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RADIOIMMUNODETECTION OF PROSTATE CANCER BY ^{111}In-LABELED MONOCLONAL ANTIBODY AGAINST PROSTATIC ACID PHOSPHATASE

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Purified human prostate acid phosphatase (PAP) was used to generate a specific monoclonal antibody (FC 3001) for detection of PAP expressed by some prostatic carcinomas. DTPA derivatives of MoAb-F(ab')2-fragments were labeled with indium-111 chloride. This labeled antibody was tested in 15 prostate cancer patients who underwent staging pelvic lymphadenectomy; 9 of them received labeled antibody alone whereas 6 received simultaneous injections of labeled and unlabeled antibody with two dose levels (40 or 80 mg). Biodistribution data obtained by direct blood measurements and imaging procedures indicated that simultaneous injection of unlabeled antibody reduced both the blood elimination rate and the accumulation in the liver. Accumulation of the radionuclide in pelvic lymph node metastases was observed in some patients but in a couple of patients accumulation was noted also in normal lymph nodes. The method cannot in its present design replace staging pelvic lymphadenectomy and further studies are needed for elaboration of clinically useful radioimmunodetection methods.

Prostatic cancer is the second common type of cancer among men, with 150 000 new cases per year seen in the Western world (1). It can be successfully treated with radical prostatectomy or radiotherapy if the disease is diagnosed at an early stage (2). Staging pelvic lymphadenectomy (SPL) has been the only accurate method to assess the degree of pelvic lymph node involvement. However, as a diagnostic tool operative staging is comparatively laborious and not free from complications.

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Routinely used conventional radiologic imaging methods (CT, transrectal US) have not the sufficient accuracy for an adequate staging (3, 4). Monoclonal antibodies and specific markers have been utilized to differentiate between malignant and benign prostatic tissue. Several monoclonal antibodies (MoAb) have been developed for use against prostate cancer cell antigens, i.e. KR-P8, 7E11-C5, D83.21, P6.2, PEQ 226, Turp 27 (5-13) and also against androgen-receptors (14). Clinically, these radiolabeled antibodies have mainly been used to detect distant metastases, and some immunolymphoscintigraphic approaches have been used for staging purposes (15, 16).

Detection of pelvic nodal metastases with ^{111}In-labeled anti-PSA antibody, using intralymphatic injections, has shown poor sensitivity as regards bone and soft tissue lesions in prostatic carcinoma (17). Radioimmunolocalization of prostate cancer using polyclonal antibodies against prostatic acid phosphatase (PAP) has been reported in two patients (18). ^{99m}Tc- and ^{111}In-labeled F(ab')2-fragments of MoAb against PAP have been used for staging after intraprostatic injection (19-21). The sensitivity of such attempts, using ^{123}I-labeled PAP-MoAb, has been reported by Leroy et al. (22) to be 100% and the specificity 83% (22). However, lack of specificity following intraprostatic

injections of ^{111}In-PAP-MoAb has also been reported in some studies (23, 24) and we have therefore now used a different scintigrafic approach.

The main aim of our study was to evaluate the effect of simultaneously injected unlabeled antibody on the biodistribution and pharmacokinetics of intravenously given ^{111}In-PAP-MoAb.

Material and Methods

Patients. The present report is based on a multicenter study on prostate cancer patients subjected to SPL. Until now, 15 patients have been studied. Nine patients received labeled antibody only and 6 patients either 40 or 80 mg of unlabeled antibody just before the ^{111}In-PAP-MoAb injection. The individual serum concentrations of ^{111}In were measured in 6 patients, of whom 3 received labeled PAP-MoAb alone and 3 both unlabeled and labeled PAP-MoAb.

MoAb. Human purified PAP was used to prepare a specific monoclonal antibody FC-3001 (25). DTPA-derivatives of the antibody F(ab')2-fragments (1 mg) were labeled with 185 MBq ^{111}In-chloride. ^{111}In was chelated by the DTPA moiety attached to the antibody fragment. The unlabeled antibody was given slowly intravenously (30 min) to the individual patients at three different doses (0 mg, 40 mg and 80 mg/100 ml) diluted in physiological saline to 100 ml. The labeled reagent was administered as a slow (1 min) i.v. injection 60 min after initiating the unlabeled antibody infusion.

Biodistribution. Whole-body scans and spot images, both anterior and posterior views, were taken at 6, 24, 72 and 168 h after the ^{111}In-PAP-MoAb injection. The activity in blood pool (heart, left ventricle, aorta), kidneys, liver, bone marrow, spleen, prostate, and possible metastases was imaged using an ordinary gamma camera (Genensys, ADAC) and analysed by estimating counts per pixel in the main organs. Regions of interest (ROI) over different organs were drawn similarly in all individual scintigrams. The background accumulation of ^{111}In-PAP-MoAb was subtracted before comparing organ activities between patients. The area below the liver was used as background region. Conventional methods were used as control methods for detection of possible metastases: bone scintigraphy, ultrasonography (US) or computerized tomography (CT). In two patients single photon emission tomography (SPET) studies were also performed.

Pharmacokinetics. Serum samples were taken after the labeled antibody injection at 0, 5, 10, 15, 20, 60, 120 and 240 min and at 26, 50, 74 and 170 h. The ^{111}In concentrations in serum of each patient were measured to conceive the half-life. Pharmacokinetics of ^{111}In-PAP-MoAb in serum was mathematically evaluated by approximately fitting the values to the sum of two or three exponential terms according to the formulas:

$$y = A \times e^{-at} + B \times e^{-bt}$$

or

$$y = A \times e^{-at} + B \times e^{-bt} + C \times e^{-ct}$$

where A, B and C are constants.

Results

No allergic reactions to the antibodies were seen and the procedure was well tolerated. Only the rather long time needed for gamma camera imaging, especially on the seventh day, was inconvenient to some patients.

The apparent final half-life (>20 h postinjection) was 10 h for the patients receiving no unlabeled antibody. For the patients given unlabeled antibody the apparent final half-life for ^{111}In-PAP-MoAb was 24 h in serum (Fig. 1). The individual ^{111}In concentrations in serum are shown on Fig. 1 for the six patients investigated. The results of exponential fitting indicated that the sum of three exponential terms was more accurate in our model than that of two exponential terms.

The spot images and the whole-body images showed that the accumulation in the liver was lower in patients receiving unlabeled antibody. The liver-to-blood ratio of the patients given no unlabeled antibody was higher than in the patients receiving unlabeled antibody (Figs 2 and 3). The difference between the groups was best seen on the spot images (Fig. 2) and when no background subtraction was made before estimation of the liver-to-blood ratio.

SPET images of one patient who received 185 MBq ^{111}In-PAP-MoAb and unlabeled antibody is shown in Fig. 4. Four sagittal slices (lateral views) demonstrate structures with high level of tracer in the urogenital area: bladder, prostate, urethra and penis. The prostate was clearly visible only when at least 185 MBq of tracer was injected.

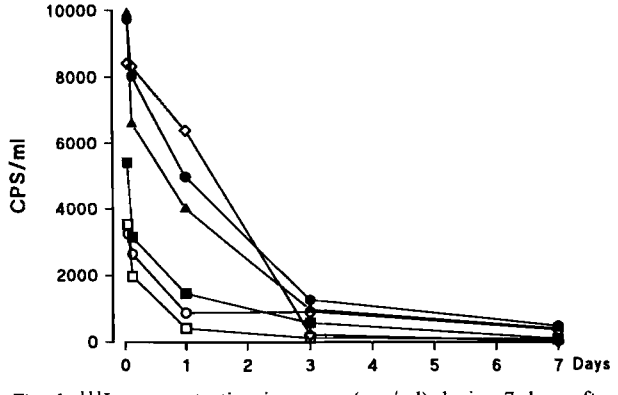

Fig. 1. ^{111}In concentration in serum (cps/ml) during 7 days after ^{111}In-MoAb injection. Patients 2, 3 and 4 (■, ▲, ●) were given 40, 80 and 40 mg of unlabeled antibody respectively. Patients 1, 8 and 9 (○, ◇, □) received no unlabeled MoAb.

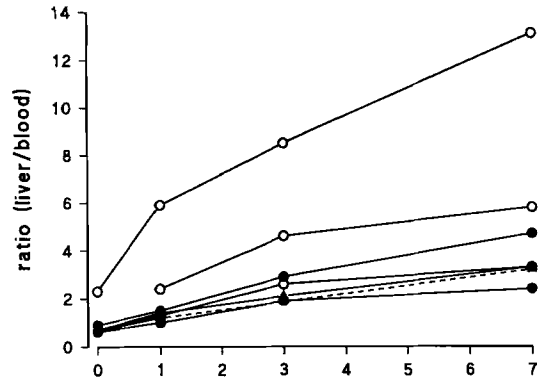

Fig. 2. Liver to blood ratio according to SPOT-imaging data. Patients 1, 6 and and 10 (○) were given no unlabeled MoAb. Patients 4 and 11 (●) received 40 mg and patients 5 and 12 (▲) were given 80 mg of unlabeled MoAb.

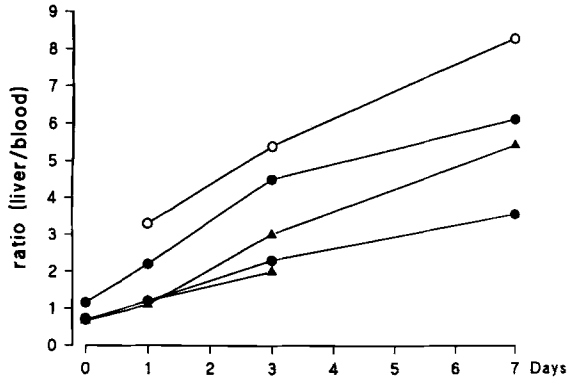

Fig. 3. Liver to blood according to whole body imaging data. Patient 1 (○) were given no unlabeled MoAb, patients 2 and 4 (●) were given 40 mg and patients 3 and 5 (▲) 80 mg unlabeled MoAb.

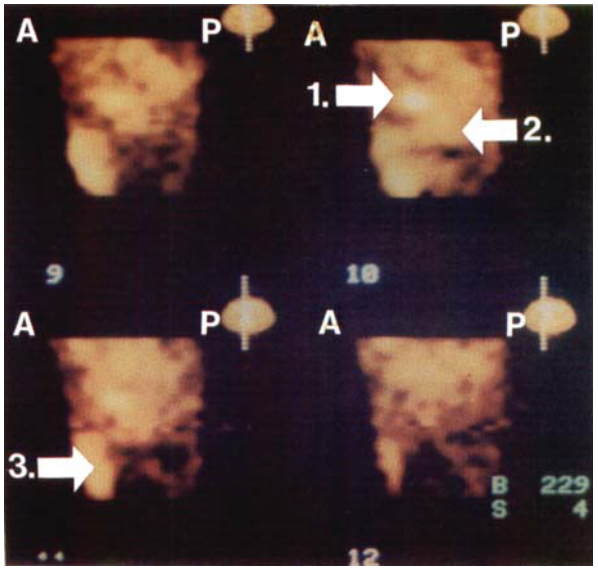

Fig. 4. SPET scan 72 h after injection of ^{111}In-PAP-MoAb (185 MBq) and unlabeled antibody. Four continuous sagittal slices (lateral views). Normal distribution of MoAb in urogenital area: high level of tracer in bladder (1), prostate (2), urethra and penis (3).

The method was able to visualize prostatic cancer metastases in bone or soft tissues including lymph nodes in 5 out of 9 patients receiving ^{111}In-PAP-MoAb alone and in 3 out of 6 patients receiving both labeled and unlabeled antibody. However, most of the metastases that were detected with conventional bone scintigraphy could not be found by ^{111}In-PAP-MoAb. False positive accumulation in normal pelvic lymph nodes were also detected in two patients and one of them had received 80 mg of unlabeled antibody. In two patients with detected lymph node involvement the ratio of activity in the metastases-to-blood was 1.3–1.6. This was seen in the spot images of days 3 and 7. However, a larger number of patients with verified lymph node metastases would be necessary to confirm the increased uptake of ^{111}In-PAP-MoAb in prostatic cancer nodal metastases.

Discussion

In our study the liver-to-blood ratio was lower, and the plasma clearance slower in the patients who received unlabeled antibody then in those who reveived labeled antibody only. Perälä-Heape et al. (26) used the same ^{111}In-PAP-MoAb and investigated the effect of unlabeled MoAb in a nude mouse model. The liver-to-blood ratio decreased slightly when unlabeled antibody was used. The blood concentration of tracer increased significantly and the liver concentration decreased simultaneously when unlabeled MoAb was used. Our results in prostatic cancer patients are the same.

The mechanism of liver uptake of ^{111}In-labeled MoAb has not been clearly identified. Blockage of the reticulo-endothelial system does not affect the liver uptake of ^{111}In-PAP-MoAb, revealing that the Kupffer cells do not play a major role in the uptake of ^{111}In by the liver (27). Much of the uptake of the label by the liver is probably related to the formation of antigen-antibody complexes (28).

In several clinical trials, a tendency towards prolonged serum half-lives and increased tumor uptakes of labeled MoAb have been observed when unlabeled MoAbs are simultaneously administrated (29, 30). Eger et al. (31) used three different doses of unlabeled MoAbs and found that both plasma clearance and liver accumulation decreased with increasing doses of unlabeled MoAbs. Babain et al. used five different doses of anti-PAP MoAb and found a definite relationship between the dose of antibody and the detection rate of metastatic disease (32). In our study the serum activity of the tracer was higher in the patients receiving unlabeled antibodies, but we could not see any considerably increased accumulation of ^{111}In-PAP-MoAb in the tumor tissue. We had the impression, however, that pelvic lymph node metastases were more clearly visualized on days 3 to 7 when unlabeled antibody had been given. Due to some false positive results and a small number of patients with verified pelvic lymph node involvement in the

present study it remains uncertain whether the concentrations of unlabeled antibody used can improve the detection of metastases in prostatic cancer patients.

Both ^{111}In-labeled anti-PSA- and anti-PAP-MoAbs have shown poor sensitivity in limited clinical radioimmunoimaging trials (13, 22, 32, 33). Our results, using anti-PAP-MoAb, are similar. It should be noticed, however, that poorly differentiated tumors are more likely to spread and give metastases and are less likely to be detected compared to well-differentiated ones. By using autoradiography in a patient we could, in a previous study, show that poorly differentiated tumor without glandular structures had significantly lower uptake of anti-PAP-MoAb than well-differentiated ones (23).

To conclude, the use of unlabeled antibody before the injection of labeled antibody seems to enhance the amount of radioactive antibody in the blood with a simultaneous decrease in the liver accumulation. However, whether this method can be used for improvement of radioimmunologic detection of pelvic prostate cancer metastases is still an open question.

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