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Evaluation of ^{111}In labeled antibodies for SPECT imaging of mesothelin expressing tumors

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Abstract

Introduction: Mesothelin is expressed in many cancers, especially in mesothelioma and lung, pancreatic and ovarian cancers. In the present study, we evaluate ^{111}In labeled antimesothelin antibodies as an imaging bioprobe for the SPECT imaging of mesothelin-expressing tumors.
Methods: We radiolabeled the antimesothelin antibodies mAbMB and mAbK1 with ^{111}In using the p-SCN-bn-DTPA chelator. The immunoreactivity, affinity (K_d) and internalization properties of the resulting two ^{111}In labeled antibodies were evaluated in vitro using mesothelin-expressing A431K5 cells. The biodistribution and microSPECT/CT imaging studies with ^{111}In labeled antibodies were performed in mice bearing both mesothelin positive (A431K5) and mesothelin negative (A431) tumors.
Results: In vitro studies demonstrated that ^{111}In-mAbMB bound with a higher affinity (K_d=3.6±1.7 nM) to the mesothelin-expressing A431K5 cells than did the ^{111}In-mAbK1 (K_d=29.3±2.3 nM). ^{111}In-mAbMB was also internalized at a greater rate and extent into the A431K5 cells than was the ^{111}In-mAbK1. Biodistribution studies showed that ^{111}In-mAbMB was preferentially localized in A431K5 tumors when compared to A431 tumors. At the low dose, the peak A431K5 tumor uptake of 9.65±2.65% ID/g (injected dose per gram) occurred at 48 h, while at high dose tumor uptake peaked with 14.29±6.18% ID/g at 72 h. Non-specific localization of ^{111}In-mAbMB was mainly observed in spleen. ^{111}In-mAbK1 also showed superior localization in A431K5 tumors than in A431 tumors, but the peak uptake was only 3.04±0.68% ID/g at 24 h. MicroSPECT/CT studies confirmed better visualization of A431K5 tumors with ^{111}In-mAbMB, than with ^{111}In-mAbK1.
Conclusion: SPECT imaging of mesothelin expressing tumors was demonstrated successfully. Our findings indicate that the antimesothelin antibody mAbMB has the potential to be developed into a diagnostic agent for imaging mesothelin-expressing cancers.

Keywords: Radiolabeled antibody; Mesothelin; Biodistribution; In-111; SPECT/CT

1. Introduction

Malignant mesothelioma, pancreatic cancer and ovarian cancer are all characterized by low survival rates. Pancreatic cancer has the highest mortality of all cancers, with a 5-year survival rate of only 5%, while those of mesothelioma and ovarian cancer are also bad with 9% and 30%, respectively [1,2]. These low survival rates can be attributed to late diagnosis and the cancers' propensity for early metastasis, both of which significantly reduce the chance of a cure. Early detection can increase the survival rates by allowing the identification of tumors, at a time when they are still amenable to surgical resection or other therapeutic approaches. Imaging has a pivotal role to play in the detection and treatment planning of these cancers [3–5]. Better imaging of the tumor sites would also permit more accurately targeted drug or radiation delivery for subsequent management of the disease [6]. Although improved imaging methods are needed to assist in detection and treatment, few agents have been designed to image the molecular targets expressed by these cancers [7–10].

A number of studies have shown that mesotheliomas, pancreatic and ovarian cancers overexpress mesothelin, a cell surface glycoprotein which can potentially be useful as a target for development of novel imaging and treatment strategies [11–14]. Mesothelin, a 40 kDa glycosylphosphatidyl-inositol (GPI) anchored cell surface glycoprotein, is expressed at low levels by a restricted set of normal adult

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tissues, but is overexpressed in 50-65% of mesotheliomas, ovarian cancers, pancreatic cancers, and some lung adenocarcinomas [13,15]. Overexpression of mesothelin has been associated with increased proliferation, migration, and tumor volume [16]. Mesothelin specific targeting for imaging and therapy thus holds great promise in the management of these cancers. One way to do this is to use monoclonal antibodies specific to tumor-associated antigens and use them as delivery vehicles for radionuclides to visualize or treat tumors [17]. An antibody to mesothelin, when labeled with a radionuclide for imaging, would be valuable as a molecular imaging bioprobe, especially given its limited expression in normal tissues and high expression in specific cancers.

The first anti-mesothelin antibody mAbK1 (IgG1) was isolated from mice immunized with an ovarian cancer cell line [18]. Subsequently, Hassan et al. showed in a biodistribution study the targeting potential of ^{111}In-labeled mAbK1 in mice bearing mesothelin positive and mesothelin negative tumors [19]. Since then many new anti-mesothelin antibodies have been reported and become commercially available. However, there has not been much progress in the molecular imaging of mesothelin-expressing cancers, with only one recently published study using ^{64}Cu-radiolabeled antibody fragments for the PET imaging of mesothelioma in mice [9]. Onda et al. reported two new antibodies, mAbMB and mAbMN, and compared their anti-mesothelin affinity characteristics with the other available antibodies including mAbK1, showing that mAbMB (IgG2A) has the best affinity for mesothelin [11].

Our lab is interested in the imaging and potential treatment of mesotheliomas and pancreatic cancer with radiopharmaceuticals. For this purpose, we recently performed a biodistribution study with mAbK1 antibody radiolabeled with ^{99m}Tc (unpublished data). Using the direct labeling method [20], 0.2 mg of mAbK1 was radiolabeled with 90 MBq of ^{99m}Tc and then injected into SCID mice with mesothelin-expressing NCI-H226 tumors. The tumor uptake at 8 hours was only 0.8% ID/g showing a further decrease at subsequent time points. Compared to Hassan et al.'s reported 53% ID/g uptake in mesothelin-expressing A431K5 tumors with the ^{111}In-labeled mAbK1 antibody [19], this was very low. In the current study, we firstly sought to identify the reasons for this difference in tumor uptake by evaluating ^{111}In labeled mAbK1 (^{111}In-mAbK1) in the same A431K5 tumor model reported previously by Hassan et al. Secondly, with the aim of developing a radiotracer for imaging mesothelin-expressing tumors, we tested ^{111}In labeled mAbMB antibody (^{111}In-mAbMB) for its potential to selectively accumulate at a tumor site and to be imaged by SPECT/CT. We also compared the characteristics of ^{111}In-mAbMB to those of ^{111}In-mAbK1 in order to find the optimal one for imaging. Herein, we describe the radiolabeling of the mesothelin antibodies mAbMB and mAbK1 and report their immunoreactivity, affinity, internalization characteristics, and biodistribution. The SPECT/CT imaging properties of the two antibodies, which has not been reported previously, was also evaluated in tumor-bearing mice.

2. Materials and methods

2.1. Materials

All chemicals and reagents were purchased from Sigma-Aldrich (Oakville, Ontario, Canada). The mAbMB antibody was purchased from Rockland Immunochemicals (Gilbertsville, Pennsylvania, U.S.A.) and mAbK1 from Abcam (Cambridge, Massachusetts, U.S.A.). ITLC strips were obtained from Biodex (Cat# 150−771; Shirley, New York, U.S.A.). The cell culture media and supplements were obtained from Invitrogen (Burlington, Ontario, Canada). $^{111}InCl_3$ was obtained from MDS Nordion (Vancouver, British Columbia, Canada) and p-SCN-bn-DTPA from Macrocyclics (Dallas, Texas, U.S.A.). Ultracel 100K centrifugal filters were purchased from Millipore Corporation (Billerica, Massachusetts, U.S.A.). Reagents for electrophoresis were purchased from BIO-RAD Laboratories (Mississauga, Ontario, Canada). Activity measurements were carried out using a Packard Cobra II gamma counter (Perkin-Elmer, Waltham, Massachusetts, U.S.A.).

2.2. Radiolabeling

Antimesothelin antibodies mAbMB and mAbK1 were purified and buffer-exchanged into 0.1 M sodium bicarbonate, pH 8.5 using 100 kDa cut-off Ultracel centrifugal filters, and incubated on an Eppendorf shaker for 21 hours at room temperature with p-SCN-bn-DTPA in 0.1 M sodium bicarbonate at a molar ratio of 1:5. After 21 hours, unreacted DTPA (diethylene triamine pentaacetic acid) was removed by centrifugation through the centrifugal filters and antibody buffer-exchanged into 0.15 M ammonium acetate buffer, pH 5.5. For ^{111}In-labeling, $^{111}InCl_3$ (3.7 MBq/20 μg of antibody) was added to mAbMB and mAbK1 antibody, respectively, and incubated for 30 minutes on an Eppendorf shaker at room temperature. The radiolabeled antibodies were purified using the centrifugal filters and buffer-exchanged into phosphate buffered saline, pH 7.4 (PBS) for injection into mice or in vitro testing. Radiochemical purity was determined using ITLC-SG plates after DTPA challenge (0.05 M). Saline was used as the mobile phase. In this ITLC system, ^{111}In DTPA moves to R_f=1 and ^{111}In mAb to R_f=0. The number of DTPA molecules conjugated to the antibody was determined by trace labeling with $^{111}InCl_3$ [21].

2.3. Cell culture

A431K5 and A431 cell lines were generously provided by Dr. Ira Pastan (NCI, Bethesda, Maryland, U.S.A.) and Dr. Marcel Bally (Advanced Therapeutics, British Columbia Cancer Agency, Vancouver, Canada), respectively. Both cell lines were cultured in Dulbecco's Modified Eagle medium