Toxicity to neuroblastoma cells and spheroids of benzylguanidine conjugated to radionuclides with short-range emissions

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Summary Radiolabelled meta-iodobenzylguanidine (MIBG) is selectively taken up by tumours of neuroendocrine origin, where its cellular localization is believed to be cytoplasmic. The radiopharmaceutical [¹³¹I]MIBG is now widely used in the treatment of neuroblastoma, but other radioconjugates of benzylguanidine have been little studied. We have investigated the cytotoxic efficacy of beta, alpha and Auger electron-emitting radioconjugates in treating neuroblastoma cells grown in monolayer or spheroid culture. Using a no-carrier-added synthesis route, we produced [¹²⁹I], [¹³¹I] and [²¹¹At]-labelled benzylguanidines and compared their in vitro toxicity to the neuroblastoma cell line SK-N-Be(2)c grown in monolayer and spheroid culture. The Auger electron-emitting conjugates ([¹²⁹I]MIBG and [¹³¹I]MIBG) and the alpha-emitting conjugate ([²¹¹At]MABG) were highly toxic to monolayers and small spheroids, whereas the beta-emitting conjugate [¹³¹I]MIBG was relatively ineffective. The Auger emitters were more effective than expected if the cellular localization of MIBG is cytoplasmic. As dosimetrically predicted however, [²¹¹At]MABG was found to be extremely potent in terms of both concentration of radioactivity and number of atoms administered. In contrast, the Auger electron emitters were ineffective in the treatment of larger spheroids, while the beta emitter showed greater efficacy. These findings suggest that short-range emitters would be well suited to the treatment of circulating tumour cells or small clumps, whereas beta emitters would be superior in the treatment of subclinical metastases or macroscopic tumours. These experimental results provide support for a clinical strategy of combinations ('cocktails') of radioconjugates in targeted radiotherapy.

Keywords: meta-iodobenzylguanidine; neuroblastoma; targeted radiotherapy; astatine

Meta-iodobenzylguanidine (MIBG) is a structural analogue of the neuroadrenergic blocking drugs bretyllium and guanethidine. It is selectively accumulated by an active mechanism in cells of neural crest origin. Radiolabelled MIBG allows the scintigraphic imaging of neural crest tumours (Weiland et al, 1980) and [¹³¹I]MIBG is used in the treatment of neuroblastoma and pheochromocytoma (Hartmann et al, 1987; Mastrangelo, 1987; Schwabe et al, 1987; Voute et al, 1988). Clinical experience since 1984 has demonstrated its potential, with an objective response rate of 35% in patients with progressive heavily pretreated disease (Hoenfagel, 1994). Clinical studies are now evaluating the role of [¹³¹I]MIBG at an earlier stage in therapy, either as a first line treatment or in combination with other treatment modalities (DeKraker et al, 1995; Gaze et al, 1995; Mastrangelo et al, 1995).

Although many patients show clinical benefit to [¹³¹I]MIBG treatment, a significant number subsequently relapse from previously undetected tumour sites (Sisson et al, 1989). This suggests that microtumours below the limit of clinical detectability have survived [¹³¹I]MIBG therapy. A possible explanation for the relative sparing of micrometastases is related to the microdosimetry of [¹³¹I] targeted radiation. [¹³¹I] emits β particles with a mean path length of 800 μm. Therefore, in addition to targeted cells, neighbouring cells will be irradiated by crossfire. In large tumours, this may be advantageous, as underdosage due to heterogeneity of uptake could be circumvented. However, as tumour size decreases the fraction of energy absorbed by the tumour becomes progressively smaller and more of the energy is deposited outside the target (Humm, 1986). Mathematical modelling studies predict that, for [¹³¹I], the optimal diameter range for curability is 2.6–5.0 mm (Wheldon et al, 1991; O’Donoghue et al, 1995). Tumours below this size are operationally resistant to [¹³¹I] β-emissions as the fraction of energy they absorb is greatly reduced. Experimental evidence supporting these predictions has been provided by our in vitro investigation using a multicellular spheroid model (Gaze et al, 1992) and independently by the recent studies of Weber et al (1996). The possible sparing of small micrometastases by a single agent [¹³¹I]MIBG provides a rationale for the incorporation of [¹³¹I]MIBG in combined-modality treatments (Gaze and Wheldon, 1996). In addition to underdosing of small tumour deposits, long-range β-emissions may also damage surrounding normal tissues. A large proportion of the patients treated with [¹³¹I]MIBG suffer significant haematological toxicity, which may be partially due to radiation crossfire to haemopoietic cells from MIBG-targeted neuroblastoma cells infiltrating the marrow (Gelfand, 1993).

Because of these limitations of [¹³¹I], alternative radiohaloconjuga-
gates of benzylguanidine have been proposed. Short-range emitters with therapeutic potential include [¹²⁹I], [¹³¹I] and [²¹¹At]. [¹³¹I] and [¹²⁹I] decay by electron capture and internal conversion. These processes result in the emission of low-energy 'Auger' electrons, which have ultra-short range (< 1 μm) and are densely ionizing.
Auger electron emitters deposit highly localized energy, resulting in severe damage to molecular structures in the immediate vicinity of the decay site. This has important consequences for the treatment of micrometastases. Firstly, the efficacy of Auger electron-emitters will be unaffected by the same size constraints as $^{131}$I, with the result that the energy they deposit will be more fully absorbed in small tumour volumes. In addition, crossfire to adjacent non-target sites will be negligible, resulting in the sparing of surrounding normal tissues.

$^{[23]}$I-MIBG has been shown to be toxic to neuroblastoma cells in vitro (Bruchelt et al., 1988; Guerreau et al., 1990; Senekowitsch et al., 1992). In contrast, in vivo data from mice bearing microscopic disease demonstrated no difference in tumour survival between control and $^{[123]}$I-MIBG-treated animals (Rutgers et al., 1994). Despite these conflicting laboratory findings, $^{[123]}$I-MIBG has been used to treat stage III and IV neuroblastoma patients with bone marrow involvement with encouraging results (Sisson et al., 1990, 1991, 1996; Hoefnagel et al., 1991).

Another radiohalogen with potential for the treatment of micrometastatic disease is $^{211}$At. Its $\alpha$-decay particles have a range of 50–100 $\mu$m in tissues, corresponding to a few cell diameters. These emissions have high linear energy transfer quality and hence strong biological effectiveness. Several investigators have shown that astatinated compounds are extremely toxic to experimental neoplasms, including melanoma and osteosarcoma (Link and Carpenter, 1992; Vergote et al., 1992; Larsen et al., 1994). In addition, it has been shown to be a particularly effective means of eradicating metastatic disease due to the short range and potency of its $\alpha$-particles (Link and Carpenter, 1990; Link et al., 1996). It has been suggested that $^{211}$At-labelled benzylguanidine (MABG) may be effective in the treatment of neuroblastoma micrometastases (Shapiro and Gross, 1987; Mairs et al., 1991). This radiopharmaceutical has been synthesized, and its uptake and toxicity have been characterized in vitro (Vaidyanathan and Zalutsky, 1992; Strickland et al., 1994). With respect to kinetics of accumulation and retention MABG was identical to MIBG. However, MABG was much more toxic to neuroblastoma cells than MIBG.

The advent of chemical syntheses that produce no-carrier-added (n.c.a) radiohalogenated benzylguanidines (Vaidyanathan and Zalutsky, 1993) has facilitated the evaluation of the potential of short-lived radionuclides such as $^{123}$I and $^{211}$At for neuroblastoma therapy. We therefore undertook a comprehensive in vitro study in neuroblastoma cell monolayers and multicellular spheroids to examine the toxicity of four different n.c.a preparations of benzylguanidine: $^{[123]}$I-MIBG, $^{[123]}$I-MIBG, $^{[211]}$I-MIBG and $^{[211]}$At-MABG.

**MATERIALS AND METHODS**

**Cell lines**

The human neuroblastoma cell line SK-N-BE (2c) was used for these studies. This cell line was derived from the bone marrow of a patient with progressive neuroblastoma following treatment with radiotherapy and chemotherapy (Beidler et al., 1978). The MCF-7 cell line was used as a negative control. This is a human breast cell line derived from the pleural effusion of a breast carcinoma patient (Soule et al., 1973).

**Culture conditions**

Cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, penicillin/streptomycin (100 IU ml$^{-1}$), fungizone (2 $\mu$g ml$^{-1}$) and glutamine (200 $\mu$m) (all Gibco, Paisley, UK). Cells were grown in a 5% CO$_2$ atmosphere at 37°C.

**No-carrier-added synthesis of radiohaloanalogues of MIBG**

Chemicals were purchased from Aldrich Chemical Company (Dorset, UK). HPLC-grade solvents were obtained from Rathburn Chemicals (Peeblesshire, UK). Carrier-free sodium $^{[23]}$Iodide and sodium $^{[23]}$Iodide were purchased from Amersham International (Buckinghamshire, UK). Carrier-free sodium $^{[123]}$Iodide was purchased from Cygne (Holland). No-carrier-added MIBG was synthesized by iododesilylation of trimethylsilylbenzylguanidine (Vaidyanathan and Zalutsky, 1993) and purified by HPLC and solid-phase extraction (Mairs et al., 1994a). $^{[211]}$At-MABG was synthesized as previously described (Vaidyanathan and Zalutsky, 1992).
Kinetics of MIBG uptake in SK-N-BE(2c) and MCF-7 cells

To determine the appropriate incubation period for cytotoxicity studies, experiments were undertaken to determine the kinetics of uptake of MIBG and sodium iodide (NaI). Cells were seeded in six-well plates at an initial density of \(0.5 \times 10^5\) cells and cultured for 48 h. They were then assayed for MIBG or NaI uptake at 30-min intervals for a 5-h period. For the determination of MIBG incorporation, the cells were incubated with 7 kBq of \(^{[131]I}\)MIBG (specific activity 45–65 MBq mg\(^{-1}\), Dupont Radiochemicals, Hertfordshire, UK). Non-specific uptake was measured in the presence of 1.5 mM desmethyliquipramine (DMI) (Sigma-Aldrich, Dorset, UK). For NaI uptake, the cells were incubated with 7 kBq of \(^{[131]I}\)NaI (Amersham International, UK). After incubation for the appropriate time, medium was removed, the cells were washed with phosphate-buffered saline (PBS) and radioactivity was extracted using two aliquots of 10% (v/v) trichloroacetic acid. The activities of the extracts were then measured in a gamma-well counter. Specific uptake of MIBG, expressed as c.p.m. per \(10^5\) cells, was calculated by subtracting values obtained in the presence of DMI from total uptake.

Clonogenic assays

Cells were seeded in 25-cm\(^2\) flasks at \(2.5 \times 10^5\) cells per flask. After 2 days, medium was removed and replaced with 4 ml of fresh medium containing the appropriately labelled benzylguanidine at the desired radioactivity concentration. After incubation for 2 h, medium was removed and the cells were washed three times with PBS. Cells were then trypsinized and counted. For each radioactivity concentration, three flasks were seeded at 1000 cells per flask. For greater radioactive concentrations (> 400 kBq ml\(^{-1}\)), additional flasks were seeded at higher cell numbers to compensate for the potential increase in toxicity. Flasks were equilibrated with 5% carbon dioxide and then incubated at 37°C. After 14 days, medium was removed and the colonies were fixed and stained with carbol fuchsin. Colonies of more than 50 cells were counted using an automated colony counter (Artek Systems Corporation).

Spheroid studies

In this study, we used two sizes of spheroid of approximately 240 \(\mu\)m and 400 \(\mu\)m diameter. These were grown by the continuous stirring of \(3 \times 10^6\) SK-N-BE(2c) cells in Techne stirrer flasks. Visible spheroids developed in 4–6 days.

For cytotoxicity studies, aliquots of spheroids of the required diameter were transferred to 20-ml universal containers and suspended in 1 ml of RPMI containing the appropriate concentration of radiolabelled drug. Spheroids were then incubated for 2 h at 37°C. This incubation time was chosen because it has previously been shown that MIBG accumulation by spheroids is close to maximal after 2 h (Mairs et al, 1991). The medium was then removed and the spheroids washed three times with PBS. Spheroids were then transferred to Petri dishes and individually pipetted into agar-coated wells containing 1 ml of RPMI medium. One 24-well plate was used per treatment. These were incubated at 37°C in a 5% carbon dioxide atmosphere. Growth of the spheroids was monitored over the next 2–3 weeks by measurement of their cross-sectional area using a semiautomated image analysis system coupled via a television camera to a microscope. From these measurements the median volume of the spheroids was calculated to allow the construction of spheroid regrowth curves.

RESULTS

Kinetics of uptake of MIBG and NaI in SK-N-BE(2c) and MCF-7 cells

Preliminary experiments were undertaken to determine the rate of uptake of MIBG and NaI in SK-N-BE(2c) and MCF-7 cells. As Figure 1 shows, accumulation of MIBG by neuroblastoma cells was maximal after about 2 h. This incubation period was used in all subsequent experiments. MCF-7 cells demonstrated no ability to actively accumulate MIBG over the time period studied. Neither cell line showed any accumulation of NaI.
Effect of $^{[123]}$I-, $^{[125]}$I- and $^{[33]}$I-MIBG on SK-N-BE(2c) neuroblastoma monolayers

Clonogenic assays of neuroblastoma cell monolayers demonstrated that the two Auger-emitting conjugates were more toxic to neuroblastoma cells than $^{131}$I-labelled drug (Figure 2). Even at radioactive concentrations of 1000 kBq ml$^{-1}$, the surviving fraction for $^{131}$I-labelled material was only 0.4. In contrast, using $^{[123]}$I-MIBG, a 1 log cell kill was achieved at a concentration of 600 kBq ml$^{-1}$. $^{[123]}$I-MIBG was even more potent than $^{[123]}$I-MIBG. 1 log cell kill was apparent after exposure to approximately 300 kBq ml$^{-1}$ drug. It should be noted that the expression activity per ml does not give any information about the absorbed dose to cells. Consequently, no inference can be made about the shape of these survival curves and the type of radiation to which these cells have been exposed.

To control for the effects of non-specific irradiation by the isotopes used, monolayers were incubated with identical activities of unconjugated radioactive sodium iodides for 2 h. As Figure 3 shows, none of these was toxic. In addition, to demonstrate that the observed toxicity was the result of specific uptake of the labelled drug, the cell line MCF-7 was used as a negative control. This cell line does not express the noradrenaline transporter (Mairs et al., 1994b) and consequently has no capacity for active uptake of MIBG (see Figure 1). Again, none of the species tested caused inhibition of colony formation (Figure 4). It is concluded that the observed toxicity to neuroblastoma cells was due to the specific incorporation of labelled MIBG.

Effect of $^{[123]}$I-, $^{[125]}$I- and $^{[33]}$I-MIBG and $^{[131]}$AtMABG on small (240 μm) neuroblastoma spheroids

The relative effectiveness of the radiolabelled MIBG analogues was also determined using small (240 μm) spheroids. Determination of efficacy was derived from the regrowth part of the volume vs time plot (Wheldon et al., 1985). A result similar to

Figure 5  Growth curves of 240 μm-diameter SK-N-BE(2c) spheroids after treatment with (A) $^{[123]}$I-MIBG, (B) $^{[125]}$I-MIBG, (C) $^{[33]}$I-MIBG and (D) $^{[131]}$At MABG.

- control; ○, 0.1 MBq ml$^{-1}$; □, 1 MBq ml$^{-1}$; ■, 2 MBq ml$^{-1}$; ▲, 3 MBq ml$^{-1}$; ◦, 4 MBq ml$^{-1}$. For MABG ○, control; ○, 0.24 kBq ml$^{-1}$; □, 0.48 kBq ml$^{-1}$; ■, 0.74 kBq ml$^{-1}$; ▲, 1.24 kBq ml$^{-1}$; ◦, 1.51 kBq ml$^{-1}$. The ordinate is the common logarithm of the spheroid volume in units of (μm)$^3$. Each point represents the median of 24 measurements.
that obtained with cell monolayers was observed. Both $^{125}$I MIBG and $^{125}$I MIBG inhibited spheroid growth in a dose-dependent manner. $^{125}$I MIBG was the more effective radiopharmaceutical when compared on a radioactivity per ml basis: growth inhibition, defined as failure to regrow after 20 days, was apparent at 0.1 MBq ml$^{-1}$ $^{125}$I MIBG, whereas 1 MBq ml$^{-1}$ $^{125}$I MIBG was required to achieve a similar effect. $^{131}$I MIBG was again the least effective of the iodinated conjugates – significant growth delay was only apparent at a concentration of 3 MBq ml$^{-1}$. $^{131}$At MABG was the most toxic of all the conjugates investigated: treatment with 0.48 kBq ml$^{-1}$ was sufficient to inhibit growth (Figure 5).

**Effect of $^{131}$I, $^{125}$I and $^{131}$I MIBG on large (400 μm) neuroblastoma spheroids**

In contrast to the toxic effects of $^{125}$I MIBG and $^{125}$I MIBG on monolayer and small spheroids, $^{125}$I- and $^{125}$I-labelled conjugates were completely ineffective in the treatment of large (400 μm) spheroids. At the radioactive concentrations tested, no inhibition of growth was observed and all spheroids regrew at rates similar to controls. Conversely, $^{131}$I MIBG induced growth inhibition at concentrations greater than 1 MBq ml$^{-1}$ (Figure 6).

To control for the effects of non-specific irradiation, spheroids of both sizes were incubated with 4 MBq ml$^{-1}$ of the appropriate radioactive sodium iodide. For the MABG studies, control spheroids were incubated with 9.5 kBq ml$^{-1}$ of sodium astatide. No growth inhibition was observed in any of the spheroids (data not shown).

**DISCUSSION**

These results describe the comparative cytotoxic efficacies of alpha, beta and Auger electron-emitting radioconjugates of the targeting agent MIBG using a neuroblastoma cell line grown in monolayer and spheroid culture. Our findings clearly demonstrate a relationship between the physical characteristics of radionuclides and their therapeutic effectiveness in experimental in vitro therapy (Table 1). The Auger electron-emitting conjugates of MIBG ($^{125}$I MIBG and $^{131}$I MIBG) were capable of killing single cells and small spheroids. However, their toxicity was reduced in larger target volumes. Incomplete drug penetration and lack of crossfire probably account for the absence of growth inhibition in the 400 μm spheroids after exposure to $^{125}$I MIBG or $^{131}$I MIBG. Incomplete penetration of MIBG in neuroblastoma spheroids whose diameter exceeds 400 μm has previously been documented (Mairs et al., 1991). Therefore, $^{131}$I MIBG and $^{125}$I MIBG may have been toxic only to the outer, metabolically most active, cell layers. Adjacent untargeted cells would have continued to proliferate once they had been treated with cell monolayers which underwent no crossfire from their targeted neighbours. Consequently, growth would be unaffected. $^{131}$At MABG has already been shown to be significantly more toxic than $^{131}$I MIBG to neuroblastoma cell monolayers (Strickland et al., 1994). The results presented here confirm this effect in small spheroids. Its efficacy in large spheroids was not determined.

For the long-range β-emitting conjugate ($^{131}$I MIBG), the size dependence of toxicity was opposite to that of $^{131}$I MIBG and $^{125}$I MIBG, i.e. small spheroids were less vulnerable to $^{131}$I MIBG than large ones. This may be due to the dissipation of more β-decay energy outside small target volumes, as predicted from microdosimetric considerations (Wheldon et al., 1991; O’Donoghue et al., 1995) and in agreement with previous experimental findings (Gaze et al., 1992). This explanation is supported by the results of our data obtained from monolayer studies which indicated that, of the radioiodine isotopes examined, $^{131}$I was the least effective inhibitor of colony formation. Because of the planar geometry of cellular monolayers, most of the decay energy would have been deposited above and below the plane of the cells. These findings are in agreement with the results of a recent study by Weber et al. (1996), who compared the therapeutic effectiveness of $^{131}$I and $^{125}$I labelled MIBG in neuroblastoma spheroids. Using a mathematical model, the radiation dose rates within small spherical tumours could be calculated. These calculations predict that higher mean dose rates can be achieved by $^{125}$I MIBG than $^{131}$I MIBG in tumour diameters up to 100 μm. However, in larger tumours, the mean dose rates achieved by $^{131}$I labelled MIBG...
predicted that the administration of low concentrations of MIBG should decrease passive, relative to active, drug uptake, thereby enhancing the therapeutic ratio (Mairs et al., 1995). Furthermore, the number of radioactive atoms administered should be as low as possible to minimize the dose to normal organs that are capable of uptake with long-term retention (e.g. thyroid).

The results of this in vitro study suggest that short-range conjugates of MIBG could be effective in the treatment of metastatic neuroblastoma. A prerequisite to examining their potential in vivo is the availability of a realistic murine model of micrometastatic neuroblastoma. Recent studies using severe combined immune deficient (SCID) mice have demonstrated dissemination of human neuroblastoma cells to the liver, adrenal glands and bone marrow. Metastases in different organs had characteristic histopathology, and those in the bone marrow presented as syncytiun-like cell aggregates – features typically seen in patients (Bogemann, 1996). Such a model could be used to investigate the efficacy of 125I- and 211At-labelled benzylguanidine conjugates in vivo.

The observation that Auger electron emitters conjugated to benzylguanidine are capable of killing SK-N-BE(2c) neuroblastoma cells raises important questions about the subcellular location of MIBG and the mechanism of cell death. Several hypotheses could explain these results. MIBG may localize in the nucleus of neuroblastoma cells. It is also possible that there are other subcellular targets for Auger electrons, such as the mitochondria. Alternatively, particles other than Auger electrons emitted during the decay of 125I and 121I may have sufficient range to penetrate into the nucleus of a targeted cell. A fourth possibility is that MIBG labelled with Auger electron emitters mediates cell kill through apoptosis triggered by a novel mechanism.

The conventional opinion is that the critical cellular target for ionizing radiation damage is nuclear DNA. Therefore, ultra-short-range radionuclides, such as Auger electron emitters, should be toxic only if delivered to the nucleus of the target cell (Charlton, 1986). This has been confirmed by in vitro studies – using extracellular Na[125I], cytoplasmic [125I]iododihydrobodamine and nuclear [125I]UDR – which demonstrated that significant toxicity was associated only with the nuclear located 125I (Kassis et al., 1987). Similar results have also been observed in vivo by Link and colleagues, who compared the therapeutic efficacy of 125I and 211At-labelled methylene blue to treat mice bearing malignant melanoma. Only 211At-labelled methylene blue produced significant therapeutic effects. As the subcellular fate of this targeting agent is cytoplasmic, it was concluded that 125I-labelled methylene blue was too far away from the genome to produce cytotoxicity (Link et al., 1989). Although subcellular localization studies have demonstrated that MIBG concentrates mainly in the cytoplasm of neuroblastoma cells (Gaze et al., 1991; Clerc et al., 1993), the fixation procedures employed in these studies may have caused a redistribution of the drug (Smets et al., 1991). The demonstration that significant cell kill can be achieved with 125I or 121I-labelled MIBG could represent evidence for a nuclear localization of MIBG. The amount of drug accumulated at this site may be undetectable by conventional means but nevertheless capable of delivering a toxic dose of radiation to the cell nucleus.

Alternatively, MIBG conjugated to Auger electron emitters could be cytotoxic through damage to the mitochondria. Subcellular localization studies have identified mitochondria as sites of MIBG accumulation (Gaze et al., 1991). Mitochondrial toxicity could result from disruption of inner mitochondrial membrane proteins or by direct effects on the mitochondrial genome. Indeed,
mitochondrial DNA is susceptible to radiation damage because of its limited capacity for repair (Tritschler and Medori, 1993).

It is also conceivable that some particles emitted during the decay of $^{121}$I and $^{125}$I may have sufficient range to reach genomic DNA of a targeted cell despite cytoplasmic or perinuclear localization. Although the entire Auger and Coster–Kronig electron spectra for these radionuclides have not been measured experimentally, calculations using theoretical transition rates and energies indicate that both isotopes emit some electrons with ranges of the order of the radius of a mammalian cell (Howell, 1992; Sastry, 1992). Assuming a cytoplasmic location for MIBG, it is possible that such emissions would deliver significant doses of radiation to the nucleus. However, this possibility is not supported by the classical experiments of Kassis and colleagues described earlier (Kassis et al, 1987).

A more exotic possibility is that Auger electron irradiation of cytoplasmic targets achieves cell kill by an indirect route, such as the triggering of apoptosis in susceptible cells. Some workers have reported that apoptosis-mediated cell death may result from membrane damage and the consequent generation of ceramide (Jarvis et al, 1994; Obeid et al, 1994) and that this process may be initiated by ionizing radiation damage to cell membranes (Haimovitz-friedman, 1994). We intend to investigate this possibility using DNA-targeted or cell-membrane-targeted Auger emitters ($^{121}$I incorporated in IUDR or conjugated to concanavin A) to treat neuroblastoma cells which differ in their capacity for apoptosis and expect to be able to determine the role of membrane irradiation in apoptosis in apoptosis competent cells. These experiments should provide a definitive evaluation of the role of apoptosis and of membrane irradiation in cell kill by Auger electron-emitters.

Whatever the detailed mechanisms, it is clear that these alternative isotopes have potential in the treatment of neuroblastoma. Short-range Auger electron emitters are suited to the treatment of single cells or small clumps and longer range $\beta$-emitters to larger cell masses. Alpha-emitters are of very high molecular potency in treating single cells or small neuroblastoma spheroids, though their efficacy in the treatment of larger cellular aggregates is not yet evaluated. These results suggest that ‘combination cocktails’ of radionuclides may be beneficial in the treatment of disseminated cancer where a targeting agent exists for which alternative radioconjugates can be made. Benzylguanidine targeting of radiolabels to neuroblastoma cells may be the first example of a strategy with wide application in targeted radiotherapy.

CONCLUSION

The experiments reported demonstrate the theoretically expected relationships between radiotoxicity, radionuclide emission characteristics and the geometrical configuration of the target cell kill population, while posing some questions about mechanisms of cell kill by Auger electron emitters. These results suggest that the combined use of short-range and long-range particle-emitting radionuclide conjugates of benzylguanidine could enhance the therapeutic efficacy of targeted radiotherapy in neuroblastoma patients with disseminated disease.

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