Radioimmunoscintigraphy of ocular melanoma with ^{99m}Tc labelled cutaneous melanoma antibody fragments

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SUMMARY The possibility of using radiolabelled monoclonal antibody fragments to image uveal melanomas has been assessed in a pilot study. ^{99m}Tc labelled F(ab')_2 fragments of MoAb 225.28S raised against cutaneous melanomas were used. Initially 10 patients were imaged. In five patients the clinical findings were typical of uveal melanoma. Immunoscintigraphy was positive in all five cases. In a further five patients there was doubt about the diagnosis. One was thought to have a choroidal haemangioma but failed to respond to treatment and immunoscintigraphy was positive, suggesting a diagnosis of melanoma. Two patients were assigned a diagnosis of choroidal haemangioma, one of melanocytoma or possible retinal pigment epithelium carcinoma, and one of metastasis. Immunoscintigraphy was negative in all these four cases. In combination with established diagnostic tests immunoscintigraphy may have a part to play in differentiating uveal melanoma from other similar tumours.

Malignant melanoma is the most frequently encountered primary intraocular neoplasm. Improved standards of eye care have resulted in more frequent discovery of asymptomatic lesions. These can be detected so early in their natural history that it may be difficult to distinguish between a small melanoma and a large choroidal naevus. A small symptomatic melanoma in turn may be similar in appearance to a choroidal haemangioma or a choroidal metastasis, particularly when relatively amelanotic. Because of the hazards of biopsy within the eye the ophthalmologist must differentiate between these lesions on clinical grounds aided by special investigations.

At present both radical and conservative options for treatment of uveal malignant melanoma rest largely on a clinical diagnosis. When the radical option is exercised or when a melanoma is treated by local excision, a tissue diagnosis is obtained, albeit retrospectively. On the other hand when an ocular melanoma is treated conservatively by photocoagulation or radiation, a tissue diagnosis may never be obtained.

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Although radioactive tracers have been used since 1952¹ to localise choroidal melanomas, no single nuclear imaging test has helped to answer these diagnostic problems specifically. The ^{32}P test has been used extensively in this context,^{2,3} but the limitations of this test have restricted its popularity.⁴⁻⁸ The test is more accurate for large tumours, for which the diagnosis is rarely in doubt, than for small lesions which may be difficult to diagnose. The short range in tissue of beta particles emitted by ^{32}P means that a surgical operation is required to place the probe over the tumour and over an unaffected area of the choroid for comparison. Several preclinical studies have identified other radiopharmaceuticals as likely agents to localise melanomas.⁹ Agents with melanin affinity—for example, quinolines,¹⁰ melanin precursors such as thiouracil,^{11,12} and non-specific tumour localising agents such as gallium¹³—have been evaluated and have had limited success.

Nuclear medicine has added a new dimension for localising tumours in the last decade by means of radiolabelled monoclonal antibodies (MoAb) against tumour associated antigens for diagnostic and therapeutic purposes.¹⁴ This study follows preliminary in-vitro work with monoclonal antibodies (MoAb) raised against cutaneous melanoma to recognise

antigenic similarity in ocular melanomas.¹⁵ The results were sufficiently encouraging to proceed to the use of these antibodies in vivo. It has been established that $F(ab')_2$ fragments are more effective for radioimmunodetection than whole MoAb, since non-specific uptake by the spleen, liver, and bone marrow is reduced and they are more rapidly cleared from the blood.¹⁶ Comparative studies on biodistribution of $F(ab')_2$ fragments with different radioisotopes (¹³¹I, ¹²³I, ¹¹¹In, and ^{99m}Tc) indicate that ^{99m}Tc- $F(ab')_2$ fragments are the most suitable for radioimmunoscintigraphy when there is rapid tumour uptake, because of the high signal generated from the target through the injection of a high activity short-lived radionuclide (half life of 6 h). ^{99m}Tc is also readily available, cheap, and has favourable dosimetric consideration.¹⁷ Based on these parameters it was decided to use ^{99m}Tc labelled $F(ab')_2$ fragments of MoAb 225.28S (provided by Sorin Biomedica) for the initial work.

Materials and methods

PATIENTS

Ten patients were studied serially. Five patients had a known primary uveal melanoma, and five had a doubtful clinical diagnosis of melanoma. The study has been accepted by the City and Hackney District Ethical Committee.

RADIOLABELLING

The antibody was provided in the form of an instant labelling kit Technemab-K-1 of Sorin Biomedica. Labelling was carried out by the addition of sterile ^{99m}Tc pertechnetate to a lyophilised preparation containing 350 µg of $F(ab')_2$ fragments of MoAb 225.28S, which is against a high molecular weight-melanoma associated antigen (HMW-MAA). After thorough mixing the solution was incubated for 15 minutes at room temperature. Free ^{99m}Tc was then removed by ion exchange column chromatography through sterile pyrogen free DEAE-Sephadex A25. This method gave 85–90% labelling, and residual free ^{99m}Tc was <5%. The labelled preparation was injected within one hour of being prepared.

IMAGING PROTOCOL

Prior to the study the procedure was explained to the patient and written informed consent obtained in each case. Before the intravenous injection of labelled antibodies the patient's skin was tested for hypersensitivity to mouse immunoglobulins. No reaction was noted in any patient. 400 mg of potassium perchlorate was given orally 30 minutes prior to the injection to block the thyroid. A large field of view gamma camera (GE 400AT) with a

general purpose collimator and an on-line computer (DEC Gamma 11) was used. The patient was placed supine with head fixed in a foam support. The camera was placed over the head in the anterior Waters view (caudal tilt of 15–30°). This position was chosen for the head to optimise separation of the orbits from interfering background, such as the nasal cavity and paranasal sinuses. An 18 gauge cannula was placed in an antecubital vein connected to a three-way stopcock. Between 340 and 370 MBq of ^{99m}Tc $F(ab')_2$ was placed through the three-way stopcock and flushed with 20 ml of 0.9% normal saline. Dynamic images were acquired on a 64×64 matrix on the computer at a frame rate of 1 second/frame for 60 frames followed by 30 s/frame for 18 frames with the camera placed over the head in the anterior Waters view. Static images were acquired on a 128×128 matrix on the computer with the camera over the head, chest, and pelvis for 400 000 counts each at 20 minutes, 1 h, 3 h, 5 h, and 6 h.

Results

Patient 1 was a 57-year-old white male with a large inferolateral choroidal malignant melanoma in the right eye measuring 18 mm in maximum diameter and 11 mm in thickness on ultrasound. Immunoscintigraphy was positive (Figs. 1a, 2). The eye was subsequently enucleated and histopathology

Fig. 1a

Fig. 1 Immunoscintigraphy images at 5 h. (T)= Tumour. (a) Choroidal melanoma right eye. (b) Haemangioma right eye. (c) Melanocytoma right eye.

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Fig. 1b

Fig. 1c

revealed a lightly pigmented, vascular, predominantly spindle cell malignant melanoma.

Patient 2 was a 35-year-old Asian male with a large ciliochoroidal melanoma in his right eye measuring 13 mm in maximum diameter and 9 mm in thickness and situated temporally. Immunoscintigraphy was strongly positive for the right eye but was also weakly so for the left (Table 1). The right eye was enucleated, and histopathology of the specimen

showed a heavily pigmented, mixed cell melanoma arising in the ciliary body. The patient had heavily pigmented fundi, but a possible flat choroidal naevus was identified in the left eye.

Patient 3 was a 43-year-old white female with a small, lightly pigmented malignant melanoma measuring 7 mm in maximum diameter and 3 mm in thickness and situated above the left macula. Growth of the lesion had been recorded photographically.

Fig. 2 Immunoscintigraphy images of patient 1. (T)= Tumour. (a) Static image at 20 minutes. (b) Static image at 6 h.

Table 1 Clinical and immunoscintigraphy data

Patient no	age	Clinical diagnosis	Immunoscintigraphy			
			Dynamic L	R	Static L	R
1	57	Melanoma R eye	-ve	-ve	-ve	+ve
2	35	Melanoma R eye	-ve	-ve	+ve	+ve
3	43	Melanoma L eye	-ve	-ve	+ve	-ve
4	34	Melanoma L eye	-ve	-ve	+ve	-ve
5	27	Melanoma R eye	-ve	-ve	-ve	+ve
6	35	Melanoma R eye	-ve	-ve	-ve	+ve
7	69	Haemangioma R eye	-ve	+ve	-ve	-ve
8	50	Melanocytoma R eye	-ve	-ve	-ve	-ve
9	53	Haemangioma L eye	-ve	-ve	-ve	-ve
10	45	Metastasis R eye	-ve	-ve	-ve	-ve

Immunoscintigraphy was positive, and the tumour was treated conservatively with a ^{106}Ru scleral plaque.

Patient 4 was a 34-year-old Asian female with a large ciliary body malignant melanoma invading the iris root nasally in the left eye. The tumour measured 12 mm in maximum diameter and 6 mm in thickness. Immunoscintigraphy was positive. Enucleation was advised but was refused. The patient was treated with ^{106}Ru plaque but enucleation was once more advocated for extrascleral extension and persistent glaucoma. Histopathology of the enucleated eye confirmed the presence of a heavily pigmented totally necrotic melanoma arising in the ciliary body.

Patient 5 was a 27-year-old Asian female with a large ciliochoroidal malignant melanoma situated temporally in the right eye and associated with a total serous retinal detachment. The tumour measured 18 mm in maximum diameter and 12 mm in thickness. Immunoscintigraphy was strongly positive. The right eye was enucleated. Histopathology of the enucleated eye revealed a pigmented, vascular, predominantly spindle cell malignant melanoma.

Patient 6 was a 35-year-old white male with a small non-pigmented mass superotemporal to the right macula and measuring 6 mm in maximum diameter and 3 mm in thickness. There was an extensive associated serous retinal detachment. The patient was in good health, and comprehensive systemic examination revealed no evidence of tumour elsewhere. Ultrasound and fluorescein fundus angiography were equivocal for choroidal melanoma or haemangioma. There was a family history of cutaneous haemangioma, and a tentative diagnosis of choroidal haemangioma was made. Neither laser photocoagulation nor subsequent lens sparing treatment with external beam radiotherapy to a dose of 15 Gy resulted in resorption of subretinal fluid. Immunoscintigraphy became available and was positive. The diagnosis was revised to choroidal

Fig. 3 *Patient 7. Non-pigmented choroidal mass nasal to the right optic disc.*

melanoma and conservative treatment was undertaken with ^{106}Ru scleral plaque.

Patient 7 was a 69-year-old white female with a non-pigmented mass nasal to the right optic disc considered by the referring ophthalmologist to be an amelanotic malignant melanoma (Fig. 3). The lesion measured 7 mm in maximum diameter and 2 mm in thickness on ultrasound. Fluorescein angiography showed very early, uniform (Fig. 4a), and subsequently intense (Fig. 4b) choroidal filling which was more suggestive of a choroidal haemangioma. Immunoscintigraphy showed a vascular blush in the involved eye on the initial dynamic images but the static images were negative (Fig. 1b). The diagnosis was revised to choroidal haemangioma, and treatment by laser photocoagulation was instituted.

Patient 8 was a 70-year-old white female with an extremely heavily pigmented mass arising just above and partially obscuring the right optic disc (Fig. 5). The maximum diameter of the lesion was 12 mm and it was 6 mm thick. Ultrasound also suggested that there was superficial invasion of the optic nerve. The differential diagnosis was considered to be between a melanocytoma, a juxtapapillary melanoma, and a carcinoma of the retinal pigment epithelium. Immunoscintigraphy was negative (Fig. 1c), and management was by serial observation with photography and ultrasound.

Patient 9 was a 53-year-old white male with a pale lesion at the superior margin of the left optic disc (Fig. 6). The tumour measured 9 mm in maximum diameter by 2 mm in thickness. Fluorescein fundus

Fig. 4a

Fig. 4b

Fig. 4 Patient 7. (a) Arteriovenous phase fluorescein angiography. (b) Late venous phase fluorescein angiography.

angiography was equivocal for a choroidal haemangioma or a melanoma. Immunoscintigraphy was negative. A diagnosis of choroidal haemangioma was made. There was no associated serous retinal detachment, and management was by serial observation with photography and ultrasound.

Patient 10 was a 45-year-old white male with a pale lesion immediately temporal to the right macula

measuring 7 mm in maximum diameter and 3 mm in thickness (Fig. 7). Fluorescein angiography was atypical of a choroidal haemangioma and the differential diagnosis lay between an amelanotic melanoma and a metastasis. Immunoscintigraphy was negative (Fig. 8). The patient, a non-smoker, was clinically well, but a small opacity was seen at the right hilum on plain chest x-ray, and subsequently a

Fig. 5 Patient 8. Heavily pigmented mass arising just above and partially obscuring the right optic disc.

Fig. 6 Patient 9. Pale choroidal mass superior to the left optic disc.

Fig. 7 Patient 10. Pale choroidal mass temporal to the right macula.

cystic lesion was demonstrated on CT. Bronchoscopic biopsy showed an adenocarcinoma.

The best scintigraphic images for tumour detection were obtained between 3 h and 6 h after injection (Fig. 2b); during this time interval the tumour/background ratio was highest. In some cases the tumour was visualised at 1 h after injection. The nasal cavity, heart, liver, kidneys, and bladder were visualised in all cases on the static images. In three patients

some splenic uptake were also noted. Maximum uptake was seen in the kidneys. In two cases, patients 1 and 4, cell impressions of the resected tumour were obtained. These were subjected to immunofluorescence staining; both specimens also gave a strong positive immunofluorescence with MoAb 225.28S in vitro.

Discussion

The HMW-MAA is a combination of two non-covalently associated glycopolypeptides. One has an apparent molecular weight of 280 K; the other is composed of a heterogeneous array of glycopolypeptides with molecular weights ranging from 300 K to 700 K.^{18,21} The HMW-MAA has not been detected in normal tissues except for clusters of Malpighian cells in epidermis and hair bulbs.^{21,22} Although the HMW-MAA carries the determinants for more than one MoAb, a subpopulation of HMW-MAA expresses only the determinant defined by MoAb 225.28S, and this subpopulation is present in primary and metastatic melanomas and benign skin naevi.²³ The results of imaging cutaneous melanomas in 43 patients with F(ab')₂ MoAb 225.28S indicate that this technique has a sensitivity of 77% and a specificity of 99%.²⁴

The use of an antibody raised against cutaneous melanoma for imaging ocular melanomas is potentially specific. It has been shown that the ocular melanomas have some similarity to the cutaneous melanomas in their antigenic features.¹⁵ This sharing of antigenicity can be used for diagnostic purposes.

Fig. 8 Immunoscintigraphy images of patient 10. (a) Static image at 20 minutes. (b) Static image at 6 h.

This small series covers some of the puzzling cases which present to the ophthalmic surgeon as a diagnostic dilemma, and it is considered that this technique has complemented other diagnostic methods in this context. A major problem is to differentiate a choroidal melanoma from a choroidal haemangioma. In the case of ocular melanoma this is achieved when a focal area of increased uptake is seen to develop with time, whereas a haemangioma shows an early blush decreasing with time. It is not yet clear whether a negative scan rules out the diagnosis of melanoma. Serial image subtraction, image enhancement techniques, and kinetic analysis with probability mapping²⁵⁻²⁸ are expected to allow even smaller ocular melanomas to be detected.

Another possible application of this imaging method would be for imaging metastatic lesions from ocular melanomas. Because there is high uptake in the kidneys which interferes with the image a small metastasis in an area of the liver overlying the kidney would be missed. But metastases at other sites should be detected. At this stage only ^{99m}Tc F(ab')₂ fragments of the MoAb 225.28S have been evaluated. In-vitro staining characteristics of this antibody were less marked when compared with MoAb 763.24T and 376.96S.¹⁵ Whether these other antibodies or their fragments labelled with ^{99m}Tc or ^{125}I would also improve the image remains to be explored.

The specificity for radioimmunoscintigraphy procedures as a whole is high. However, the sensitivity varies in accordance with the tumour size, lesion site, and the rate and amount of antibody uptake. In this study we had one false positive result in patient 2. This may have resulted from the presence of a naevus in the second eye, and the immunoscintigraphic response of the choroidal naevi remains to be investigated. As with any diagnostic imaging test for tumours, the possibility of a false negative or false positive result is always present, but it is hoped that this test will ultimately supplement and complement other established diagnostic tests and help the ophthalmic surgeon in the management of patients with ocular tumours.

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