Initial Tumor Targeting, Biodistribution, and Pharmacokinetic Evaluation of the Monoclonal Antibody PAM4 in Patients with Pancreatic Cancer¹

Giuliano Mariani,² Nicola Molea, Daniela Bacciardi, Ugo Boggi, Gino Fornaciari, Daniela Campani, Piero A. Salvadori, Pier Cristoforo Giulianotti, Franco Mosca, David V. Gold, Robert M. Sharkey, and David M. Goldenberg

Nuclear Medicine Service, DIMI, University of Genoa, Genoa, Italy [G. M.]; Consiglio Nazionale delle Ricerche Institute of Clinical Physiology [G. M., M. A. S.]; Regional Center of Nuclear Medicine [N. M., D. B.]; Institute of General and Vascular Surgery [U. B., P. C. G., F. M.]; and Institute of Morbid Anatomy of the University of Pisa [G. F., D. C.]; Pisa, Italy; and the Garden State Cancer Center, Center for Molecular Medicine and Immunology, Newark, New Jersey 07103 [R. M. S., D. V. G., D. M. G.]

Abstract

This pharmacokinetic study was performed to assess the potential usefulness of the murine monoclonal antibody (MoAb) PAM4-IgG₁ as an immunotargeting agent for pancreatic cancer imaging or therapy. This MoAb reacts specifically with mucin purified from human pancreatic cancer.

¹¹I-labeled PAM4-IgG₁ was injected i.v. into five patients with suspected pancreatic cancer. Whole-body scans and spot views of the abdominal area were recorded with a computed gamma camera, and specific regions of interest were drawn over the liver and spleen to define the kinetics of activity in these organs. Blood samples taken from 0.1–144 h after injection served to define the kinetics of plasma distribution and removal of activity from the body.

Surgery confirmed pancreatic cancer in four of the five patients, whereas chronic pancreatitis was present in the fifth patient; in all four pancreatic cancer patients, immunostaining with the MoAb PAM4 demonstrated the presence of the specific antigen, with a cytoplasmic and endoluminal/secretory pattern of distribution. Nonspecific radioactivity accumulation in the liver, spleen, and bone marrow was low, linked essentially to the blood pool effect of circulating activity in these organs. The overall quality of scintigraphic maps recorded over the abdomen was quite satisfactory for the low liver and spleen activity, with good scintigraphic demonstration of the pancreatic cancers (either primary or metastatic); the patient subsequently found to have pancreatitis failed to show PAM4 targeting. Except in one patient with widespread peritoneal metastases (in whom these tumor implants were detected scintigraphically already 24–48 hours after tracer injection), scintigraphic evidence of the tumor lesions was usually late, starting at about 72–96 h after tracer injection.

The results obtained in this preliminary study indicate the potential usefulness of MoAb PAM4 for immunoscintigraphy in patients with either primary and/or recurrent pancreatic cancer while also suggesting that the use of the faster-clearing Fab fragments of this MoAb probably would result in improved immunoscintigraphic properties.

Introduction

The murine MoAb¹ PAM4 is an IgG₁ produced by immunization of mice with mucin purified from the xenografted RIP1 human pancreatic carcinoma, originally a mucinous, moderately differentiated tumor in the head of the pancreas (1). The antigenic determinant of the mucin defined by MoAb PAM4 does not cross-react with carbohydrate epitopes defined by MoAbs B72.3 and G4 (reactive with the core oligosaccharides) or CA19.9 and anti-Leα (reactive with terminal epitopes defined by MoAbs B72.3 and G4 (reactive with the

¹ Presented at the “Fifth Conference on Radioimmunodetection and Radioimmunotherapy of Cancer,” October 6–8, 1994, Princeton, NJ. Supported in part by USPHS Grants CA39841 and CA43218/44225 from the NIH, Bethesda, Maryland.
² To whom requests for reprints should be addressed, at Nuclear Medicine Service, DIMI, University of Genoa, Viale Benedetto XV, n,6, I-16132 Genoa, Italy. Fax: +39-10-353232.
³ The abbreviation used is: MoAb, monoclonal antibody.

is not specific for the peptide core of mucin, whereas it may be reactive with a conformational-dependent peptide epitope.

Immunohistochemical studies have shown that PAM4 is reactive with a normal adult gastrointestinal-specific mucin epitope that is absent from the normal pancreas but expresses itself in pancreatic carcinoma. In fact, this MoAb was reactive with 85% of all 25 pancreatic cancer specimens tested, whereas it reacted with about one-half of the gastrointestinal cancers examined and was, in general, unreactive with pancreatitis specimens (1). All data obtained so far suggest that PAM4 recognizes a unique and novel epitope preferentially expressed by a high proportion of pancreatic cancers and, therefore, may be of value in the diagnosis and detection of this aggressive neoplasia, which is often difficult to diagnose due to its location and silent growth.

A preliminary investigation performed at The Garden State Cancer Center in two patients with pancreatic cancer indicated the favorable tumor-targeting potential in vivo of MoAb PAM4 radiolabeled with¹¹I.¹ The present pilot investigation was carried out with the purpose of evaluating the tumor targeting, tissue biodistribution, and pharmacokinetics of the radioiodinated MoAb PAM4.

Materials and Methods

Patients. The study group consisted of five patients (three men and two women ages 56 to 67 years, all of whom had denied any present or past allergic condition), who were scheduled for surgery or who were undergoing an extensive diagnostic work-up and staging because of a strong suspicion of either primary or recurrent pancreatic cancer. All five patients gave their consent to participate in the protocol and were hospitalized during the study; their baseline blood chemistry (including differential blood cell count) was evaluated both the day before and approximately 1 week after completing the biodistribution study. Vital signs were recorded immediately on tracer injection, then daily throughout the pharmacokinetic investigation, paying special attention to any adverse effects following administration of the radioiodinated tracer. Routine preparation of the patients included blockade of thyroid radioactive iodide uptake by the administration of Lugol’s solution (10 drops twice daily) for 10 days, starting 3 days prior to the injection of the MoAb tracer.

Pancreatic cancer was confirmed surgically in four of the five patients, whereas chronic pancreatitis was found at surgery in the fifth patient. Immunostaining demonstrated the presence of the PAM4 antigen in all four of the pancreatic cancer patients. The antigen was expressed in a relatively high proportion of the tumor cells, generally exhibiting a secretory-like pattern of distribution located in the cytoplasm or at the luminal surface of the cells (see Fig. 1).

Tracer. The PAM4 hybridoma was maintained in the nude mouse ascites system using female BALB/c mice, 5–6 weeks old, primed by i.p. inoculation with 1 ml 2,6,10,14-tetramethylpent-a-decane, and then injected with 10⁷ hybridoma cells into the i.p. cavity, as described previously (1). The MoAb IgG₁, purified as follows. Ascites fluids diluted 2-fold with 0.1 mM sodium phosphate buffer (pH 8.1) were centrifuged at 20,000 X g for 30 min, filtered through glass wool, and passed through an Affi-Gel 15-protein A affinity column. The purified MoAb PAM4 was labeled with¹¹I by the Iodo-Gen technique to obtain a tracer with a specific activity of approximately 185

¹ R. M. Sharkey et al., unpublished observations.

5911s
TARGETING OF MoAb PAM4 IN PANCREATIC CANCER

Results

Pharmacokinetics. The typical plasma disappearance curves of 
$I^{131}$-labeled PAM4-IgG, showed a pattern of decay characterized by two exponentials, with half-life values equal to about 42-50 h (terminal component) and 4-6 h (early component). Fig. 2 depicts the plasma clearance curves of four of the five patients studied. One of these patients (case CE04) had widespread peritoneal metastases in addition to the primary pancreatic cancer; the pronounced, early drop in plasma activity observed in this patient corresponded to a clear tracer uptake by the metastatic lesions within the first few hours after injection; although starting from about 36-42 h after injection, the slope of plasma disappearance curve was virtually identical to those in the other three patients.

The whole-body activity retention curves were monoeponential in all patients, with half-life values similar to those of the terminal slope of the corresponding plasma disappearance curves; about 70, 35, and 12% of injected activity were still retained in the body 24, 72, and 144 hours, respectively, after tracer injection.

It was apparent that the radioactivity over time curves generated for the liver and spleen were not due to true binding but, rather, to the activity in the blood circulating in these two organs at each given time point, i.e., about 7-8 and 2-3% of the total plasma pool at zero time for the liver and spleen, respectively, with a subsequent decline paralleling that of the plasma disappearance curve. Similarly, the activity estimated in the bone marrow was so low and was subject to such a large degree of experimental error that it was, for all practical purposes, useless.

Scintigraphic Results. The scintigraphic maps showed a pattern of prolonged retention in the plasma space; therefore, a significant "blood pool" effect occurred until some time after the tracer injection, while there was minimal radioactivity accumulation in the reticuloendothelial system (liver, spleen, and bone marrow), a finding consistent with the presence of activity in the blood rather than significant nonspecific binding at these sites.

Experimental Protocol. After the i.v. injection of approximately 75-185 MBq (2-5 mCi) $^{131}$I-labeled PAM4-IgG, as a single bolus, heparinized venous blood samples were taken at variously timed intervals from each patient for 6 days to determine the radioactivity disappearance curve.

A fully computerized large-field-of-view gamma camera (GE400 Starcam) equipped with a high-energy, parallel-hole collimator was used to record digital images at daily intervals, both in the whole-body mode using the conjugate-view approach and as spot views of the abdomen (taking anterior as well as oblique views to distinguish free $^{131}$I accumulated in the gastrointestinal lumen from specific uptake in the pancreas). The energy level was set at the 364-keV emission peak of $^{131}$I, with a 20% window. Regions of interest were drawn over the recorded scintigraphic maps to estimate the accumulation curves over time for the liver, spleen, and bone marrow (choosing the midfemur and iliac crest as reference regions for the bone marrow).

Scintigraphic Evaluation. For each patient, the whole-body and spot scans obtained at various times were read first in a blind fashion by two specialists in nuclear medicine who were unaware of the site of the primary tumor or its recurrences. A second evaluation of the scans was then undertaken, in which sites of specific abnormal uptake consistent with the presence of tumor lesions were searched for by the same two physicians as well as by a surgeon, now armed with full knowledge of the results of the extensive diagnostic work-up of the patients.

Fig. 1. Immunostaining with the MoAb PAM4 of a tumor histological section from patient GM01. A relatively high proportion of cancer cells stain positive for the PAM4 antigen, both with a cytoplasmic type of distribution and/or with a typical secretory-like endoluminal distribution pattern.

Fig. 2. Plasma disappearance curves obtained in four of the patients studied after the bolus i.v. injection of $^{131}$I-labeled PAM4-IgG,. The early drop in plasma radioactivity observed in patient CE04 is associated with an earlier scintigraphic detection of some of the tumor lesions (the peritoneal metastatic implants) than in the other patients.

MBq/mg (or 5 mCi/mg). The tracer was injected within 24 h after radiolabeling, and gel chromatography on Sephadex G-25 columns showed that the proportion of free $^{131}$I never exceeded 5% of the total radioactivity and was usually <3% at the time of tracer injection.
The procedures were performed in a totally blind fashion and with full knowledge of the patients' data. Immunoscintigraphy demonstrated clear tumor uptake in all four patients with pancreatic cancer, whereas there was no tracer uptake in the pancreatic region in the patient who, at subsequent surgery, was found to have a fibrous-like plaque proven histologically to be chronic pancreatitis. Significant uptake of radioiodinated PAM4-IgG1 in the tumor lesions was observed at relatively late times, starting about 72–96 h after tracer injection (see Fig. 3 for some examples), except in the patient with widespread peritoneal metastases (see below). The tumor lesions detected by immunoscintigraphy ranged in size from bulky lesions occupying virtually the entire pancreas, as in patients GM01 (see Fig. 3A) and FL05, to liver metastases about 1–2 cm. The tumor implants in the peritoneal space of patient CE04 were detected in the scans as early as 24 h after tracer injection (at laparoscopy, these lesions ranged in size from 1–2 to 3–4 cm), whereas the primary pancreatic cancer was outlined clearly only in the scans recorded at 96–120 h after injection. In the patient with liver metastases, these lesions appeared as "cold" filling defects in the early scans (up to 48–72 h after injection), filling in at later times (see Fig. 3B).

Unexpected Patterns of Tracer Distribution. No severe adverse clinical reactions of any type were observed in the patients during the study, nor were any abnormal results found in the routine blood chemistry tests performed at the end of the study. However, one case of minor adverse clinical reaction was recorded. About 15–20 min after tracer injection, patient MA03 (subsequently found to be affected by chronic pancreatitis) started complaining of vague malaise and chest discomfort, without, however, any shortness of breath; his arterial blood pressure levels were unchanged, and there was only a mild increase in the heart rate with respect to baseline (from 68/min to 78–82/min, without extrasystolic beats). The symptoms disappeared promptly after the i.v. administration of 500 mg hydrocortisone given as a bolus. The scans recorded at various times after tracer injection showed an unexpected pattern of distribution characterized by an immediate, marked retention of radioactivity in the lungs (not unlike a conventional perfusion lung scan), which decreased, however, with time and disappeared completely by 19 hours after injection (see Fig. 4A). In quantitative terms, activity retained in the lungs corresponded to about 23.7% of injected activity at 0.5 h, 16.8% at 2 h, and 12.4% at 3 h. The radioactivity plasma disappearance curve observed in this patient showed a much lower zero time value than in the other patients (about 5% injected dose/liter plasma versus an average 27% in the other patients), plasma radioactivity decreasing further to a minimum 2.3% at 20 min, increasing again to peak at about 6.1% at 4 h, then declining progressively to the end of the study (see Fig. 4B). The final slope of the plasma clearance curve observed in patient MA03 was considerably faster than in the other patients (half-life, about 20 h versus about 46 h), as was the whole-body radioactivity retention curve (half-life, about 24 h versus about 48 h in the other four patients).

On stringent questioning, the patient remembered vaguely a previously denied allergic condition (with seasonal asthma-like symptoms) suffered when he was younger. A plausible explanation for the events observed in this patient is that the monoclonal tracer adhered to the
Fig. 4. A, Whole-body scintigraphic maps obtained in patient MA03 at 0.5, 2, 3, and 19 h after injection of \(^{131}\text{I}\) labeled PAM4-IgG1 (incomplete blockade of thyroidal uptake of free radioiodide, due to the fact that Lugol’s solution was administered starting only 1 day before the study). Remarkable radioactivity retention in the lungs, clearly evident in the early scans, progressively decreasing until normalization of the scintigraphic pattern at 19 h. This finding makes it unrealistic that macroaggregates have been formed in the tracer injected, although this possibility cannot be ruled out definitely on the basis of our data. B, Plasma radioactivity curve observed in patient MA03, showing a pattern of distribution consistent with early binding of the tracer in the pulmonary circulation, followed by virtually complete release back into the systemic circulation.

Pulmonary endothelium almost immediately after injection, thus resulting in the peculiar scintigraphic pattern of distribution and altered plasma disappearance curve, with the peak in plasma activity at 4 h corresponding to the subsequent complete release from the binding sites in the lungs. Alternatively, the scintigraphic pattern observed in this patient could be due to the presence of protein macroaggregates present in the injectate and taken up by the lungs, then catabolized rapidly with \(^{131}\text{I}\) release and subsequent uptake by the thyroid, stomach, and kidneys. Although the tracer was injected some 15–20 min after gel chromatography on a Sephadex G-25 column (making it unlikely that particulates had formed during this short period of storage in the same conditions as in all other studies), the possibility still exists that particulates were formed on injection due to the presence of, e.g., cold agglutinins circulating in the patient. However, it should be considered that the time of disappearance of radioactivity from the lungs observed in this patient (within 19 h) is much shorter than it would be implied by the half-life of radioactivity retention in the lungs observed after injection of, e.g., radioiodinated
serum albumin macroaggregates used for perfusion lung scintigraphy (about 76 h).

Discussion

Pancreatic cancer is almost invariably fatal, due to the fact that it is one of the most aggressive malignant tumors, and its location and silent growth make it difficult to diagnose early by traditional methodologies. Therefore, any diagnostic procedure leading to the earlier detection of either primary or recurrent pancreatic cancer may have value in the management of patients with this type of cancer. Immunoscintigraphy with radiolabeled antibodies has shown a considerable clinical usefulness in the noninvasive localization of tumor masses (2–4). The present pilot study was undertaken to explore the potential clinical usefulness of a MoAB that has been developed recently against a novel antigenic determinant purified from mucin produced by the human pancreatic cancer xenograft RIP1. This new MoAb, termed PAM4, shows a high specificity and intense reactivity with pancreatic carcinoma tissue, suggesting that it may prove useful for in vivo targeting of diagnostic and, possibly, therapeutic agents.

The results obtained in this study demonstrate a very satisfactory pattern of distribution of the $^{131}$I-PAM4-IgG$_1$ tracer, with minimal, if any, nonspecific uptake in the liver, spleen, and bone marrow, in which most of the activity was due to the blood pool. The prolonged retention of the tracer in the circulating plasma is probably responsible for the fact that scintigraphic evidence of the tumor lesions appeared late after injection (starting at about 72–96 h), with the single exception of one patient with widespread peritoneal metastases, in whom these lesions were detectable 24–48 h after injection (although the primary tumor exhibited the same time-related pattern of scintigraphic evidence as in the other patients). It is also worthwhile mentioning that the tumor lesions detected by this radiiodinated tracer varied widely in size, with the smaller lesions being in the 1–2-cm range, despite the fact that the radioisotope used for this pharmacokinetic study ($^{131}$I) is less than optimal for imaging with the gamma camera.

The overall results encourage further clinical studies with the MoAb PAM4. In particular, the use of Fab fragments of this antibody may result in a significant improvement in the immunoscintigraphic detection of tumor lesions, due to the combined effect of faster blood clearance (and, therefore, better tumor:nontumor ratios earlier after tracer injection) and higher tissue diffusion of the smaller fragments with respect to the whole immunoglobulin molecule. Therapeutic studies with radiolabeled PAM4 are also planned.

References

Initial Tumor Targeting, Biodistribution, and Pharmacokinetic Evaluation of the Monoclonal Antibody PAM4 in Patients with Pancreatic Cancer

Giuliano Mariani, Nicola Molea, Daniela Bacciardi, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/55/23_Supplement/5911s

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.