¹²³I metaiodobenzylguanidine (MIBG) scintigraphy of retinoblastoma: preliminary experience

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SUMMARY ¹²³I metaiodobenzylguanidine (MIBG) is a radiopharmaceutical used for imaging neural crest tumours. The possibility of using ¹²³I MIBG for imaging retinoblastomas has been assessed in this pilot study. Ten patients were studied, nine with clinically and histologically proved retinoblastomas and one with Coats's disease. ¹²³I MIBG scintigraphy correctly identified the neoplasm in eight patients but gave a negative result in two, one of whom had Coats's disease and the other a retinoblastoma which proved to be extensively necrotic on histological examination. These preliminary results suggest that ¹²³I MIBG scintigraphy may have a role in differentiating retinoblastomas from lesions that simulate them.

Retinoblastoma is the commonest primary intra-ocular malignant tumour of childhood. It is a poorly differentiated neuroblastic neoplasm which may arise in any of the nucleated layers of the retina. The clinical diagnosis of retinoblastoma is rarely difficult when there is a family history of the tumour, when the ocular media are clear, and when the retina is not detached. Large tumours are more difficult to diagnose, but in bilateral cases examination of the second eye may help by demonstrating a typical early lesion. In unilateral retinoblastoma, and especially when this occurs in an older child, it may be difficult to distinguish a retinoblastoma with an exophytic growth pattern from conditions characterised by an exudative retinal detachment such as Coats's disease, and to differentiate a retinoblastoma with an endophytic growth pattern with copious vitreous seeds from inflammatory or nematode endophthalmitis.¹ A more serious though less frequent problem is failure to suspect retinoblastoma when it is present. In their analysis of 618 histologically proved cases Stafford, Yanoff, and Dornell² showed that almost 15% of cases had been misdiagnosed initially. The mortality rate is significantly increased by delay in enucleation of eyes with advanced tumours. It is also increased by attempts to establish the diagnosis by an intraocular biopsy procedure. This dilemma might be resolved

by the development of a diagnostic imaging test which is sensitive and specific for retinoblastoma.

Table 1 Comparison of clinical, scintigraphic, and histopathological data in patients with retinoblastomas

Patient	Clinical diagnosis	¹²³ I MIBG scintigraphy	^{99m} Tc-MDP scintigraphy	Histology	Grimelius staining	Tumour necrosis
1	Retinoblastoma	++ve	-ve	-ve	II	
2	Retinoblastoma	++ve	-ve	-ve	I	
3	Retinoblastoma	+ve	-ve	-ve	I	
4	Retinoblastoma	++ve	-ve	-ve	I	
5	Retinoblastoma	+ve	-ve	-ve	II	
6	Retinoblastoma	++ve	+ve	NP	NP	NP
7	Retinoblastoma	-ve	-ve	-ve	III	
8	Coats's disease	-ve	-ve	-ve	-	
9	Ectopic intracranial retinoblastoma	++ve	-ve	NP	NP	NP
10	Ectopic intracranial retinoblastoma	+ve	-ve	NP	NP	NP

NP=not performed.

MIBG=metaiodobenzylguanidine.

MDP=methylene diphosphonate.

-ve=grade 0 (no uptake).

+ve=grade 1 (minimal uptake).

++ve=grade 2 (intense uptake).

Grade I=tumour mostly viable with little necrosis.

Grade II=much viable and necrotic tissue.

Grade III=tumour mostly necrotic with little viable tissue.

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Radioactive tracers like ³²P³ and ¹³¹I chloroquine⁴ have been used for localising these tumours, with variable results. In the last decade radioiodinated metaiodobenzylguanidine (MIBG) has been used extensively to image pheochromocytomas, paragangliomas,⁵ neuroblastomas,^{6,7} carcinoids,⁸ and other neural crest tumours containing cytoplasmic neurosecretory granules.⁹ MIBG is an analogue of guanethedine and shares many cellular transport properties with noradrenaline. It is transported across the cell membrane via the neuronal uptake-one system which is an active process and is dependent on the sodium pump¹⁰ and by a sodium-dependent pathway which is probably passive diffusion.¹¹ From the cell cytoplasm MIBG is transported across the granular membrane of the neurosecretory granules via a proton pump¹² and stored in the neurosecretory granules.¹³ Since retinoblastomas are of neural origin,¹⁴ we postulated that these tumour cells would share the metabolic pathway of specific MIBG uptake and storage with other neural tumours and could thus be imaged. The study was undertaken to evaluate this hypothesis.

Materials and methods

PATIENTS

Ten patients, aged 6–48 months, were included in this study. Five patients had a known primary retinoblastoma, three had a doubtful clinical diagnosis of retinoblastoma, and two patients had bilateral disease with ectopic intracranial retinoblastoma (EIR) (Table 1). In the two patients with EIR the ocular tumours were treated by external beam radiotherapy, and at the time of imaging there was no evidence of active ocular disease. Each patient underwent extensive ophthalmological and systemic examination to evaluate the stage of the disease. ^{99m}Tc-methylene diphosphonate (MDP) radionuclide bone imaging was carried out as part of the routine staging procedure in each case. In eight patients enucleation of the involved eye was undertaken.

IMAGING PROTOCOL

Prior to injection of ¹²³I MIBG a drug history was taken to rule out the possible use of drugs which interfere with tracer uptake.⁵ Each patient received 400 mg of potassium perchlorate to block the uptake of free iodine by the thyroid. Between 74 and 185 MBq (2–5 mCi) of ¹²³I MIBG (Amersham International PLC) was given intravenously over a period of 20–30 seconds with the patient lying supine. Images were obtained with a large field of view gamma camera (Siemens ZLC) with a high-resolution low-energy collimator and an on-line computer (PDP 11/34). The patient was

placed supine with the head fixed in a foam head support, and static images of 400 000 counts each were obtained for the skull in the anterior Waters view, anterior chest, anterior abdomen, posterior chest, and posterior abdomen at 4 h and 24 h after injection. Additional oblique and lateral images were obtained when needed. In all subjects mild sedation was required for the 24 h study to avoid patient movement, since obtaining each image took approximately 20–30 minutes, and movement of the head during this time interval would have degraded the image.

^{99m}Tc-MDP bone scanning was carried out 72–96 h after ¹²³I MIBG study in each case. Approximately 20 MBq/kg body weight of ^{99m}Tc-MDP was injected intravenously, and static images were obtained three hours after the injection, as in the ¹²³I MIBG study.

HISTOLOGICAL TECHNIQUE

The enucleated eye was immediately fixed in 10% formalin. The formalin fixed tissue was routinely embedded in paraffin wax, and sectioned at 4 µm. Sections were all stained with haematoxylin and eosin and for the purposes of this study by the argyrophil Grimelius method.¹⁵ The sections were examined by light microscopy. The numbers of viable cells in the tumour sections were scored on a scale of I–III: I=tumour mostly viable, with little necrosis; II=much viable and necrotic tissue; III=tumour mostly necrotic, with little viable tissue. Assessment of all tissue sections was carried out without prior knowledge of the ¹²³I MIBG scintigraphy results by an experienced pathologist (DAL).

DATA ANALYSIS

Image processing of the 4 h and 24 h digitised computer images was carried out by the unsharp masking technique.¹⁶ This technique helps to increase the detection of abnormalities by improving the signal to noise ratio. The digitised computer images (colour scale, unprocessed and processed) were analysed by four observers as a blind control study (JB, JLH, JEK, KEB). The images were classified with respect to their ¹²³I MIBG uptake intensity in the orbital region on the anterior view of the skull at 24 h into the following three groups: grade 0 no persistent uptake; grade 1 minimal, persistent, discernible uptake; grade 2 intense, persistent uptake (less than or equal to that in the adjacent salivary glands). The criteria for normal and abnormal distribution of the tracer in other organs were the same as described previously.¹⁷

Results

Of the 10 patients imaged ¹²³I MIBG scintigraphy was

Fig. 1 ^{123}I MIBG processed scintigraphic image 24 h after injection (case 1). Arrow head indicates uptake by the lacrimal gland, large arrow indicates uptake by the tumour, small arrow indicates uptake by the salivary gland.

positive in eight. Six of the eight positive patients proved to have a large retinoblastoma in one eye. ^{123}I MIBG scintigraphy correctly showed uptake in all six cases (Figs. 1, 2). The two remaining patients with positive results had previously treated bilateral ocular tumours, and currently had ectopic intracranial retinoblastoma. One of the two had both eyes removed for retinoblastoma and the other had been treated successfully with radiotherapy for tumour in both eyes. In these cases MIBG scintigraphy correctly identified the intracranial retinoblastoma (Fig. 3) but showed no orbital uptake. In two of the 10 patients scintigraphy was negative: one had a retinoblastoma and the second had Coats's disease.

Fig. 2 ^{123}I MIBG processed scintigraphic image 24 h after injection (case 5). Magnification $\times 2$. Small arrow indicates lacrimal gland uptake on the right side; the left lacrimal gland was not visualised in this case. Larger arrow indicates uptake in the left orbital region. Open arrow indicates normal salivary gland uptake.

Fig. 3 ^{123}I MIBG unprocessed scintigraphic image 24 h after injection. Left lateral view of the skull (case 9). Small arrow indicates uptake by the tumour in the pineal region. Large arrow indicates uptake by the salivary glands. Curved arrow indicates uptake by the thyroid gland due to incomplete blockage.

Normal bilateral uptake of ^{123}I MIBG was observed in the lacrimal glands of most patients (Fig. 1), except cases 3 and 5 (Fig. 2), where only one gland was visualised; no explanation could be found for this discrepancy. In cases 9 and 10 the lacrimal glands were not visualised owing to prior therapy for the orbital tumour in these two cases. The uptake was more prominent in the 4 h images than in the 24 h images. Distribution of the tracer in other organs was within normal limits.¹⁷

In three cases (1, 2, and 4) the activity was measured in the enucleated eye. The calculated activity, expressed as a percentage of the injected dose at 48 h, was 0.001%, 0.002%, and 0.007% respectively. Also at 48 h the ratio of activity in the enucleated eye to that in the same weight of blood drawn during removal of the globe was 1.29, 1.57, and 1.98 respectively.

On analysis of the images there was good agreement among the four observers in detecting the lesion with a mean value of 90% sensitivity. Of the 20 images analysed disagreement occurred on two unprocessed images in two subjects (3 and 5). All observers labelled these as negative, but called them positive on the corresponding processed images (Table 1). The overall sensitivity of ^{123}I MIBG for tumour detection was 90% and specificity was 100% in this limited series. $^{99\text{m}}\text{Tc}$ -MDP bone scan images showed normal distribution of the tracer in all patients except case 6, where uptake was seen in the left orbital region (Table 1).

Eight patients underwent enucleation. In seven of

these eight patients histological sections showed the tumours to be composed of sheets of small, densely packed, round cells with focal rosette formation and various degrees of necrosis and calcification. In some cases the bulk of the tumour was necrotic while in other cases there was relatively little necrosis. In one case (case 8) the findings were typical of Coats's disease, with exudative changes and cholesterol crystals present in the section. Grimelius staining was consistently negative on all cases (Table 1).

Discussion

This study has shown that retinoblastoma can be imaged with ¹²³I MIBG. Uptake of ¹²³I MIBG by the tumour shows a wide spectrum from no detectable activity to high tracer uptake. Overall uptake by retinoblastoma was low compared with other neural crest tumours.^{5,9,18} The uptake of ¹²³I MIBG by the tumour appears to be specific, since a non-specific agent like ^{99m}Tc-MDP showed no uptake in the orbital region in any patient except case 6, where uptake was seen. This was attributed to the dense calcification in necrotic tumour cells.

The absence of neurosecretory granules, as reflected by the negative Grimelius staining of tissue sections, may imply that the storage system for ¹²³I MIBG in these tumours is absent or deficient but the uptake mechanism remains functional. This is in keeping with the finding that the degree of tracer uptake was related to the proportion of viable cells in the tumour sections as seen in cases 2, 3, and 4. In cases where the tumour was extensively necrotic there was either no or poor uptake. Mechanisms for uptake of ¹²³I MIBG by these tumour cells are probably a combination of an active process (uptake-one system)^{10,11}; sodium-independent pathway which is probably passive diffusion of the tracer across the tumour cell membrane and retention in the cytoplasm^{10,11}; and breakdown of the blood-retinal barrier in case of retinoblastomas and/or blood-brain barrier in case of EIR, facilitating the entry of the tracer.

Previous radiotherapy or chemotherapy also reduced tracer uptake. This was seen in case 9, where the ocular tumours had been treated with radiotherapy and showed no uptake while the EIR showed good uptake. In the case of patient 10 radiotherapy for the ocular tumours and chemotherapy for EIR was also given. There was no uptake by the ocular tumours, and uptake by the EIR was low. This is in keeping with the findings of other investigators in the context of other neural crest tumours.^{6,8,18}

Some of the practical problems experienced during this study and the precautions needed to avoid them require mentioning. Since the overall tumour size was small, with low tracer uptake, and as the tumour

was surrounded by spatially inhomogeneous high background activity in the salivary glands and the nasopharyngeal region, proper camera positioning was important to get a clear delineation of the orbit from the adjacent activity. Patient movement was another limiting factor, especially in the 24 h study, since obtaining each image took approximately 25–30 minutes, and even a slight amount of head movement caused deterioration in the quality of the image. In small infants head movement occurred in spite of mild sedation and restriction by a foam head support. These problems can be solved if a high tumour to background ratio can be achieved. In this context the possibility of improving tumour uptake by means of other radiopharmaceuticals such as radiolabelled monoclonal antibodies is being explored.^{19,20}

In this limited series the sensitivity and specificity of MIBG in imaging retinoblastomas was high, but a larger series would be required to define its precise clinical role. It is hoped that ¹²³I MIBG may ultimately provide a simple, non-invasive technique for screening the whole patient for metastatic retinoblastoma in a single procedure. MIBG may also have a role in the assessment of disease response in patients following radiotherapy. This would require that treatment be shown not only to reduce ¹²³I MIBG uptake but also to correlate with the objective evidence of tumour shrinkage, cell necrosis, or extirpation of tumour tissue.

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