Urol Res (1994) 22: 323–327

ORIGINAL PAPER

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Immunoscintigraphy with iodine-131-labelled monoclonal antibody AUA1 in patients with transitional cell carcinoma of the bladder

Received: 2 November 1993 / Accepted: 16 June 1994

Abstract The monoclonal antibody AUA1, labelled with 2 or 3 mCi iodine-131, was administered intravesically to 11 patients with known or suspected bladder carcinoma and was kept in the bladder for 1 h. All patients underwent immunoscintigraphy of the bladder at 2 h and three patients also at 20 h after instillation. Conventional histological and immunohistochemical examinations were performed on tissue samples from tumour and normal areas taken during cystoscopy, carried out 24-h after the instillation. Transitional cell carcinoma of the bladder was present in nine patients whereas dysplastic and normal urothelium was found in the remaining two patients, respectively. Six out of nine tumours were successfully imaged at the 2-h scan. Normal urothelium showed no uptake while dysplastic urothelium was positive on imaging. Successful detection was correlated with size and grade of tumour in almost all cases. Tumors with a diameter of 1 cm or less were not detected. Four out of five grade II tumours and two out of three grade III tumours were detected with this method. The method is a promising one although further studies using more suitable isotopes and/or monoclonal antibodies are required to increase its sensitivity.

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Key words Bladder carcinoma · Monoclonal antibodies Immunoscintigraphy

Transitional cell carcinoma (TCC) of the bladder is one of the most common genitourinary tumours and is characterised by a high recurrence rate [12, 16]. Traditionally, diagnosis is established by cystoscopy and biopsies of bladder mucosa. Additional examinations such as intravenous urography, ultrasonography and urine cytological examinations may also contribute to the initial diagnosis or diagnosis of recurrence [19]. However, cystoscopy is an invasive technique requiring anaesthesia in order to examine systematically the entire bladder mucosa and perform multiple biopsies. Furthermore, cystoscopy may not reveal flat, in situ carcinoma or small solid lesions. Therefore there is a need for new diagnostic techniques which are less invasive and at least as accurate as cystoscopy.

During the past decade the use of monoclonal antibodies (mAbs) has offered a new perspective in cancer diagnosis [14, 22] and treatment [8, 10]. Radiolabelled Moabs have been proved effective in detecting metastatic disease after intravenous (i.v.) administration [5, 9, 21], whereas regional administration can be effective in detecting malignant lesions less than 1 cm in diameter, in certain cases [17]. A panel of mAbs has been found to react with TCC of the bladder [1, 11]. One of them, AUA1, showed a strong reaction with tumour cells of bladder tumours, in particular of high-grade malignancies [1]. Recently, radiolabelled AUA1 mAb has been intravesically administered in patients with TCC of the bladder [2, 24]. Selective localisation of the antibody was found in the tumours with little or no uptake by the normal urothelium.

In this paper we report the final data of a pilot immunoscintigraphy study, using intravesical administration of radiolabelled mAb in patients with TCC of the bladder. The aim of the study was to assess the potential of this application as an effective tool in the detection of

malignant lesions of the bladder. We also correlated the diagnostic accuracy of this method both with other conventional techniques and with the findings obtained by histological and immunohistochemical examination of the tumours.

Materials and methods

Material

Monoclonal antibody AUA1 is an IgG1 mouse immunoglobulin which recognises as 35-kDa glycoprotein [7] present in certain types of normal epithelia and most human carcinomas [23]. It reacts with the majority of bladder carcinomas and particularly high-grade transitional cell carcinomas [1].

Patients

Eleven [11] patients undergoing cystoscopy for suspected or known bladder carcinoma gave their informed consent before entering the study. Patients with other abnormalities of the bladder (e.g. urinary stones, bladder diverticula) or benign prostatic hyperplasia were excluded from this trial. All patients were studied in the Urology Department of Tzanio Hospital and the Nuclear Medicine Department of Metaxa Cancer Hospital.

Methods

Labelling

The *N*-bromosuccinamide (NBS) technique was used [13]. Briefly, 10 mCi Iodine-131 (50 µl), 3 mg AUA1 (500 µl) and 17 µl of a 10% NBS solution were mixed together by gently shaking for 15 min. Labelling efficiency was then estimated by paper chromatography. If it was below 75%, 5 µl NBS solution was added and the mixture was allowed to react for a further 15 min. This method resulted in a labelling efficiency of consistently higher than 95%, so gel filtration for removing free iodine was unnecessary. Four millilitres of phosphate-buffered saline containing 1% human serum albumin was added to stop the reaction. The final volume was sterilised through a micropore filter and collected in a sterile vial. There was no significant loss of immunoreactivity of the antibody after iodination, as shown by enzyme-linked immunosorbent assay and indirect radioimmunoassay.

Administration of mAb imaging

A quantity of 2 or 3 mCi ^{131}I-AUA1 (1 mg) was diluted in 50 ml normal saline and administered intravesically through a Foley 16-Ch catheter. The mAb was maintained in the bladder for 1 h, during which time the patients were rotated in position. The bladder was then emptied and lavaged thoroughly 4–5 times with normal saline. The Foley catheter was then substituted with a Nelaton 12-G catheter and the bladder was filled with 100–150 ml normal saline for imaging. Gamma camera scans were taken at 0 h (immediately after the administration), 2 h (immediately after lavage and refilling of bladder with normal saline) and in three cases also at 20 h after the administration of the radiolabelled antibody, using a Gammatome-2 (Sofa Medical, France) camera. Anterior views of the bladder were taken with 150 000–200 000 counts/image and the median period to obtain an image was 25 min. Image interpretation was performed by two nuclear medicine physicians without knowledge of the cystoscopy or the CT scan results. Criteria for positive, negative and equivocal findings were as follows: (1) positive findings – clearly defined hot area in the bladder, (2) negative findings – no hot area in the bladder; similar to an immunoscan of normal bladder and (3) equivocal findings – no clearly defined hot area.

Histological and immunohistochemical examinations

From the 11 patients studied tissue specimens of malignant lesions and normal urothelium were taken during cystoscopy or transurethral resection of the tumour, 24 h after the administration of the antibody, and fixed in 10% formalin. They were then processed in the conventional way and embedded in paraffin. Five-micrometre serial sections were cut and used for conventional histological or immunohistochemical examinations. Haematoxylin-eosin staining was performed to confirm the presence of tumour in the samples and to define the grade of tumour according to the three-grade system accepted by the WHO [15]. Indirect immunoperoxidase staining using AUA1 was used to study the antigenic expression of AUA1 in tumour and normal tissues. For categories were defined, based on the percentage of cells positive for AUA1: (1) no staining, (2) weak staining (5–10%), (3) moderate staining (10–50%) and (4) intense staining (more than 50%).

Results

Histology and immunohistochemistry

Tumours were confirmed in 9 out of the 11 cases studied. One patient had grade I, five patients grade II and three patients grade III TCC of the bladder. In the remaining two cases normal and dysplastic urothelium was found, respectively. Regarding the immunohistochemical findings, AUA1 mAb stained positively in eight out of the nine tumours (Fig. 1). The staining was correlated with the grade of disease (Table 1). Grade I tumours showed weak staining of the tumour surface (surface of the papillae) while staining of grade II tumours extended towards the middle part of the neoplasms. Grade III tumours showed staining of most of the cells, with some heterogeneity of the staining intensity. Normal urothelium showed weak staining of the epithelial surface, in contrast to the dysplastic urothelium, which showed intense staining.

Imaging

Table 1 shows the immunoscintigraphy results and their correlation with the findings of CT scanning, cystoscopy, histology and immunohistochemistry. Figure 2 shows the scans of patients 1, 8, 10 and 11 at 2 h after the instillation of the radiolabelled mAb. The immunoscan of the patient whose bladder mucosa was proved to be normal by means of cystoscopy and histology shows no specific localisation of radioactivity (Fig. 2a). The remaining three immunoscans in Fig. 2 are immunoscans of patients with urothelial tumours diagnosed by means of cystoscopy and histology and show localisation of radioactivity (arrows) at tumour sites as was also confirmed by CT scan (Figs. 3, 4).

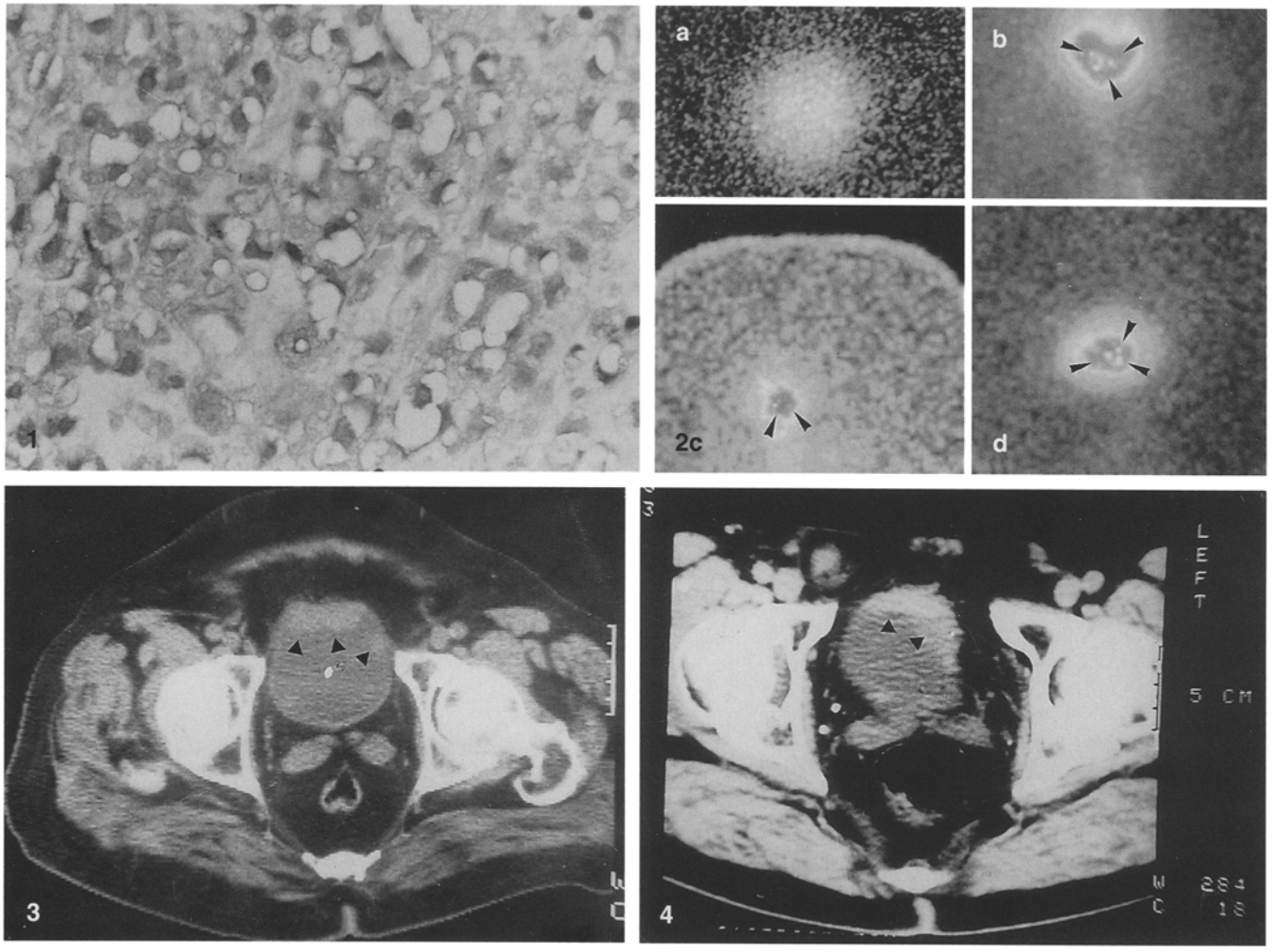

Fig. 1 Expression of the antigen recognised by AUA1 in a grade III TCC of the bladder. The indirect immunoperoxidase method was used. Intense staining of tumour cells is seen, ×400

Fig. 2a–d Immunoscans (*Is*) of patients 1, 8, 10 and 11 at 2 h after the instillation of ^{131}I-labelled AUA1 (anterior views). **a** Is of patient 1 where no tumour was found during cytoscopy. **b** Is of patient 8. Hot area in the lower part of bladder (*arrows*). **c** Is of patient 10. Hot area in the middle and left part of the bladder (*arrows*). **d** Is of patient 11. Hot area in the lower right and left part of the bladder (*arrows*)

Fig. 3 Computed tomography scan of patient 8. View of the lower part of the urinary bladder. The tumour is localised mainly at the anterior but also at the left lateral and posterior wall of the bladder (*arrows*). The tumour is seen extending all around the bladder neck

Fig. 4 Computed tomography scan of patient 10. The tumour is localised at the anterior and left lateral bladder wall (*arrows*)

At 2 h after the administration, in six out of nine cases immunoscintigraphy detected a well-defined area corresponding to the site of the disease, as shown by CT scan and cystoscopy. In one case (patient 4), the findings were described as equivocal because the hot area was not clearly defined. In patients 2 and 3 there was not visualisation of the tumours by immunoscintigraphy. These tumours were less than 1 cm in diameter, while all the detected tumours were equal to or larger than 2 cm in diameter. In patients with no evidence of TCC, the immunoscan was negative. In contrast, the patient with a histological confirmed dysplastic urothelium showed both a positive immunoscan and intense immunohistochemical staining (patient 5). Finally, the tumours of three patients with positive scans at 2 h could not be deteced at 20 h.

Table 1 Correlation of immunoscintigraphy findings at 2 h after intravesical administration of radiolabelled AUA1 with cystoscopic, CT scan, histological and immunohistochemical findings (*PT*, papillary tumour; *ST*, solid tumour; *TCC*, transitional cell carcinoma; *ND*, not done)

Patients	Cystoscopy findings	Histological findings	Immunohisto-chemical findings	CT scan findings	Immunoscan
1	No evidence of neoplasm	Normal epithelium	Weak staining	ND	Negative
2	PT: diameter 1 cm	TCC grade I	No stainng	ND	Negative
3	PT: diameter 0.5 cm	TCC grade II	Moderate staining	ND	Negative
4	PT: diameter >2 cm	TCC grade III	Intense staining	ND	Equivocal
5	No evidence of neoplasm	Dysplastic epithelium	Intense staining	ND	Positive
6	PT: diameter >3 cm	TCC grade II	Weak staining	ND	Positive
7	PT: diameter 2 cm	TCC grade II	Intense staining	ND	Positive
8	PT: diameter >2 cm	TCC grade II	Weak staining	Tumour extending around the bladder neck	Positive
9	PT: diameter >3 cm	TCC grade II	Moderate staining	Tumour at the left lateral bladder well	Positive
10	ST: diameter >3 cm	TCC grade III	Intense staining	Tumour at the anterior and left lateral bladder wall	Positive
11	PT: diameter >2 cm	TCC grade III	Moderate staining	Tumour at the right anterior and left lateral bladder wall	Positive

Discussion

Immunoscintigraphy has been widely used for the detection of human malignancies and with promising results in many cases [3, 5, 20, 21]. There is evidence that TCC can be detected by systemic administration of mAbs [4]. Nevertheless, bladder cancer seems an ideal model for diagnostic and therapeutic approaches using regional administration of mAbs. AUA1 was selected for this study because it has been shown to be localised specifically at tumour sites after intravesical administration, with minimal uptake by the normal urothelium and with no circulating radioactivity [2, 24]. Therefore by using intravesically AUA1 mAb a high specificity due to selective accumulation of radioisotopes at tumour sites and early imaging due to the absence of systemic activity is expected.

This study shows that immunoscintigraphy following intravesical administration of ^{131}I-labelled-AUA1 can result in successful detection of tumour sites as early as 2 h after the administration of the antibody in 66% of cases. There was a correlation between the positive findings of immunoscintigraphy and the size of the tumours in almost all cases. All tumours with a diameter of 2 cm or larger were successfully detected except in one case, which gave equivocal findings. Two tumours with a diameter of 1 cm or less could not be detected by this method. No hot areas were detected in the patient with normal urothelium. However, dysplastic urothelium showed a positive scan. This could be attributed to the high antigenic expression in this particular case as this was confirmed by immunohistochemistry. Considering that conventional histology is unable to define the benign or malignant nature in such cases until true malignant transformation occurs, it would be interesting to follow up cases with dysplasia where immunoscintigraphy is positive. Finally, all scans performed at 20 h failed to image the tumour; this finding could be attributed either to deiodination or to progressive clearance of the antibody-radioisotope complex from the bladder, as this has already been shown in a previous study [24].

Antigen expression seems to be an important factor in the success of immunoscintigraphy. All but two cases with a positive immunoscan had moderate or intense staining with AUA1. All positive scans were obtained from tumours of low or moderate differentiation (grade II or III). Assessment of the sensitivity of this method in grade I tumours is not possible, since only one grade I tumour was included in our material. Nevertheless, definite conclusions cannot be drawn from the available number of patients. A prospective study which assess the contribution of intravesically administered mAbs in the detection of small, low-grade TCC is needed. It would also be of crucial importance to obtain an impression of the false-positive rate by studying in significant number of people without bladder tumour.

The expected major contribution of studies with intravesically administered radiolabelled mAbs is not only to detect TCC but mainly to use them for regional therapy. Although the successful localization of AUA1 in grade II and III tumours is promising, the potential therapeutic of mAbs has not yet been established. An earlier study has shown that the amount of the antibody, localized in the tumours, is not sufficient to deliver a cytotoxic dose of a radioisotope suitable for radioimmunotherapy [2]. We have obtained similar results, using ^{131}I-AUA1 [24]. Inadequate penetration of the tumours by the radiolabelled antibody might represent a major factor in poor

localisation. The simultaneous use of different antibodies, recognising antigens expressed by tumour cells [6], could facilitate antigen-antibody interaction. Additionally, antibody fragments have been proved more effective in the penetration of tumours than intact antibodies [18] and could result in increased tumour uptake if they were used in this approach.

In conclusion, immunoscintigraphy using intravesical administration of ^{131}I-labelled-AUA1 can result in successful localisation of bladder carcinomas 2 cm or more in diameter. The method is simple, safe and well tolerated by the patients and shows a high sensitivity in grade II and III tumours, where it could represent a useful adjunct to conventional cystoscopy. Pilot clinical studies should be performed, to confirm the usefulness of this approach, especially in the cases where non-invasive procedures result in inconclusive evidence of the presence or absence of tumour in the bladder. Further studies using different antibodies and isotopes are also required to improve the sensitivity of this method for lower-grade and smaller-sized tumours.

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