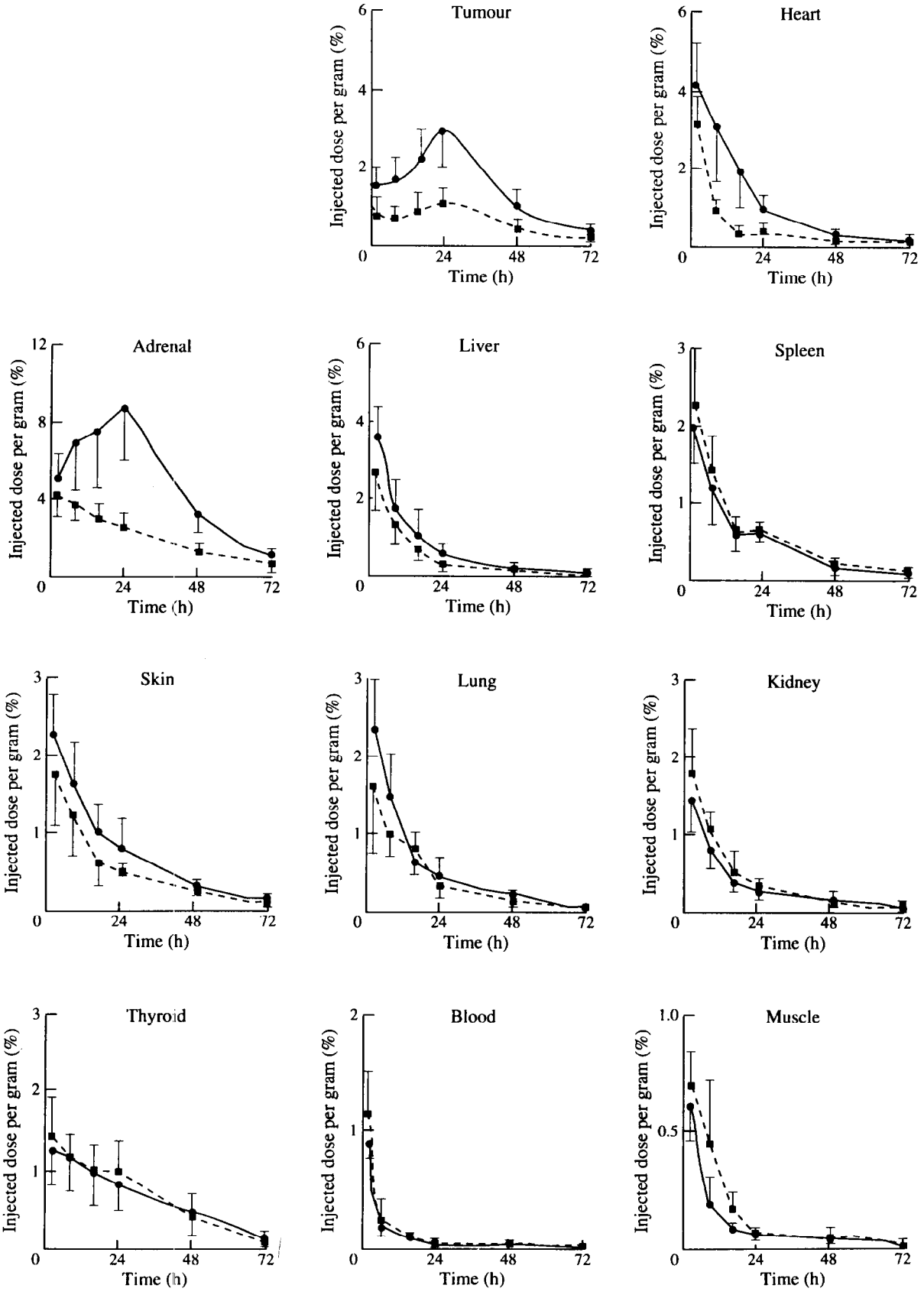

Figure 2. Time-dependent uptake of n.c.a. [^{131}I]MIBG (•) and ex.[^{131}I]MIBG (■) in tumour and normal tissues of nude mouse xenografts. Means and standard deviations of 7 determinations.

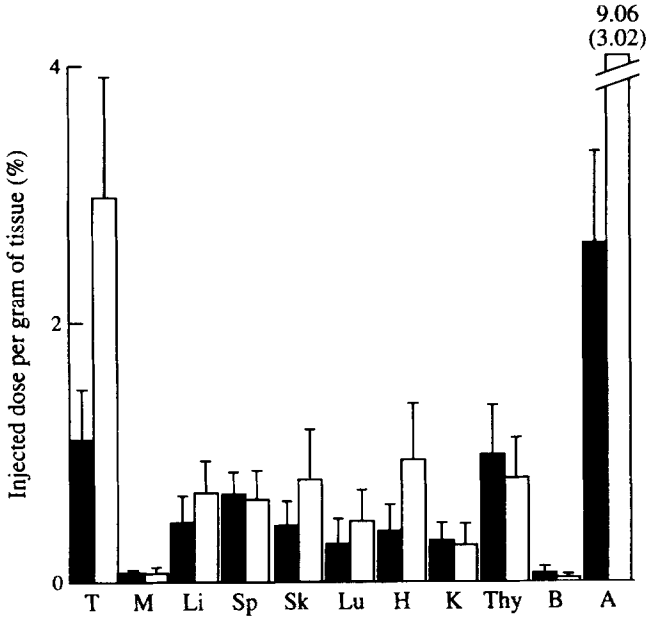

Figure 3. 24 h biodistribution in nude mouse xenografts of n.c.a. [^{131}I]MIBG (open bars) and commercial therapy preparation [^{131}I]MIBG (solid bars). T, tumour; M, muscle; Li, liver; Sp, Spleen; Sk, skin; Lu, lung; H, heart; K, kidney; Thy, thyroid; B, blood; A, adrenal. Means and standard deviations of 7 determinations.

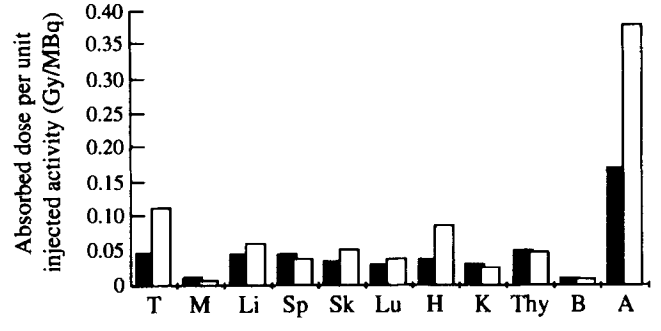

Figure 4. Calculated values of absorbed radiation dose per unit injected activity (Gy/MBq) to tumour and normal organs for n.c.a. (open bars) and exchange (solid bars) preparations of [^{131}I]MIBG. These were calculated by estimating the areas under the time-activity curves, corrected for radionuclide decay, and multiplying by the equilibrium dose constant for ^{131}I. T, tumour; M, muscle; Li, liver; Sp, spleen; Sk, skin; Lu, lung; H, heart; K, kidney, Thy, thyroid; B, blood; A, adrenal.

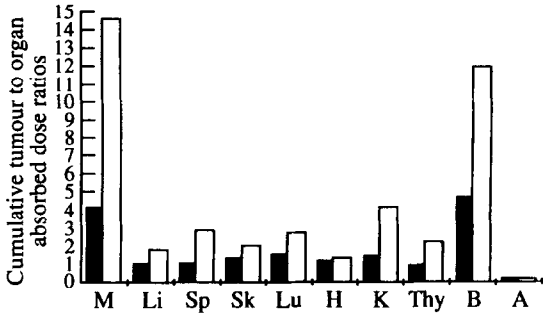

Figure 5. Calculated values of cumulative tumour to non-tumour absorbed dose ratios. These were produced by calculating the ratio of absorbed dose per unit injected activity for the tumour to that for normal tissues for either n.c.a. (open bars) or exchange (solid bars) preparations of [^{131}I]MIBG. The data indicate that n.c.a. [^{131}I]MIBG produces equal or greater tumour to non-tumour absorbed dose ratios compared with the exchange preparation for all normal organs examined. M, muscle; Li, liver; Sp, spleen; Sk, skin; Lu, lung; H, heart; K, kidney; Thy, thyroid; B, blood; A, adrenal.

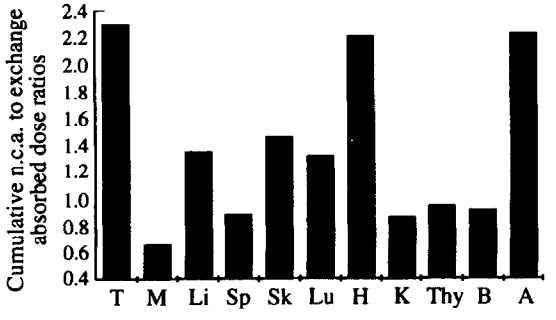

Figure 6. Cumulative n.c.a. [^{131}I]MIBG preparation to exchange [^{131}I]MIBG preparation absorbed dose ratios. These were produced by calculating the ratio of absorbed dose per unit injected activity for n.c.a. [^{131}I]MIBG to that for ex. [^{131}I]MIBG for tumour and all organs. These data indicate that the tumour dose is predicted to be greater by a factor of approximately 2.3 for n.c.a. compared with exchange preparations. Absorbed doses to heart and adrenals are also higher by a similar factor. The radiation doses to other organs are in the range (+45% to −35%) compared with the exchange preparation. T, tumour; M, muscle; Li, liver; Sp, spleen; Sk, skin; Lu, lung; H, heart; K, kidney; Thy, thyroid; B, blood; A, adrenal.

at a range of low specific activities was undertaken in PC-12 (pheochromocytoma) and SK-N-SH (neuroblastoma) xenografts in nude mice [29]. The highest specific activity studied (1.5 GBq/mg) was equivalent to that of commercial, exchange preparation [^{131}I]MIBG used for therapy. In accordance with our results, uptake by PC-12 tumours was proportional to specific activity, whereas SK-N-SH tumour uptake was unaffected by a drop in specific activity from 1.5 GBq/mg to 15 MBq/mg. Since neurosecretory granules are absent from SK-N-SH cells but are responsible for retention of MIBG in PC-12 cells [30], it is possible that the mechanism of storage influences the effect that plasma drug concentration has upon tumour concentration of MIBG. It remains to be established whether the observed relationship between specific activity and SK-N-SH tumour uptake will also be obtained at the higher specific activities achievable using n.c.a. [^{131}I]MIBG.

In clinical practice, injected activities of MIBG are usually restricted by bone marrow toxicity. If the absorbed dose to the bone marrow is directly proportional to blood dose, the n.c.a. preparation would deliver a slightly reduced bone marrow dose. This suggests that approximately the same or perhaps slightly higher activities of the n.c.a. preparation could be administered for therapy. Obviously, the biodistribution of n.c.a. [^{131}I]MIBG will have to be assessed in patients before any definitive conclusions can be drawn. However, the calculations described here suggest that an enhancement of tumour absorbed dose by a factor of 2 to 2.5 may be possible, albeit at the expense of an increased dose to the heart and adrenals.

The commercial preparation of therapeutic [^{131}I]MIBG entails the substitution of radioiodine for stable iodine on cold MIBG. This is accomplished by refluxing the reactants at 150°C in a sealed container for 1 h. The reaction is easy to perform, requires minimal handling of radioactivity and does not involve chromatographic purification of the radiopharmaceutical. These are all practical advantages over the current method used for the preparation of n.c.a. [^{131}I]MIBG. However, it would be

fortuitous if the more convenient radiosynthetic method yielded the most useful therapeutic product. Unfortunately, the commercially available preparation consists mainly of cold MIBG, necessitating the administration of about 7 mg of drug in a therapy dose of ex.[^{131}I]MIBG. To avoid substantial elevation of blood pressure, this biogenic amine is infused over at least 1 h. Thus, even if the n.c.a. preparation offered no advantage in terms of therapeutic efficacy, the same radioactive dose could be administered with only a few micrograms of total drug, thereby circumventing the possibility of pressor effects.

In conclusion, the results of these studies in human neuroblastoma spheroids and athymic mice bearing human neuroblastoma xenografts indicate significant advantages for no-carrier-added compared with commercially available [^{131}I]MIBG. Pharmacokinetic investigations are currently under way to determine whether similar advantages can be obtained in patients.

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