

Localization of melanoma with radiolabelled monoclonal antibody fragments and iodoamphetamine

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Abstract. In two melanoma patients, metastases accumulated both ^{99m}Tc-labelled monoclonal anti-tumor F(ab')₂ fragments and N-isopropyl-p-(¹²³I)-iodoamphetamine. Small metastatic deposits were localized only by labelled antibody, for which a higher target-to-nontarget ratio was observed than for radioiodoamphetamine, indicating that immunoscintigraphy may be the more sensitive method. In these two patients positive immunohistochemical staining for the antibody used was observed, whereas in a third patient, with no concentration of labelled antibody, the staining result was negative showing the specificity of the immunoscintigraphy findings. It is possible that the accumulation of radio-iodoamphetamine is due to binding to melanin but this is not certain as tissue samples from one of the two patients with positive scintigrams did not contain stainable melanin.

Key words: Immunoscintigraphy – Iodoamphetamine – Melanoma

A relatively high specificity for detection of cancer has been reported for newly developed immunoscintigraphic techniques employing monoclonal F(ab')₂ antibody fragments lacking the non-specific binding associated with F_c-reactivity (Callegaro et al. 1983). Mouse melanoma cells in-vitro have been shown to accumulate radioiodinated iodoamphetamine (Holman et al. 1983) and recently a case of malignant melanoma concentrating this radiopharmaceutical was observed (Wada et al. 1985), two findings suggesting potential clinical use of radiotracers with affinity for melanin. Here we report results from three patients with histologically verified recurrent or metastatic melanoma who underwent scintigraphy with radiolabelled melanoma antibodies and iodoamphetamine. To our knowledge this is the first report on melanoma patients studied by both methods.

Methods and case records

Methods

Immunoscintigraphy was performed with Tecnemab-1 material from Sorin Biomedica (Saluggia, Italy). The mono-

clonal antibodies, converted into F(ab')₂ fragments, were prepared from human melanoma cell cultures (Imai et al. 1981) and labelled with technetium immediately prior to use. The antibody labelling procedure, which included removal of free pertechnetate by ionic exchange chromatography, yielded 54%–67% (mean recovery 60.0% in 7 experiments) efficiency. An intracutaneous skin test was performed before administration of the labelled antibody. Scintigraphy was performed at time intervals varying from 4 h to 24 h after infusion of 350 µg of antibody labelled with 8.2 mCi–11.2 mCi (300 MBq–400 MBq) of ^{99m}Tc (^{99m}Tc-MAB). For confirmation of the specificity of the immunoscintigraphic findings immunohistochemical studies were performed on surgically removed tissue specimens using melanoma antibody material provided by Sorin Biomedica. For immunohistochemistry four µm frozen sections were cut and fixed in acetone. The biotin-avidin method (Vectastain, Vector Laboratories Inc., Burlingame, CA, USA) was used for immunostaining of melanoma antibodies.

N-isopropyl-p-(¹²³I)-iodoamphetamine (¹²³IMP) with an approximate specific activity of 5 mCi/mg was purchased from EIR (Eidg. Institut für Reaktorforschung, Würelingen, Switzerland). Scintigraphy with ¹²³IMP was performed between 2 h and 6 h after intravenous administration of 5.6 mCi–7.5 mCi (200 MBq–280 MBq) of radiopharmaceutical. Staining for melanin was performed on surgically removed tissue specimens.

Verification of the diagnosis of malignant melanoma was based on the following immunohistological criteria: positivity for monoclonal vimentin (Labsystems Corp., Helsinki, Finland) and S-100 protein (Dacopatts A/S, Denmark) and negativity for monoclonal cytokeratin PKK-1 (Labsystems Corp.).

We used a large field gamma camera equipped with a high sensitivity parallel hole low energy collimator. Computer analysis of images was performed for determination of target-to-nontarget ratios.

Case reports

The purpose of the study was explained to the patients and written informed consent was obtained in each case. The study protocol was approved by the Ethical Committee of the Helsinki University Central Hospital.

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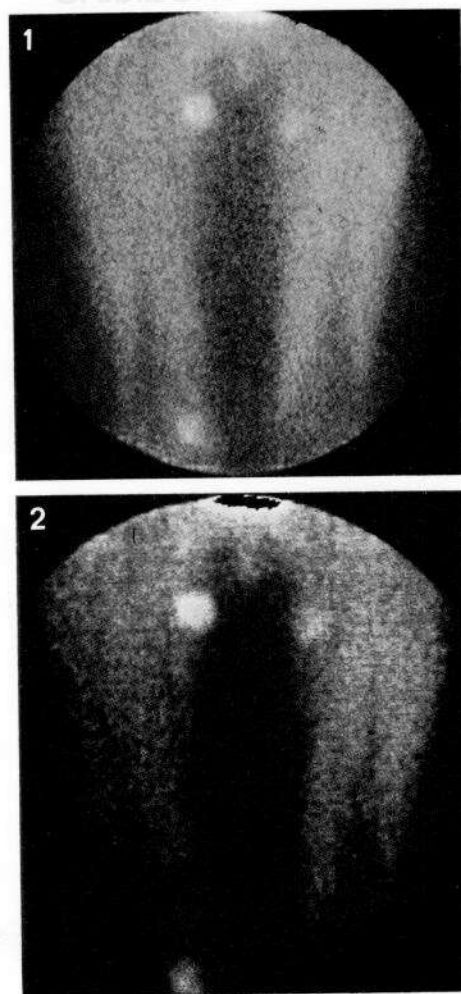


Fig. 1. Anterior scintigram from Case 1 showing accumulation of ^{99m}Tc -labelled antibody F(ab')_2 fragments at 6 h in 2 melanoma metastases in the right thigh and 1 metastasis in the left thigh

Fig. 2. Uptake of ^{123}I -amphetamine at 2 h by the same metastases visualized by immunoscintigraphy in Fig. 1

Case 1

In this female patient aged 59 a primary melanoma had been excised from the right cheek 40 years previously and postoperative radiotherapy was given. In May 1985 a large tumor was detected in the inferior part of the left lung. The operation in July 1985 was not radical because the tumor infiltrated adjacent tissues. Postoperative radiotherapy (24 Gy) of the tumor bed and mediastinum was administered in September 1985. During radiotherapy three new lesions appeared: in the medial part of the left thigh, in the chest wall operation scar and in the scalp. She had pain in the left side of the pelvis and an osteolytic lesion was detected by X-ray in the crest of the left iliac bone. In immunoscintigraphy abnormal uptake was seen in the proximal and distal parts of the right thigh and in the proximal part of the left thigh (Fig. 1). Accumulation of ^{123}I IMP was observed at the corresponding sites (Fig. 2). The target-to-nontarget ratios for the two imaging agents in the lesion located in the proximal right thigh are reported in Table 1. There were several small accumulations of ^{99m}Tc -MAB in

Table 1. Maximal target-to-nontarget uptake ratios for labelled antibody (^{99m}Tc -MAB) and radioiodoamphetamine (^{123}I IMP) in large size melanoma metastases

	^{99m}Tc -MAB	^{123}I IMP
Case 1 ^a	3.0 (6 h)	1.9 (2 h)
Case 2 ^b	2.5 (4 h)	1.4 (2 h)

^a Lesion in thigh

^b Lesion in neck

both lungs. The uptake of ^{123}I IMP by the lungs was homogeneous and therefore considered normal. Lesions were also detected by computed tomography (CT) in the subcutaneous tissue of the right and left thigh, the medial condyle of the right femur and both lungs. Lesions not visualized by immunoscintigraphy were observed by CT in the iliac crests, the right adrenal gland and a para-aortic lymph node. The diagnosis of melanoma was verified by histopathological examination of surface lesions. The melanoma cells from the metastases in the thighs showed a strong positive membrane staining for the antibody used in immunoscintigraphy, whereas the staining for melanin was negative.

Case 2

Primary Clark III melanoma was excised in May 1982 from the left side of the chest wall of this male patient aged 58. One month later recurrent lesions in the scar and metastatic axillary lymph nodes were removed. In 1984 metastases were excised twice from the scalp. A lymph node metastasis was removed from the neck in September 1985. In December 1985 a rapidly growing metastasis was observed in the left part of the neck and treatment with alpha and gamma interferon was tried without success. No other tumors could be detected by physical examination or CT. The neck tumor concentrated ^{99m}Tc -MAB (Fig. 3) and ^{123}I IMP (Fig. 4), the target-to nontarget uptake being higher for the labelled antibody than for radioiodoamphetamine (Table 1). A small focal uptake was observed in the left infraclavicular fossa by immunoscintigraphy (Fig. 3), whereas in the ^{123}I IMP scan there was only a weak accumulation of radiopharmaceutical at 4 h (Fig. 5); at 2 h the uptake was equivocal (Fig. 4). Immunohistochemical staining of the neck tumor tissue was positive for the antibody used in immunoscintigraphy; the intensity of the staining reaction varied from weak to moderate in different areas of the specimen. The tissue specimen was positive also for melanin.

Case 3

This male patient, aged 40, had a primary melanoma removed from the dorsum of the chest in September 1979. A metastatic lymph node in the neck was excised in December 1983. In November 1984 a subcutaneous metastasis was detected in the left chest wall. This metastasis was excised and the left axillary lymph nodes were evacuated. Starting in October 1985 numerous subcutaneous nodules appeared in various parts of the body. At the time of scintigraphy there were more than fifty visible or palpable subcutaneous nodules. CT from the neck to the pelvis showed subcutaneous metastases in almost every slice in addition to numerous lesions in the intra-abdominal fat tissue. The

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Received April 4, 1986/June 28, 1986