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Intravesical administration of tumor-associated monoclonal antibody AUA1 in transitional cell carcinoma of the bladder: a study of biodistribution

J. Zorzos[1], D. V. Skarlos[2], A. A. Epenetos[3], D. Pectasides[4], P. Koutsioumba[4], J. Elemenoglou[5], A. Bakiras[1], M. Likourinas[1], K. Dimopoulos[6]

[1] Department of Urology, Tzanio Hospital, Piraeus, Greece
[2] Third Department of Medical Oncology, Agii Anargiri Hospital, Athens, Greece
[3] ICRF Oncology Group, Hammersmith Hospital, RPMS, London, UK
[4] Nuclear Medicine Department, Metaxa Hospital, Piraeus, Greece
[5] Histopathology Department, Tzanio Hospital, Piraeus, Greece
[6] Department of Urology, Athens University, Athens, Greece

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Summary. Forty-five patients known or suspected to have transitional cell carcinoma of the urinary bladder underwent intravesical administration of either AUA1 tumor-associated monoclonal antibody or 11.4.1. nonspecific monoclonal antibody. Antibodies were radiolabeled with iodine-131, diluted in 50 ml normal saline and remained in the bladder for up to 1 h. During cystoscopy or transurethral resection of the tumor, tissue samples were taken from normal and malignant areas and were counted for radioactivity in a gamma counter. Blood samples were also measured for radioactivity. Mean uptake of AUA1 at 2, 20, 40 and 60 h after administration (expressed as $10^3 \times$ percentage of injected dose/gram of tissue) was: 1.77 ± 3.2, 1.28 ± 1.67, 0.72 ± 0.94 and 0, respectively in the tumor and 0.79 ± 0.83, 0.14 ± 0.34, 0.033 ± 0.06 and 0 in normal tissue. Mean uptake of 11.4.1 at 2 and 20 h was: 0.47 ± 0.42 and 0.018 ± 0.015, respectively, in tumor and 0.2 ± 0.19 and 0.013 ± 0.002 in normal samples. No remarkable radioactivity was found in blood samples. Conventional and immunoperoxidase staining were also performed. Mean uptake of AUA1 by the tumor increased as the degree of tumor differentiation decreased. Our findings indicate that intravesical administration of AUA1 results in selective immunolocalization of AUA1 in intermediate and high-grade transitional cell carcinoma. This may allow the development of a new method for bladder carcinoma treatment or prophylaxis against recurrence.

Key words: Bladder – Transitional cell carcinoma – Monoclonal antibodies

Transitional cell carcinoma (TCC) of the bladder is characterized by a high incidence of recurrence and/or progress in muscle invasive disease. The vast majority (70–80%) of the initially diagnosed bladder tumors are of high or intermediate differentiation (grade I or grade II) and early stage (Ta, T1); thus, efforts are focused on preventing recurrence or progression of the disease by means of intravesical chemotherapy and/or immunotherapy. Chemotherapeutic agents (mitomycin C, epirubicin) and factors modulating the bladder's local immune system (BCG, interferons) have been used extensively with satisfactory results; however, these agents do not lack local and/or systemic toxicity, and prophylactic intravesical therapy of superficial bladder tumors does not preclude the possibility of recurrence [6, 9, 14].

Monoclonal antibodies (Moabs) offer new prospectives in cancer diagnosis both in vitro and in vivo, and probably in cancer treatment. Moabs could exert their therapeutic potential either alone (non-armed) by means of direct or indirect cytotoxicity or conjugated with cytotoxic drugs, toxins or radioisotopes [3, 16]. Previous studies have shown that the regional administration (intrapleural, intraperitoneal) of radiolabeled Moabs could have therapeutic effects in certain types of cancer [5, 11].

Regarding malignant neoplasms of the bladder, a panel of Moabs have been found to be useful in the diagnosis of TCC [2, 7, 8]. Of particular interest is TCC detection with the Lewis X antigen, which is a cell surface differentiation antigen carried on either protein or lipid moieties, as a marker of neoplastic transformation [13].

AUA1 is a Moab which has been proved to react with most human carcinomas; it reacts with tumor cells of bladder TCC and, in particular, with cells of high-grade malignancy [1].

Our study was conducted in order to estimate the distribution of intravesically administered iodine-131-labeled AUA1 (^{131}I-AUA1) at tumor sites in patients with bladder carcinoma and in patients with normal urothelium.

Materials, patients and methods

Monoclonal antibodies

Moab AUA1 is an IgG1 mouse immunoglobulin recognizing a 35-kDa glycoprotein present on the membrane of various types of

Correspondence to: J. Zorzos, 7, M. Kikkou Street, Papagos 15669, Athens, Greece

Table 1. Uptake (mean ± SD) of radiolabeled antibodies in tumor and normal tissue

Moab	Time after administration (h)			
	2	20	40	60
AUA1				
Tumor	1.77 ± 3.2 ($n = 12$)	1.28 ± 1.67 ($n = 13$)[a]	0.72 ± 0.94 ($n = 5$)[a]	No remarkable radioactivity ($n = 3$)
Normal	0.79 ± 0.83 ($n = 15$)	0.14 ± 0.34 ($n = 15$)[a]	0.033 ± 0.06 ($n = 5$)[a]	No remarkable radioactivity ($n = 3$)
11.4.1				
Tumor	0.47 ± 0.42 ($n = 4$)	0.018 ± 0.015 ($n = 4$)	-	-
Normal	0.2 ± 0.19 ($n = 4$)	0.013 ± 0.002 ($n = 4$)	-	-

Uptake is expressed as percentage of administered dose per gram of tissue $\times 10^3$. Tumor uptake is that found at the tumor surface. *n*, Number of patients

[a] $P < 0.01$, Wilcoxon test for pair differences and unpaired measurements

epithelial cells. It reacts with a restricted number of normal epithelial tissues and with most human carcinomas, where it is expressed more intensively than in normal tissues [15].

Moab 11.4.1. is an IgG1 mouse immunoglobulin which recognizes a histocompatibility leukocyte antigen component of mouse lymphocytes and does not react with any human tissues. It was used as a negative control [10].

Labeling

The Moabs were labeled with ^{131}I using the *N*-bromosuccinamide (N-Br) technique [12]. Ten millicuries (50 µl) ^{131}I, 500 µl AUA1 (6.67 mg/ml) and 17 µl N-Br 10% were put into a sterile vial and mixed together by gentle shaking for 15 min. The labeling efficiency was consistently about 90–95% as determined by paper chromatography. When labeling efficiency was below 75%, 5 µl was added and the vial was gently shaken for another 15 min. This percentage of incorporation made unnecessary the step of gel filtration using Sephadex G-20 in a sterile 20-ml syringe in order to remove free iodine. Four milliliters of phosphate-buffered saline (PBS) containing 1% human serum albumin was added to stop the reaction. Subsequently, the final volume was passed through a micropore filter and collected in a sterile vial. There was no significant loss of immunoreactivity of the antibody after iodination as tested in an enzyme-linked immunosorbent assay and in a direct radioimmunoassay.

Patients and methods

Forty-five patients known or suspected to have bladder cancer gave written informed consent and entered the study. Cystoscopy and/or transurethral resection of bladder tumor (TUR-T) were scheduled. Before TUR-T, 1.5–3 mCi of I^{131}-labeled AUA1 or 11.4.1 was diluted in 50 ml normal saline and infused intravesically through a Foley catheter. The Moab was kept in the bladder for 1 h, during which time the patients changed position every 15 min. The bladder was then emptied and washed thoroughly with normal saline.

AUA1 was administered in 38 cases and 11.4.1. in 8 cases. In one patient AUA1 was administered initially and 11.4.1. was infused 3 months later when the tumor recurred. Thus, a total of 46 administrations were performed.

Tissue specimens from malignant lesions and normal bladder mucosa were obtained after cystoscopy or TUR-T at 2 h ($n = 19$), 20 h ($n = 19$), 40 h ($n = 5$) and 60 h ($n = 3$) after administration. They were weighed, fixed in 10% formalin, and radioactivity was counted in a gamma counter. Results were expressed as percentage of administered dose per gram of tissue $\times 10^3$.

Subsequently, tissue specimens were processed and embedded in paraffin. Hematoxylin-eosin staining and a two-step immunoperoxidase method using AUA1 were performed on paraffin sections. Blood samples were also taken at 0, 2, 6 and 20 h after administration, and circulating radioactivity per milliliter of whole blood was counted.

Data were analyzed statistically using the Wilcoxon tests for both pair differences and unpaired measurements. *P* values smaller than 0.05 were considered significant.

Results

Samples of normal bladder mucosa were taken from all 45 patients; five of them had no evidence of bladder neoplasm and were proved to be free of disease after meticulous evaluation.

Normal urothelium mean uptake of AUA1 in disease-free patients did not differ from normal urothelium mean uptake of patients with malignant lesions elsewhere in the bladder.

Most of the tumors were papillary and of early stage (Ta, T1); 41 samples from either papillary or solid tumors were taken from the surface but also from deeper parts of the tumor. Radioactivity targeted on tumor and normal samples after AUA1 or 11.4.1. administration was counted. Mean uptake of both antibodies is shown in Table 1, where tumor uptake is that found in superficial tissue samples.

The ratio tumor mean uptake of AUA1/normal urothelium mean uptake of AUA1 at 2, 20 and 40 h was, respectively, 2.2, 9 and 22. Statistical analysis showed that there is a significant difference between the uptake of AUA1 in tumor and nontumor samples at 20 and 40 h ($P < 0.01$). (Fig. 1). The ratio tumor mean uptake of AUA1/tumor mean uptake of 11.4.1 at 2 and 20 h was, respectively, 3.7 and 71. In one patient AUA1 and 11.4.1. were administered with an interval of 3 months; samples taken at 2 h showed that tumor uptake of AUA1 was 10 times higher than tumor uptake of 11.4.1. Tumor uptake of AUA1 was consistently lower in samples from deeper parts of the tumor.

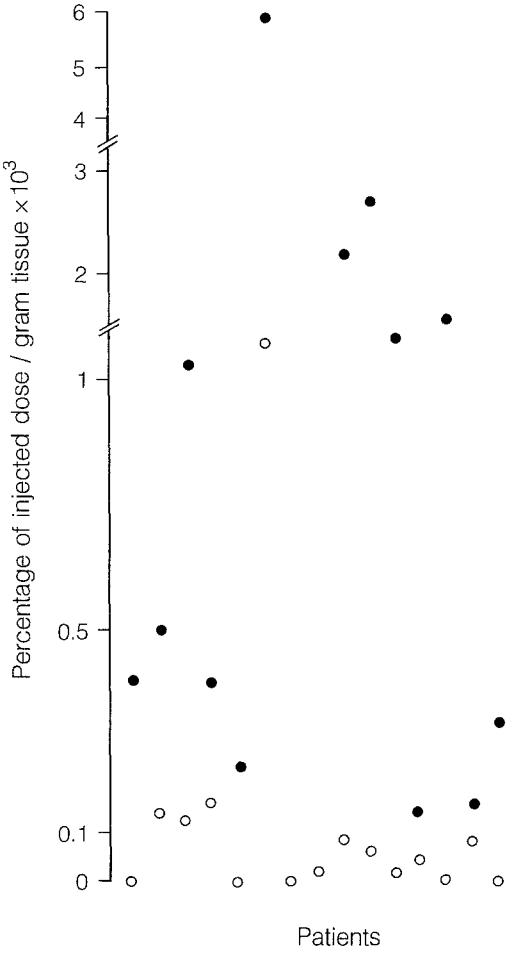

Fig. 1. Uptake of AUA1 by normal urothelium (O) and TCC (●) in samples obtained 20 h after intravesical administration ^{131}I-AUA1. The uptake is expressed as percentage of injected dose per gram tissue $\times 10^3$

An attempt to correlate tumor mean uptake of AUA1 with the TCC grade was also made; the results are shown in Table 2. Immunolocalization of AUA1 was inversively correlated with the degree of differentiation of urothelial cell carcinomas. At 2 h there was a significant difference between normal tissue uptake and grade III tumor uptake.

Both grade II and grade III tumors showed significantly higher uptake at 20 and 40 h than did normal urothelium and grade I tumors ($P < 0.01$).

Immunohistological study using AUA1 showed that intensity of staining and percentage of positive cells correlated inversively with the degree of TCC differentiation.

Radioactivity in blood samples at 2, 6 and 20 h was unremarkable.

Discussion

Bladder cancer is considered an ideal model for regional, diagnostic and/or therapeutic application of Moabs. In a previous in vitro study, Moab AUA1 was proved to react with urothelial cancer cells of high or intermediate malignancy [1]; furthermore, results of an in vivo trial, as well as our own preliminary results, indicated that AUA1 localized almost exclusively in tumor cells when given intravesically in patients with TCC [4, 17]. Our final results clearly demonstrated selective accumulation of AUA1 in bladder carcinomas of high or intermediate malignancy. This observation is in agreement with the finding that urothelial carcinomas of high or intermediate malignancy seem to carry a significantly higher number of antigenic epitopes reacting with AUA1 than do normal urothelium or carcinomas of low malignancy [1].

The maximum uptake of AUA1 by the tumor was found in tissue samples received 2 h after administration of Moab, decreasing progressively thereafter. However, tumor uptake of AUA1 remained high at 20 h, dropping remarkably at 40 h (Table 1). Therefore, it was concluded that the maximum cumulative radioactivity at tumor sites after intravesical administration of AUA1 radiolabeled with ^{131}I lasts about 18 h, from 2 h up to 20 h after administration.

Tumor uptake of AUA1 was significantly higher than uptake in normal urothelium. Furthermore, tumor uptake of AUA1 was significantly higher than tumor uptake of the nonspecific antibody 11.4.1. One should note that samples from a patient who received both AUA1 and 11.4.1., with a 3 month interval, showed 10 times higher uptake of the specific antibody. These findings indicate

Table 2. Correlation of tumor grade with mean uptake of AUA1

	Time after administration (h)		
	2	20	40
Normal	0.79 ± 0.83 ($n = 15$)	0.14 ± 0.34 ($n = 14$)[a]	0.033 ± 0.06 ($n = 5$)
Grade I	0.66 ± 0.48 ($n = 2$)	0.18 ± 0.06 ($n = 2$)	–
Grade II	1 ± 0.74 ($n = 7$)	1.1 ± 1.01 ($n = 7$)[a]	0.23 ± 0.29 ($n = 4$)
Grade III	4.33 ± 6.4 ($n = 3$)	2.36 ± 2.61 ($n = 4$)[a]	2.27 ($n = 1$)

Uptake is expressed as percentage of administered dose per gram of tissue $\times 10^3$. Tumor uptake is that found at the tumor surface. *n*, Number of patients

[a] $P < 0.01$, Wilcoxon test for unpaired measurements

the high specificity and sensitivity of the method. Interestingly, radioactivity of the blood was unremarkable.

In conclusion, our observations clearly suggest that, following improvement of the technique by the use of more appropriate isotopes and/or monoclonal antibodies, intravesical administration of radiolabeled Moabs could prove an attractive regional therapeutic approach to transitional cell carcinoma of the urinary bladder.

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