Immunoscintigraphy with three step monoclonal pretargeting technique in diagnosis of uveal melanoma: preliminary results

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Abstract

Several problems still limit the full use of the diagnostic potential of immunoscintigraphy (IS) with technetium-99m labelled monoclonal antibodies (MoAbs) 225-28S directed to high molecular weight melanoma associated antigen (HMW-MAA). The principal problem is the unfavourable ratio of tumour to non-tumour activity (T/nT), due to the poor tumour uptake and the high aspecific uptake of the tissue surrounding the tumour. Recently, it was demonstrated that using the tumour pretargeting technique based on the injection of monoclonal antibody and the avidin/biotin system (three step immunoscintigraphy), an improvement in the T/nT ratio can be obtained in patients with carcinoembryonic antigen secreting tumours. The aim of this study was to compare the diagnostic sensitivity of traditional immunoscintigraphy with that of three step immunoscintigraphy in seven patients with uveal melanoma. All the patients underwent immunoscintigraphy with MoAb 225-28S radiolabelled with technetium-99m, and a three step immunoscintigraphy 1 week later. No patients demonstrated immediate toxic effects after receiving the reagents, no matter which of the two methods was used. The traditional immunoscintigraphy had a diagnostic sensitivity of 71.4%, diagnosing five out of seven melanomas tested. The three step study detected all the melanomas examined (7/7) with a diagnostic sensitivity of 100% and showed a drastic reduction in background. The preliminary results confirm the feasibility of visualising the uveal melanoma and show that the three step immunoscintigraphy is more diagnostically sensitive than traditional immunoscintigraphy, particularly in small lesions.

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MAA), to visualise uveal melanomas.²⁻¹⁰ Even without using a specific monoclonal antibody (225-28S), immunoscintigraphy with ^{99m}Tc-225-28S proved to have a high level of specificity.²⁻¹⁰ Recent studies have demonstrated that HMW-MAA expression in uveal melanoma is more than 90%, thus confirming the validity of using MoAb 225-28S in immunoscintigraphic detection of uveal melanoma.⁸⁻¹⁰ Traditional immunoscintigraphy using directly labelled antibodies proved to have a diagnostic sensitivity which varied from 37% to 92%. These differences depend on the technique used (planar scintigraphy, single photon emission tomography (SPET), 'double pinhole' collimator, brain dedicated SPET system), and on the thickness of the tumour.³⁻¹⁰

The ability to visualise tumours using immunoscintigraphy depends on the ratio of the specific accumulation of radiolabelled antibodies in the tumour (hot spot) to the aspecific accumulation of radiotracer in the surrounding tissues (background).¹¹ This ratio in the uveal melanoma may be low owing to the weak concentration of radiotracer and to an elevated aspecific uptake in the nasopharyngeal area.³⁻¹⁰

In order to optimise the ratio between target and background, we have shown that using the immunoscintigraphy with a three step monoclonal pre-targeting technique (three step immunoscintigraphy), the amount of radioactivity tied to the tumours can be increased, and the aspecific capture of radiotracer can be decreased.¹²⁻¹⁴

The aim of this study was to evaluate the feasibility and the diagnostic sensitivity of three step immunoscintigraphy and to compare it with traditional immunoscintigraphy in patients with uveal melanoma.

Patients and methods

PATIENTS

We studied seven patients with uveal melanoma. Inclusion criteria were as follows:

Clinical diagnosis of uveal melanoma

Tumour thickness >3 mm

Normal contralateral eye.

Uveal melanoma was diagnosed clinically. All patients underwent ophthalmoscopy, ultrasonography (A and B mode), and fluorescein angiography. Tumour prominence was measured by ultrasonography (B mode). The sample included four men and three women; the mean age was 68.4 (SD 14.1) years; the mean tumour thickness was 10.62 mm (min 5.5 mm; max 14.3 mm (SD 3.28 mm)).

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In the presence of small lesions, amelanotic tumours, opaque ocular media, or retinal detachment, the diagnosis of uveal melanoma becomes problematic. In this clinical context a further instrumental assessment such as immunoscintigraphy (IS), may contribute more information in differential diagnosis.

Immunoscintigraphy with radiolabelled monoclonal antibodies (MoAbs) is a technique capable of diagnosing various types of tumours, including melanomas.¹ Several authors have demonstrated the ability of traditional immunoscintigraphy, with technetium-99m labelled MoAb 225-28S directed to high molecular weight melanoma associated antigen (HMW-

The study was approved by the local ethics committee, and informed consent was obtained from all patients.

Each patient in the sample initially underwent traditional immunoscintigraphy with MoAb 225·28S radiolabelled with ^{99m}Tc , and a three step immunoscintigraphy 1 week later.

All patients underwent enucleation. The pathological evaluation of the tumours confirmed the clinical diagnosis of uveal melanoma.

TRADITIONAL IMMUNOSCINTIGRAPHY WITH $^{99m}\text{Tc-F}(\text{AB}')_2$ 225·28S

The radiotracer was prepared as described.²⁻¹⁰ A variable amount, 1·11–1·85 GBq, of $^{99m}\text{TcO}_4^-$ freshly diluted in 1 ml saline from a $^{99}\text{Mo}/^{99m}\text{Tc}$ generator was added to a sealed glass vial containing 350 μg of lyophilised $(\text{Fab}')_2$ fragments of the anti-melanoma monoclonal antibody 225·28S purchased from Sorin Biomedica (Saluggia, Italy). The vial was then shaken at room temperature for 15 minutes and the solution was passed through a Sephadex DEAE A 25 column. Then the total amount of $^{99m}\text{Tc-MoAb}$ collected following the procedures described above (0·74–1·11 GBq in 4 ml of physiological saline), was immediately injected intravenously.

Approximately 6 hours after radiotracer injection, a tomographic study of the head was performed in each patient using a brain dedicated single photon emission tomography (SPET) system (cerASPECT, Digital Scintigraphy Inc).

THREE STEP IMMUNOSCINTIGRAPHY WITH $^{99m}\text{Tc-PAO-BIOTIN}$

The immunoscintigraphy with a tumour pre-targeting technique is based on the intravenous injection of an anti-melanoma antibody and the avidin/biotin system in three steps:

First step: Tumour pretargeting is performed by intravenous injection of 1·0 mg of biotinylated MoAb 225·28S.

Second step: 24 hours after administration of the MoAbs, when most of them tied to aspecific locations have returned into circulation, the non-radioactive avidin is injected intravenously. The avidin is injected twice: 1 mg of avidin is injected intravenously in 1 ml of physiological solution, followed by an additional 5 mg in 100 ml of albuminated physiological solution 30 minutes later. These two avidin injections are given in order to precipitate the still circulating biotinylated antibodies (reduction in the aspecific

uptake), and then to 'avidinate' the antibodies of the tumour cell surface.

Third step: 24 hours after the second avidin injection, we gave an intravenous injection of $^{99m}\text{Tc-PAO-biotin}$ (20 mCi). The avidin, targeted to the MoAb-biotin on the tumour, has several free binding sites to bind more radiolabelled biotin (amplification of signal).

Within 1 hour imaging, using a brain dedicated SPET system, was performed.

Two nuclear physicians, to whom no information had been given on the anatomical location of the tumour, separately evaluated the images obtained for each patient.

TOXICITY AND IMMUNOGENICITY

In all patients blood samples were collected before injection and 14 days after avidin administration.

The induction of human anti-mouse immunoglobulin antibodies (HAMA) was verified using an enzyme linked immunosorbent assay system.¹⁵ Avidin immunogenicity (human anti-avidin response, HAAR) was studied on micro-well plates coated with avidin or streptavidin separately. The plates were saturated for 1 hour with phosphate buffered saline and 3% bovine serum albumin. Human sera dilutions were added and incubated for 1 hour at 37°C. After five washes, the binding of human anti-avidin antibodies was revealed with horse radish peroxidase-conjugated rabbit anti-human Ig antibodies (Dako) diluted 1:1000 for 45 minutes at 37°C. After six washes, the enzymatic reaction was developed with a chromogenic substrate (*o*-phenyldiamine; Sorin Biomedica, Saluggia, Italy) for 10 minutes and blocked by addition of 1 M H_2SO_4 . The optical density reading was at 492 nm.

Results

No patient demonstrated immediate toxic effects after receiving the reagents, no matter which of the two methods were used. No patients were positive for an antibody response against mouse immunoglobulins and avidin.

The results obtained are listed and compared in Table 1. Traditional immunoscintigraphy had a diagnostic sensitivity of 71·4%, diagnosing five out of seven melanomas tested. The average thickness of the negative tumours (6·55 (SD 1·48) mm) was less than that of the positive tumours (12·24 (SD 2·02) mm).

Three step immunoscintigraphy, on the other hand, detected all the melanomas examined (7/7), with a diagnostic sensitivity of 100% (Table 1).

Figure 1 shows the comparison between the images obtained with traditional immunoscintigraphy and three step immunoscintigraphy in patient 3. In the study of directly labelled antibodies, no pathological accumulation of radiotracer was found (Fig 1A). In the image obtained with the three step immunoscintigraphy a marked pathological accumulation of radiotracer (see arrow) was found at the tumour site (Fig 1B). The same image also clearly shows

Table 1 The principal data of our sample and the results obtained with immunoscintigraphic techniques

Patient No	Tumour size (mm)	Traditional immunoscintigraphy	Three step immunoscintigraphy
1	9·9	+	+
2	14·0	+	+
3	7·6	—	+
4	14·3	+	+
5	10·4	+	+
6	5·5	—	+
7	12·6	+	+

Figure 1 Comparison between the image obtained with traditional immunoscintigraphy (A) and that obtained with three step immunoscintigraphy (B) in patient 3. The pathological accumulation of radiotracer at the tumour site (see arrow) is shown in the image obtained with the three step immunoscintigraphy alone (B). The same image also clearly shows a drastic reduction in the aspecific uptake of the tracer (A).

a drastic reduction in the aspecific uptake of the tracer.

Figure 2 compares the images obtained with traditional immunoscintigraphy and the three step immunoscintigraphy in patient 2. Both images clearly show hyperaccumulation of tumoral radiotracer (see arrows) (Fig 2A, B). In the image obtained with the three step immunoscintigraphy there is much less background activity (Fig 2B), compared with the image obtained using the directly labelled antibodies (Fig 2A).

Discussion

The immunogenicity of biotinylated antibodies and avidin was tested in all patients. None of the patients studied developed HAMA after the

injection of 1 mg of biotinylated IgG or HAAR after injection of 5–6 mg of avidin.

These data confirm that the immunoscintigraphy examination may be repeated in the future.

Numerous clinical studies using immunoscintigraphy with ^{99m}Tc -225.28S have demonstrated the high level of specificity of this technique.^{2–10} One false positive case has been reported in the literature due to uptake in a choroidal naevus.⁵ In another case, an aspecific accumulation of radiotracer was reported in a pre-existing choroidal haemorrhage simulating a uveal melanoma.¹⁰ However, the same study, carried out on a sufficiently large number of cases (101), showed up the high level of specificity (94%) of immunoscintigraphy in the diagnosis of uveal melanomas.¹⁰

Figure 2 Both images, obtained respectively with traditional immunoscintigraphy and the three step immunoscintigraphy in patient 2, clearly show the pathological hyperaccumulation of radiotracer in the tumour (see arrows). Nevertheless, in the image obtained with three step immunoscintigraphy (B) a clear reduction in background is visible (A).

Figure 3 Three step strategy. A biotinylated monoclonal antibody (225·28S) injected intravenously will bind to the tumour (first step); after 24 hours to allow the unbound antibody to be cleared from blood, avidin is injected. Avidin will recognise and bind biotin molecules present on both circulating and tumour bound monoclonal antibody (MoAb) (second step). After a sufficient interval (24 hours) to allow unbound avidin and circulating avidin-MoAb complexes to be cleared, a biotinylated radioactive tracer with fast blood clearance is injected (third step). It will be specifically recognised and bound by tumour bound avidin-MoAb complexes, therefore making it possible to convey a given label to the tumour.

Recent studies have demonstrated that HMW-MAA expression in uveal melanoma is more than 90%, and therefore confirm the validity of MoAb 225·28S, raised against HMW-MAA, in immunoscintigraphic detection of uveal melanomas.⁸⁻¹⁰ However, several problems still limit the full use of the diagnostic potential of traditional immunoscintigraphy. The principal problem is the unfavourable ratio of tumour to non-tumour activity (T/nT), owing to poor tumour uptake and high aspecific uptake of the tissue surrounding the tumour.¹¹

In a recent study the diagnostic sensitivity of traditional immunoscintigraphy showed a positive correlation with the thickness of the tumour.¹⁰ These findings suggest that the radiotracer concentration required for immunoscintigraphy visualisation depends principally on the size of the tumour. However, in tumours of the same thickness there is a different rate of immunoscintigraphy detection. This difference may be explained by the presence of a different intralésional concentration of radiotracer, varying the expression of HMW-MAA in uveal melanomas.^{8,10,16}

The aspecific radiotracer uptake in the nasopharyngeal area may reduce the detectability of uveal melanomas located nasally. However, using the SPET system this problem can be overcome.³⁻¹⁰

In order to obtain tumour signal amplification and background reduction, we recently demonstrated that using the tumour pretargeting technique based on the injection of MoAb and the three step avidin/biotin system, an improvement in the T/nT ratio can be obtained in carcinoembryonic antigen secreting solid tumours.¹²⁻¹⁴

The three step method consists of: (1) uveal melanoma pretargeting by intravenous injection of cold biotinylated anti-tumour monoclonal antibodies (225·28S); (2) removal of circulating biotinylated antibodies and 'avidination' of the

biotinylated tumour bound antibodies by an excess (at least 10-fold) of cold avidin. This is the major factor in background reduction; (3) post-labelling of the tumour by a fast clearing radioactive biotin derivative (^{99m}Tc-PAO-biotin). The amplification of the signal from the tumour is due to the fact that more than one molecule of avidin can bind to a single polybiotinylated MoAb molecule localised on the tumour and that more than one radioactive biotin can bind to an avidin molecule (Fig 3).

In this study of the diagnostic sensitivity of three step immunoscintigraphy proved superior to that of traditional immunoscintigraphy in that it visualised all the melanomas in the sample; in particular, the two melanomas with negative traditional immunoscintigraphy which were not as thick as the others (Table 1). In these two tumours the radiotracer may not have reached a concentration high enough to permit detection by gammacamera, while with the three step method the tumour signal was amplified and the background diminished. This increased the T/nT ratio, allowing visualisation of the tumour by gammacamera (Fig 1A, B).

A net reduction in the aspecific capture of radiotracer is also clearly evident in all cases (Fig 2A, B).

On the basis of these results, even though the sample was small, three step immunoscintigraphy appears to be more diagnostically sensitive than traditional immunoscintigraphy, particularly in the visualisation of smaller lesions.

Another advantage of the three step method is that it may also allow the simultaneous use of different anti-melanoma monoclonal antibodies. This could avoid the false negative due to the immunological heterogeneity of uveal melanoma.¹⁶⁻¹⁸

Finally, the drastic reduction in background and the amplification of the tumour signal

obtained by using the three step method, could lead to the use of this technique in radioimmunotherapy.

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