ORIGINAL ARTICLES

Radioimmunodetection of cervical carcinoma xenografts with ^{111}In-labeled MAb Cx-99 detected by a hand-held gamma detector

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Summary

Purpose: To establish a radioimmunodetection (RAID) system for localization of cervical cancer by labeling 111-indium (^{111}In) to a monoclonal antibody against cytokeratin 19 (MAb Cx-99), and detecting it with a hand-held gamma detector in an animal model.

Methods: MAb Cx-99 was labeled with 111-Indium by the DTPA chelating method. From the second day to the seventh day after injection of this immunoconjugate into athymic nude mice bearing cervical cancer cell line CC7T xenografts, the biodistribution ratios of tumor and non-tumor radioactivity were detected by a hand-held gamma detector. Data were also correlated with the data detected by the conventional gamma counter.

Results: The labeling efficiency of this ^{111}In-labeled MAb Cx-99 and ^{111}In-labeled MOPC was 91.6% and 95.5%, respectively. After injection, the liver, kidney and lung were initially noticed to have high radioactivity, but the localization of tumor/tissue ratios increased progressively as time passed, indicating the effect of delayed detection for distinguishing tumor from non-tumor tissues. Except for the spleen, the range of tumor/tissue ratios was 1.18-32.7 and 1.14-39.35 for the fourth day and the seventh day, respectively. The tumor/spleen ratio remained low until the seventh day after injection, thus indicating that the spleen might have a different excretion rate.

Conclusion: This study indicated the feasibility of a hand-held detection system in the localization of cervical cancer after injection of ^{111}In-labeled MAb Cx-99. The effect of delayed detection was obvious by the decreasing high bindings in the liver, spleen and kidney, with the applicable detection time being four to seven days after injection.

Key words: Radioimmunodetection, Monoclonal antibody, Cytokeratin 19, Hand-held gamma detector, Cervical cancer

Introduction

Cervical carcinoma is the second most common cancer in women worldwide and comprises approximately 12% of all cancers in women [1]. Despite progress in the conventional image exams, the diagnosis of supravaginal lesions is unsatisfactory. Magnetic resonance imaging (MRI) studies parallel computerized tomography (CT) scans, with an overall sensitivity of 50-64% and a specificity of 90-95% [2-4]. For the diagnosis of para-aortic lymph nodes, the sensitivity and specificity of CT, ultrasonography, and lymphangiography were 34% and 69%, 18.5 and 98%, and 79% and 73%, respectively, while the role of MRI has still not been confirmed [5]. Radioimmunodetection (RAID), originally called "magic bullet", is promising for cancer localization based on the affinity binding between tumor antigen and antibody [6,7]. In the diagnosis of various cancers by RAID, it has a sensitivity of 60-90% and specificity of 54-97% [6,7]. However, there have been very few reports of RAID in cervical cancers [8,9], most likely owing to lack of an appropriate monoclonal antibody (MAb). Many tumor markers have been used in the monitoring and prognostic assessment of cervical cancers, including squamous cell carcinoma antigen (SCC-Ag), cytokeratin 19 (CK19), CYFRA 21-1, carcinoembryonic antigen (CEA) and tissue polypeptide antigen (TPA), etc. [10,11]. However, there seems to be no RAID by these markers for the clinical detection of cervical cancer.

A monoclonal antibody against CK19 (MAb Cx-99) produced in our laboratory, showing a remarkable difference between cervical cancer and normal tissues, was selected for a RAID study in cervical cancer [12,13]. Preliminary immunoscintigraphy studies using ^{131}I-labeled MAb Cx-99 had promising results for the detection of cervical cancer xenografts [13], but using ^{111}In-labeled MAb, remarkably strong radioactivity of the liver, spleen and kidney was found - which was compatible with other reports - causing difficulty and limitations in identification of the tumor near these areas [14,15].

To date, the hand-held detection system has shown many advantages, for instance, on-site localization, decision-making, and convenience in use, etc. [16, 17]. Therefore the object of this study was to establish a RAID system comprised of ^{111}In-labeled MAb Cx-99 and a competent hand held detector for the detection of cervical cancer. Adams *et al.* disclosed that an intraoperative

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handheld probe was more sensitive than immunoscintigraphy [18], therefore we also want to understand if this probe system can improve the performance of RAID detected by other methods.

Materials and Methods

Cell lines

Two cervical squamous cell carcinoma cell lines, SIHA and ME180, were obtained from the ATCC (American Tissue Culture Collection) and a third cell line, CC7T, was obtained from T.M. Chang of Taipei, VGH [19]. Glioma cell line, G9T, and foreskin cell line, FS-4, were also obtained from ATCC. The SIHA cells were cultured in minimal essential medium (MEM) with 10% fetal calf serum (FCS); ME 180 was cultured in McCoy's 5A medium. All other cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FCS.

Monoclonal antibody MAb Cx-99 and preparations

MAb Cx-99 is a murine IgG1 developed in our laboratory by fusing mouse myeloma cells P3-NS1/1Ag4 (NS-1) and spleen cells of *Balb/c* mice immunized with cervical SCC cells. It was purified from mouse ascites by ammonium sulfate precipitation and DEAE ion exchange chromatography as described previously [12]. It recognizes an epitope of a glycoprotein of 37 kDa in cervical carcinoma tissue, while DNA sequences were identical with that of CK19 [12, 13]. Isotype-matched mouse immunoglobulin MOPC IgG1 (1mg/ml, Litton Bionetic Inc., Charleston, SC) was used as a control. In a pretest to select the appropriate cell line for this study, MAb Cx-99 was labeled with ^{125}I by the chloramines T method as described previously [13], and a specific activity of 12 mCi/mg was obtained. The cervical SCC-SIHA cell line showed the highest radioactivity compared with other cell lines (G9T, FS-4, CC7T, and ME180) (Figure 1). The method for labeling MAb Cx-99 with ^{111}In was described in a previous report [14]. Briefly, we used diethylenetriamine-pentaacetic acid (DTPA) as a bifunctional chelating agent for producing the DTPA-MAb complex. Then 0,4 ml of DTPA-MAb complex was mixed with 2mCi of $^{111}InCl^3$ (in 0.1 M HCl, Amersham International, Amersham, UK), dissolved in 0.5 M acetate (pH 6.5). Unbound ^{111}In was removed by Sephadex G-50 column chromatography eluted with phosphate buffered salin (PBS) containing 1% bovine serum albumin. The radioactivity in every fraction was monitored by a dose calibrator. The specific activity was 10 uCi/ug. Quality control was performed by spotting samples on instant thin-layer chromatography silica gel (ITLC-SG, Gelman). The radioactivity was monitored by a Bioscan imaging scanner. The radiolabeled MAb Cx-99 was filtrated through a 0.2 um Millipore filter before use.

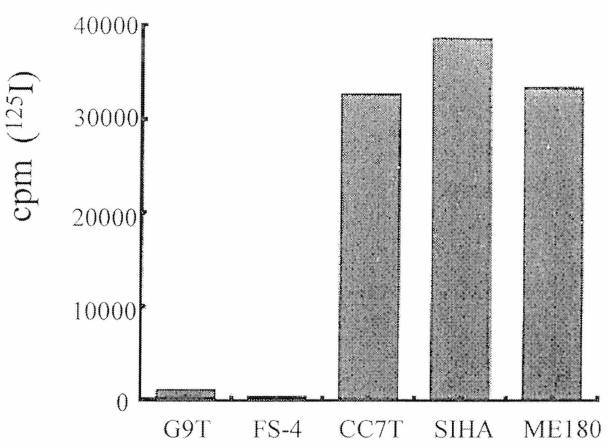


Figure 1. — Binding activity of ^{125}I-labeled MAb Cx-99 to three cervical cancer cell lines (CC7T, SIHA and ME180) and two control cell lines (G9T and FS-4).

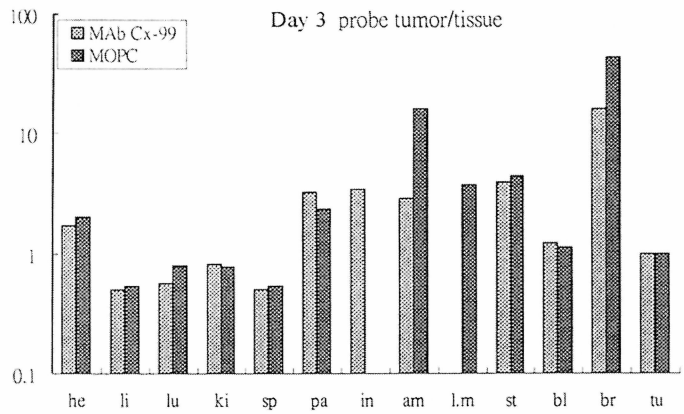
Tissue localization in nude mice

Five-week-old nude mice (BALB/c-nu/nu) (Charles River Lab., Atsugi, Japan) were inoculated subcutaneously with 4 x 10^6 SIHA cells in the flank regions. After 18-28 days, the xenografts grew to 0.3-1.8 g and were ready for use. ^{111}In-labeled normal mouse IgG1 (MOPC) was used as a control. The ^{111}In-labeled MAb Cx-99 or MOPCIgG1 (12-35 Ci/1.2-3.5 g protein) was injected intravenously into each xenograft-bearing mouse. Groups of three mice each were sacrificed under anesthesia on days 2, 3, 4, and 7 after injection. Blood was drawn, and organs were removed and weighed. Reactivity was expressed as the localization ratios of tumor/non-tumor tissues after detected by a handheld gamma detection probe (Custom Indium-111 Surgical Probe, CTC-4 Counting Unit and Optional Audible Guidance System, Radiation Monitoring Devices, Inc., Watertown, MA, USA). The probe system was used according to the manual guide. Briefly, a 20-second count of the radioactivity for each tissue specimen was duplicated. The distance between the detector head of the handheld probe and the target organs was 1 cm.

Results

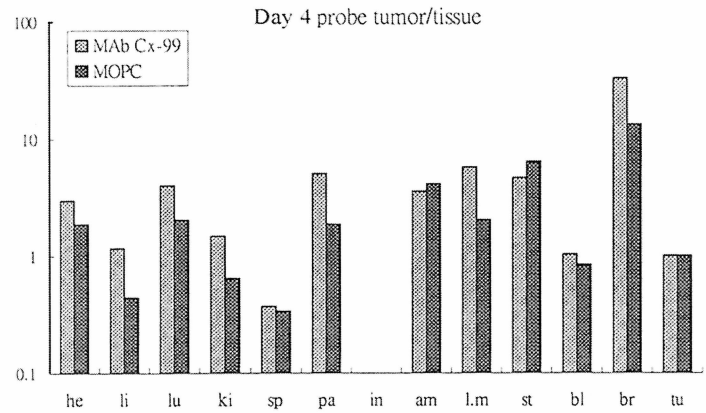
The labeling efficiency of ^{111}In-labeled MAb Cx-99 and MOPC in quality control was 91.56% and 95.5 %, respectively. With the handheld detector, the tumor/tissue ratios the second and third day after injection of ^{111}In-labeled MAb Cx-99 exhibited a similar trend, i.e., data of both days could be divided into high and low levels. The ratios the stomach, heart, pancreas, intestine, abdominal muscles, and brain tissues were at high levels, in the range of 1.37-3.57 on the second day, and 1.73-16.17 on the third day. The ratios of the liver, spleen, and kidney were at the low levels in a range of 0.39-0.79 on the second day and 0.5-0.81 on the third day (Figure 2). On the fourth day of injection of ^{111}In-MAb Cx-99, most of the tumor/tissue ratios increased to above one, including those previously high background organs, such as the liver, lung and kidney with a total range of 1.18-32.67 (Figure 3). The only exception was the spleen where the ratios remained at the same low levels of 0.37-0.5 from the second to the fourth day. Also, the ratios obtained by the ^{111}In-labeled MAb Cx-99 group were obviously higher than the controlled MOPC in most tissues including the heart, liver, kidney, pancreas, leg muscles and brain on the fourth day.

On the seventh day of injections, ratios obtained by ^{111}In-MAb Cx-99 of all tissues except the spleen were above one and the levels were higher than that of MOPC, with the ranges of 1.14-39.35 and 0.27-5.7, respectively (Figure 4). The tumor/spleen ratios of ^{111}In-labeled MAb Cx-99 increased from 0.37 on the fourth day to 0.91 on the seventh day, apparently higher than



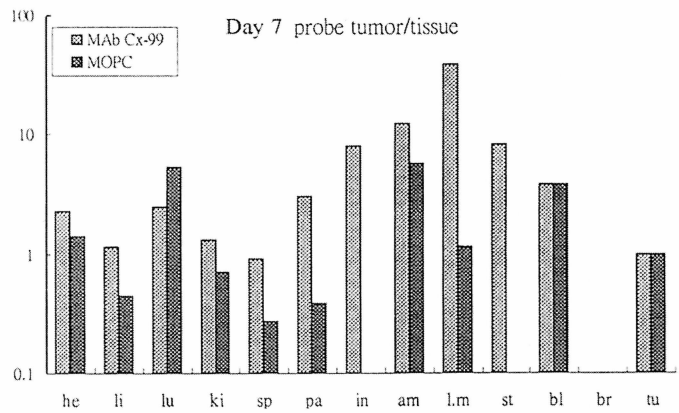
he = heart; li = liver; lu = lung; ki = kidney; sp = spleen; pa = pancreas; in = intestine; am = abdominal muscle; l.m = leg muscle; st = stomach; bl = blood; br = brain; tu = tumor

Figure 2. Localization of tumor/tissue ratios on the third day after injection of ^{111}In-labeled MAb Cx-99 into nude mice.



he = heart; li = liver; lu = lung; ki = kidney; sp = spleen; pa = pancreas; in = intestine; am = abdominal muscle; l.m = leg muscle; st = stomach; bl = blood; br = brain; tu = tumor

Figure 3. — Localization of tumor/tissue ratios on the fourth day after injection of ^{111}In -labeled MAb Cx-99 into nude mice.



he = heart; li = liver; lu = lung; ki = kidney; sp = spleen; pa = pancreas; in = intestine; am = abdominal muscle; l.m = leg muscle; st = stomach; bl = blood; br = brain; tu = tumor

Figure 4. — Localization of tumor/tissue ratios on the seventh day after injection of ^{111}In -labeled MAb Cx-99 into nude mice.

0.27 of the control MOPC. The effect of delayed detection was more evident in the liver, as the tumor/liver ratios of ^{111}In-labeled MAb Cx-99 uptake increased progressively from the second to the fourth day (0.39, 0.5 and 1.18, respectively), in contrast to the steady levels of ^{111}In-labeled MOPC (0.53, 0.44 and 0.45 on the third day, the fourth day and the seventh day, respectively).

Discussion

In this study, the cervical cancer xenografts could be distinguished from other background tissues by detection with a handheld gamma detector, from the fourth day to the seventh day after injection of ^{111}In-labeled IgG1 MAb Cx-99. These findings revealed the major issues necessary for RAID: an antibody, radionuclide and scanning process [20].

^{111}In-labeled MAb Cx-99 had a high binding activity with all of the three cervical cancer cell lines shown in this study. Previous studies also showed that MAb Cx-99 reacted with cancerous tissues from all of the cervical cancer patients by immunohistochemistry and western blot analysis [12,13]. The elicited antigen CK19 was present extensively, i.e., more than 70% of the cancerous areas, in 16 of 22 cervical squamous cell carcinomas, but the antigen expression in the normal tissues was limited to a few specific foci: basal cells of the normal cervical epithelium, ductal and glandular epithelial cells of the liver and kidney, while there was no reactivity in the stromal or blood cells [12]. Van Trappen et al used a fully quantitative, real-time reverse-transcriptase PCR assay and suggested the useful value of CK-19 in correlation to clinicopathological features [21]. These characteristics provided MAb Cx-99 the basis for RAID of cervical cancer. In the literature, CYFRA 21-1, a fragment of CK19, has been taken as a valuable serum marker for cervical carcinoma, head and neck cancer and non small cell lung cancer (NSCLC), etc. [11,22,23]. In SCC of the head and neck and NSCLC of the lung, this marker has been found to be better than the SCC-antigen owing to the high sensitivity and specificity [22,23]. However, there has not yet been a report using CK19 as the target antigen in RAID of cervical cancer except for a few preliminary reports from this laboratory [13,14]. The immunoscintigraphy study by ^{131}I-labeled MAb Cx-99 had promising results for detection of cervical cancer xenografts [13]. ^{111}In has been considered as a second generation radionuclide for RAID, it has lower energy, more stable binding and shorter half-life of only 2.83 days, and is more specific and reliable for tumor imaging compared with ^{131}I [8,24].

On the second day (one day after injection of ^{111}In-labeled MAb Cx-99) and the third day of the early distribution period, the tumor/tissue ratios detected by ^{111}In-labeled MAb Cx-99 were at a similar levels with those of the control ^{111}In-labeled MOPC in most tissues with this handheld probe. The tumor/tissue ratios were low in the liver, lung, kidney and spleen due to high uptake of immunoconjugate in these normal tissues, which was not an unexpected finding, as ^{111}In or Tc-99m have this drawback in application [25]. Beatty et al. showed that liver could absorb 20% of an injected dose of ^{111}In-MAb [24]. The mechanisms of liver uptake include sequestration of the immunoconjugate in the reticulo-endothelium system and ^{111}In is absorbed especially by the liver [24]. The liver uptake in such a high proportion by ^{111}In-MAb causes difficulties in differentiation from liver metastasis. Collier *et al.* reported that RAID by ^{111}In-MAb was inferior to a CT scan, i.e., 38% vs. 88% in the diagnosis of hepatic metastasis [25].

In this study, the tumor/liver ratios of ^{111}In-labeled MAb Cx-99 uptake increased progressively from the second day to the fourth day, compared to the steady levels of ^{111}In-labeled MOPC. This depicted a possible way to solve the problem of high background in the liver, kidney and lung by delaying detection time. Delayed detection may also be helpful in the selective concentration method reported by Divgi *et al.* for the differential diagnosis [26]. Utilizing Fab or F(ab')2 fragment of MAb is another way for diminishing background uptake due to faster clearance of the small-size immunoconjugate [15,27]. Also, ^{125}I can be a substitute for ^{111}In for the same purpose because it does not accumulate in the liver, spleen or kidney and it has been frequently used in the handheld detector system [28, 29].

The spleen is also of interest because the effect of delayed detection was not seen until the seventh day as compared with ^{111}In-labeled MOPC. This finding was different from one report where the radioactivity in the spleen and kidney decayed rapidly [24]. Our report also indicated that the excretion rate of immunoconjugates from the spleen was different from that of the liver or kidney. Therefore, the rationale is to use the delayed detection methods at different intervals to differentiate the spleen from the other background tissues.

The validity of a handheld probe system in RAID has shown many promising ways in clinical application: use for on-site detection and excision of cancer lesions, differentiation of suspicious foci, determination of tumor-free margins during and after surgery and lymph node mapping [16,17, 30-32]. Cumulative data showed that the sensitivity, specificity, positive prediction value (PPV), and negative predictive value (NPV) of the probe system for cancer detection were 71.4-90%, > 90%, 100% and 94%, respectively [17,28,29], while the PPV for lymphoid tissue was lower (46.5%) [28]. Additional lesions were detected by the probe system ranging from 36.8% to 64.7% of the cancer patients compared with conventional surgeries, which resulted in changing the stage and surgical plan [16,17,30,33]. For assisting ex vivo detection of lymph nodes, Nabi *et al.* found that this system led to a twofold to fourfold increase in the number of metastatic lymph nodes [31]. Furthermore, patients with RIGS-positive tissues remaining after surgery had a much poorer survival [30,32].

Also, it has become feasible now to perform sentinel lymph node dissection (SLND) with a handheld probe utilizing radiolabeled colloid or blue dye in cervical cancers, while preliminary results showed high sensitivity and negative predictive value of 90% and 97.4%-100%, respectively [34,35]. RAID, with the theoretically higher binding affinity to cancer will very likely improve the specificity for SLND.

In conclusion, this study confirmed that ^{111}In-labeled MAb Cx-99 is valid for RAID application by the surgical probe system. There were high non-specific bindings

in the liver and kidney, which however could be diminished through a delayed detection method. The appropriate number of days for cancer detection by the surgical probe system was between day 4 and day 7 after injection of ^{111}In-labeled MAb Cx-99.

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