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CHARACTERIZATION AND UPTAKE OF RADIOLABELLED META-IODOBENZYLGUANIDINE (MIBG) IN A HUMAN NEUROBLASTOMA HETEROTRANSPLANT MODEL IN ATHYMIC RATS

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Cells from an established human neuroblastoma cell line, SH-SY5Y, were demonstrated to grow and form solid tumours in nude rats. This cell line, which is an adrenergic subclone of the SK-N-SH cell line, has previously been used in differentiation model studies. The tumours retained the neuronal phenotype of the cultured cells, as evidenced by the expression of neuron-specific enolase (NSE) and chromogranin A + B. The transcription factor Isl-1, a protein expressed in subsets of neurons and endocrine cells as well as in neuroblastoma cells, was also expressed in the transplanted tumours, thus further verifying the retained phenotype of the cells under in vivo conditions. At scintigraphy utilizing ^{123}I-MIBG the optimal tumour/background ratio was obtained 20 h after injection. The assessment of tissue/serum ratios showed the highest uptake in the spleen (0.067% per gram of inj. activity), neuroblastoma tumours (0.067% per gram of inj. activity) and in the adrenals (0.065% per gram of inj. activity).

During the last decade increased interest has been focused on the use of radionuclides in the management of cancer (1, 2). Many different tumour-seeking radiopharmaceuticals are being used both for tumour localization and treatment. Radiolabelled meta-iodobenzylguanidine (MIBG) has been implemented in the treatment of neuroblastomas (3, 4). This malignancy is mainly a disease of early childhood. The tumour cells are derived from the peripheral nervous system and are generally thought to represent sympathetic neuroblasts (5, 6), which are frozen at different stages of neuronal differentiation (7).

The introduction of experimental systems utilizing immunodeficient animals, to which human tumour tissues or cell lines can be transplanted, has facilitated the evaluation of new treatment modalities. Such animal studies have also been performed recently with radioiodinated MIBG (8, 9). A number of neuroblastoma cell lines have been established over the years and have recently been subclassified into neuroblastomas and neuroepitheliomas (peripheral neuroblastomas), based on proto-oncogene expression and chromosomal 11:22 translocation in the latter groups of tumours (10). Cells from a number of neuroblastoma cell lines can be induced to differentiate further (11).

The aim of the present investigation was to establish an in vivo experimental system using the cell line SH-SY5Y, which is a subclone of the SK-N-SH cell line, established from a bone marrow aspirate of a patient with a thoracic neuroblastoma (12). Unlike most cultured human neuroblastoma cells, SH-SY5Y cells lack amplification of the N-myc gene (13), as does the major group of malignant neuroblastoma tumours. Furthermore, SH-SY5Y cells have the capacity to undergo neuronal differentiation in vitro (11, 14–16), thus being a suitable model for further studies with respect to MIBG uptake in relation to differentiation. In most in vivo studies nude mice have been used. Rats were chosen for our investigation since the

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possibilities of administrating MIBG and sampling serum are greater than would be the case with mice.

Material and Methods

Cell culture conditions. The adrenergic cell clone, SH-SY5Y derived from the human neuroblastoma cell line SK-N-SH (12, 17) was kindly provided by Dr June Biedler, Memorial Sloan-Kettering Institute, New York. In vitro the cells were routinely grown in Eagle's MEM-FCS supplemented with antibiotics as described (18).

Animals. Nude rats (Rowett RNu/RNu, ZfV, Hannover, Germany), a total of 10 rats/group, were kept in cages with filter tops and provided with autoclaved feed pellets, water ad libitum and sterile wood granulate bedding in a temperature- and light-controlled unit. Neuroblastoma cells (20×10^6) were injected subcutaneously with a 23 gauge syringe into the flanks of the animals. The size of each tumour was measured with a microcalliper and the volume was calculated with the help of Tomayko's formula (19). In the MIBG experiments the animals were anaesthetized with chloralhydrate and ^{123}I-labelled MIBG (Mallinckrodt, Petten, the Netherlands) at a dose of 2 MBq per animal was administered intraveneously in the tail vein. Thyroid blocking agents were not administered.

Tumour tissues. The animals in one group were sacrificed under general anaesthesia by bleeding 20 h after injection and tumours and normal organs were excised. In the initial experiments on neuroblastoma characterization, tumour tissues from the nude rats were divided and one half was snap frozen in liquid nitrogen and the other half fixed in formalin and paraffin-embedded. In the MIBG uptake quantitation experiments, tumour tissues and normal organs were analyzed for radioactivity in a well counter.

Immunoblotting. Freshly frozen tumour tissue and in vitro cultured SH-SY5Y neuroblastoma cells were solubilized in SDS sample buffer and thereafter subjected to SDS-polyacrylamide gel electrophoresis under reducing conditions, as previously described (20). After blotting on nitrocellulose filter, immunostaining was performed using a polyclonal rabbit antiserum raised against the Isl-1 protein. The detection system was based on alkaline phosphatase-conjugated antibodies (BioRad), as previously described (20). The Isl-1 antiserum (21, 22) was a kind gift of Dr Stefan Thor, Dept of Microbiology, Umeå University, Sweden.

Quantitation of tumour markers. Tumour tissue and in vitro cultured SH-SY5Y neuroblastoma cells were stored at $-70°C$ pending further use. Tissues and cultured cells were homogenized by 20 strokes in a 10 mM tris-HCl, at a pH of 7.4, and 5 mM $MgCl_2$ buffer using a Dounce homogenizer (pestle B). After centrifugation at $15\,000 \times g$ for 5 min at $+4°C$, the cleared homogenates were analyzed with radioimmunoassays for neuron-specific enolase (NSE) (NSE RIA kit, Pharmacia, Uppsala, Sweden) and chromogranin A + B (23). The NSE and chromogranin A + B levels were related to the total protein content, which was measured by a modified Lowry procedure (18).

Gamma camera imaging. Planar scintigraphic images from the posterior aspect were collected with a gamma scintillation camera (Nuclear Diagnostics, Hägersten, Sweden and London, UK) using a low-energy, general-purpose collimator. The uptake and distribution were monitored by consecutive scintigraphic examinations of each animal at 0, 12, 20, and 26 h after injection (hpi). Data were processed utilizing the region of interest (ROI) technique. ROIs were drawn over the tumour, whole body, lung and the right kidney in each animal. The right thigh was tumour-free in every animal included and was therefore regarded as representative of background activity. After correcting for decay, tissue/whole body activity ratios were calculated for each animal and plotted against the time from injection.

Results

Characterization of heterotransplanted tumours. The heterotransplanted SH-SY5Y cells grew rapidly in the nude rats, producing tumours with an average diameter of 10 ± 4 mm within 20 days of inoculation. Necrosis was visible in the central areas of the tumours after volumes greater than 2.5 cm^3 had been attained. The tumours macroscopically investigated in this study had not undergone necrosis. The transcription factor Isl-1, a protein expressed in subsets of neurons and endocrine cells (21, 22), was utilized as a marker of neuroblastoma cells. Tumour tissue and in vitro cultured cells were subjected to SDS-polyacrylamide gel electrophoresis under reducing conditions. The results obtained from the immunoblotting experiment are shown in Fig. 1; three distinct bands were obtained in the tumour tissue material as was also the case with the in vitro cultured cells, and these corresponded to molecular weights in the 38–40 kD range. The specificity of the antiserum and the location of the polypeptide bands verified the identity of the Isl-1 proteins. The results from the quantification of NSE and chromogranin A + B and the characteristics of the tumours are shown in Table 1: The hetero-transplanted tumour tissue was also investigated morphologically, see Fig. 2; the tissue exhibited typical histopathological features of human neuroblastoma with small cells which possessed a high nuclear/cytoplasm ratio.

Uptake of ^{123}I-MIBG. The distribution of radioactivity was monitored with a gamma camera in the first set of ten animals. The ROI-data, presented as tissue/whole body activity, are reproduced in Fig. 3, where 0 h post injection refers to the first minute after injection. No tumour uptake was seen at 0 h post injection. The activity in the kidney/adrenal region was intense and remained unchanged throughout the study. In the second set of ten animals, sacrificed 20 h post injection, the radioactivity was assessed as a percentage of the injected activity per gram tissue and was highest in the spleen, the adrenals and the tumours, see Fig. 4.

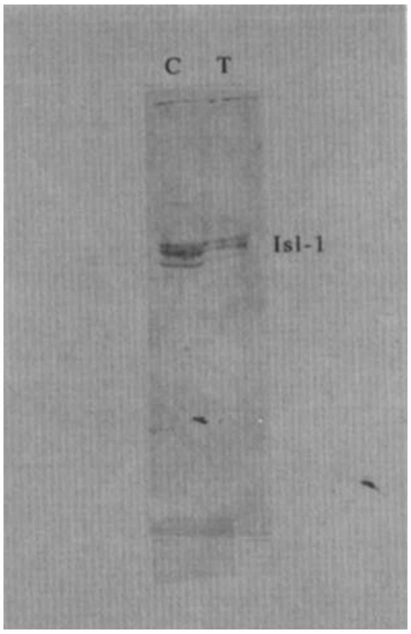

Fig. 1. Detection of tumour Isl-1 by immunoblotting. SH-SY5Y tumour tissue (T) and cultured SH-SY5Y cells (C) were solubilized in sample buffer, proteins were separated by SDS-polyacrylamide (10%) gel electrophoresis under reducing conditions and transblotted onto a nitrocellulose filter. The Isl-1 immunoreactivity was visualized by alkaline phosphatase-conjugated secondary antibodies and the Isl-1 protein triplet was identified by its molecular weight.

Table 1

Analysis of neuron-specific enolase (NSE) and chromogranin A + B in lysates from in vitro cultures SH-5SY cells and from tumour heterotransplants. n denotes the number of specimens

	NSE		Chromogranin A + B	
	n	ng/mg protein	n	ng/mg protein
SH-SY5Y cells	4	617 ± 37	8	> 1000
Tumour tissue	8	558 ± 281	8	> 1000

Table 2

Uptake of ^{123}I-MIBG in different tissues presented as a percentage of the injected activity per gram tissue together with the observed ranges. Average values of 10 rats

Tissue	Average value	Range
Lung	0.029	(0.021–0.040)
Blood	0.009	(0.08–0.12)
Heart	0.060	(0.56–0.65)
Spleen	0.067	(0.52–0.81)
Muscle	0.014	(0.13–0.17)
Pancreas	0.020	(0.016–0.022)
Liver	0.018	(0.014–0.023)
Kidney	0.021	(0.023–0.019)
Prostate	0.035	(0.028–0.043)
Adrenal	0.065	(0.060–0.072)
Tumour (SH-SY5Y)	0.067	(0.061–0.077)

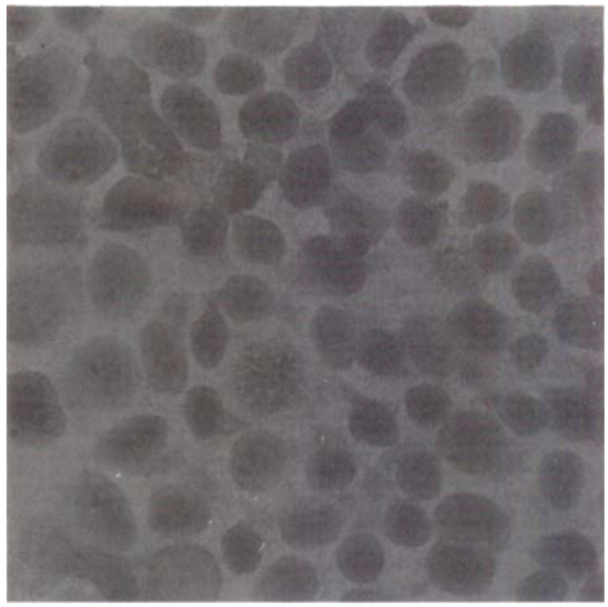

Fig. 2. Human neuroblastoma SH-SY5Y cells grown as xenografts in athymic (nude) rats. The specimen was formalin-fixed and paraffin-embedded and thereafter stained with eosin.

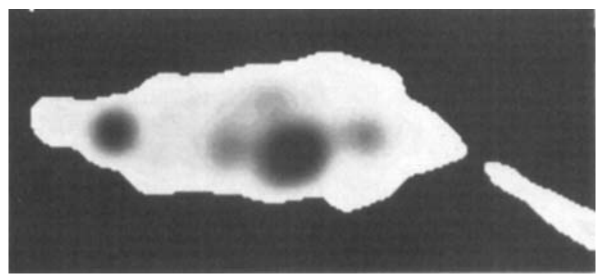

Fig. 3. Scintigraphic images of a rat, inoculated s.c. in the left flank with the human neuroblastoma cell line SH-SY5Y, 20 h from injection of ^{123}I-MIBG. A distinct tumour uptake can be seen in the left flank corresponding to the tumour. A marked uptake in the thyroid is also visible in the left part of the image.

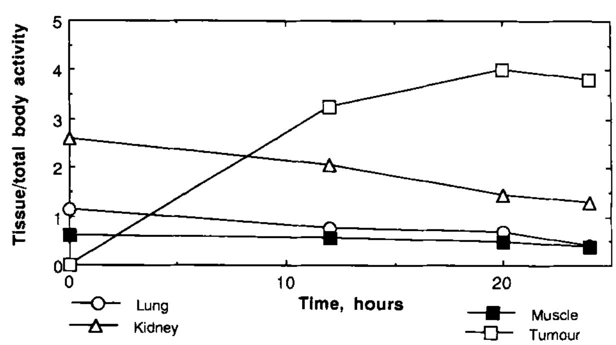

Fig. 4. Tissue/whole body activity ratios after 2 min and 12, 20 and 26 h post injection of ^{123}I-MIBG, calculated from region of interest (ROI) data in the scintigraphic images. The highest tumour/background ratio was seen 20 h after injection.

Discussion

MIBG targets the human neuroblastoma cell line SH-SY5Y cell line in vivo. These results are in agreement with previously reported data on neuroblastoma cells xenografted to athymic mice (8, 9, 24). The rat model enables an easier administration of MIBG intravenously in a tail vein compared with a mouse model. This offers the possibility of studying early uptakes, in contrast to the intraperitoneal administration route commonly used in mice models. Repeated serum samples can also be more easily drawn and in larger volumes than in a mouse system, thereby facilitating the monitoring of tumour markers that reflect treatment response. One important question that arises when attempting to translate the in vitro characteristics of a cell line to the in vivo situation is whether the phenotypic characteristics have been altered. The results obtained in this study support the assumption that the characteristics of the neuroblastoma cells have not altered in a major way, as judged from the general phenotypic appearance of the tumour and since high levels of NSE and chromogranin A + B in the tumours still could be detected after the transplantation. Further support for this assumption of an unaltered phenotype is the expression of the transcription factor Isl-1, which is comparable in both the in vitro cell line and in the transplanted tumours. Unlike most cultured human neuroblastoma cells, SH-SY5Y cells lack amplification of the N-myc gene, as does the major group of malignant neuroblastoma tumours. The experimental tumours, in this respect, more closely resemble the clinical situation and may therefore be of potential interest in further studies, as regards systemic radiotherapy. The SH-SY5Y cell line, an adrenergic clone of the SK-N-SH, has the capacity to undergo neuronal differentiation in vitro. However, it is still too early to speculate whether the SH-SY5Y tumour model is suitable for differentiation experiments in vivo. Such studies are under way at our laboratory.

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