Intravesical Administration of Radiolabeled Antitumor Monoclonal Antibody in Bladder Carcinoma

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ABSTRACT

Tumor associated AU A1 monoclonal antibody and 11.4.1. nonspecific monoclonal antibody, which does not react with human tissues, were radiolabeled with $^{111}$In and administered intravesically to 23 patients undergoing cystoscopy for bladder carcinoma. The antibody solution remained in the bladder for 1 h and then was washed out prior to cystoscopy. Tumor and nontumor samples were obtained during cystoscopy and were counted in a gamma counter. Conventional and immunoperoxidase staining with both antibodies were also performed. AU A1 reacted with all bladder carcinomas while 11.4.1. was negative in all cases. The mean uptake of AU A1 at 2, 24, and 48 h after the instillation (expressed as $10^3$ × percentage of injected dose/g of tissue) was: 6.12 ± 5.50 (SD), 1.70 ± 2.57, 0.30 ± 0.17 in the tumors and 0.32 ± 0.50, 0.22 ± 0.30, 0 in the nontumor areas, and for 11.4.1. it was: 0.075 ± 0.075, 0.025 ± 0.025 in the tumors and 0.30 ± 0.42, 0.15 ± 0.26 in the nontumor areas. The uptake of AU A1 by the tumors correlated with the tumor grade. There was no radioactivity in the blood at 2 h, and at 1, 2, and 3 days after the instillation. Our results indicate that intravesical administration of radiolabeled monoclonal antibody AU A1 targets selectively to tumor tissue without any significant normal tissue uptake. This finding might allow the development of a nontoxic and specific therapeutic approach for superficial bladder carcinoma.

INTRODUCTION

Bladder carcinoma accounts for 2% of all human malignancies. Ninety% originate from transitional epithelium and usually are papillary and multicentric. Superficial bladder carcinomas (TIS, Ta, T1) account for 70% of all bladder carcinomas at the time of diagnosis and most of them can be controlled by transurethral resection. However, recurrences will occur in 80% of cases, and 10% will progress to a higher grade carcinoma with a poorer prognosis (1). Because of the high recurrence rate, careful and frequent endoscopic surveillance of the bladder is necessary. This results in inconvenience for the patients and an increase in the work load of a urology department.

Intravesical administration of several chemotherapeutic agents (thiotepa, Epirubicin, Epodyl, mitomycin C) has been used, in addition to endoscopic resection, in order to reduce recurrence in patients with superficial bladder carcinoma (2–5). Apart from chemotherapeutic drugs, immunotherapy using Bacillus Calmette-Guérin (6) has also been used with good results. However, intravesical therapy can cause significant toxicity in the bladder. The absorption of the drugs from the urothelium, especially of thiotepa and Bacillus Calmette-Guérin, can result in systemic toxicity. Therefore new forms of more effective and less toxic treatment are desirable.

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During the last decade the production and application of monoclonal antibodies opened new avenues in cancer immunotherapy. Imaging of human tumors with monoclonal antibodies has been, in many cases, successful (7, 8), but distribution studies have shown that the absolute amount of antibody delivered to the tumor after i.v. administration was low and below the desired therapeutic dose (9). On the other hand, regional administration of radiolabeled antibodies (intracavitary, intrathecal, intraarterial, intrapleural, intralymphatic) could achieve therapeutic levels with tolerable toxicity (10–13).

In this study, AU A1, a tumor associated monoclonal antibody, was administered intravesically in patients with superficial bladder carcinoma in order to estimate the distribution at tumor sites and consequently to assess this possibility as a selective form of treatment.

PATIENTS AND METHODS

Patients. Twenty-three patients undergoing cystoscopy for known or suspected bladder carcinoma entered the study. Each patient gave written consent before entering the study. Four patients were studied twice and one three times: a total of 29 instillations were performed.

One hundred fifty to 200 µg of AU A1 or 11.4.1. monoclonal antibody, labeled with 0.3–5.0 mCi of $^{111}$In and diluted in 50 ml of 0.9 g/100 ml NaCl solution were administered intravesically through a catheter. AU A1 was administered in 22 and 11.4.1. in 7 cases. The radiolabeled antibody was kept in the bladder for 1 h, during which patients were encouraged to change position every 15 min. The bladder was then emptied and washed twice with 50 ml of 0.9 g/100 ml NaCl solution.

AU A1 Monoclonal Antibody. This is an IgG1 mouse immunoglobulin recognizing a M, 35,000 glycoprotein present on the membrane of various types of epithelial cells. It reacts with a restricted number of normal epithelial tissues and most human carcinomas, where it is expressed more intensely than in normal tissues (14).

11.4.1. Monoclonal Antibody. This 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 (15).

DTPA Coupling of Monoclonal Antibodies. The monoclonal antibodies were coupled with DTPA (Sigma, United Kingdom) using the method of Hnatowich et al. (16). Briefly, DTPA was added to the antibodies (10 mg/ml) at a 10:1 molar ratio. The reaction was carried out at room temperature and at pH 8.0 for 10 min. Free DTPA was removed by using a Sephadex G50 column. Protein containing fractions were collected, Millipore filtered, and stored at −70°C.

Radiolabeling. $^{111}$In (Amersham, United Kingdom) was added to DTPA coupled antibodies and reacted for 30 min at room temperature at pH 6.5. Free $^{111}$In was removed by using a Sephadex G50 column. Sixty to 70% of $^{111}$In was bound to AU A1 and 85–90% to control antibody. The specific activity ranged from 2 to 3 mCi/mg.

Immunoreactivity and in Vivo Stability. Immunoreactivity of AU A1 before and after administration into the bladder was tested by using an ELISA and competition RIA. Briefly, HT29 cells (17), which react with

The abbreviations used are: DTPA, diethylenetriaminepentaacetic acid; ELISA, enzyme linked immunosorbent assay; RIA, radioimmunoassay.
AU A1, as confirmed by immunocytology and flow cytometry, were grown in 96-well plates (Falcon, United Kingdom), for 2 days (10^4 cells/well). The cells were then fixed with 0.25% glutaraldehyde for 15–20 min. The plates were stored at 4°C with phosphate buffered saline with 0.02% NaN₃.

For ELISA, ^111_In labeled AU A1, before and after instillation, reacted with the cells for 2 h at 37°C, in 1:2 serial dilutions starting from 200 ng/well. Unlabeled AU A1 and 11.4.1. at the same concentrations were used as negative and positive controls, respectively. This was followed by a 1-h incubation with a sheep anti-mouse antibody conjugated with peroxidase (Amersham, United Kingdom). The color reaction was developed with 2,2'-azino-di(3-ethylbenzthiazoline sulfonate and the absorbance at 405 nm was measured by an ELISA plate reader machine (EFLAB, Finland).

In RIA, cells were incubated with labeled AU A1, after the instillation, in 1:2 serial dilutions, starting from 100 ng/well, or with the same amounts of labeled AU A1 mixed with a standard amount of 100 ng of unlabeled AU A1/well. After a 2-h incubation at 37°C plates were washed with phosphate-buffered saline, wells were cut, and radioactivity was counted in a gamma counter.

In vivo stability of both antibodies was tested by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, under nonreducing conditions and gel autoradiography.

Tissue and Blood Samples. Cystoscopy was performed at 2 h (n = 14), 24 h (n = 12), and 48 h (n = 3) after instillation. Samples from tumors as well as from normal areas were taken during cystoscopy. They were fresh frozen or fixed in Methacarn. Samples were weighed and then counted in a gamma counter for radioactivity. They were then processed and embedded in paraffin. Blood samples were obtained at 2 h, and 1, 2, and 3 days after the instillation and were counted for radioactivity.

Histology and Immunocytochemistry. Hematoxylin-eosin and a two step immunoperoxidase method using AU A1 and control antibody were performed on paraffin and frozen sections.

Statistical Analysis. Data were analyzed statistically with the Student’s t-test.

RESULTS

ELISA and RIA showed that instillation into the urinary bladder caused no loss of AU A1 immunoreactivity (Fig 1). Autoradiography of the gel after instillation showed that practically all ^111_In was bound to the antibodies (Fig. 2). There was also a minimal amount of a component with fast mobility which most possibly represents free ^111_In, but the possibility of chelated ^111_In or ^111_In bound to small peptides cannot be excluded. Nevertheless, the amount of this component was low and it is unlikely that it interfered with the dosimetry results.

Tumor was found in 20 of 29 cases studied (T1, 1; T2, 17; T3, 1; T4, 1; Grade I, 9; Grade II, 6; Grade III, 4). Eighteen of the tumors were papillary, one was anaplastic, and one was in situ carcinoma. The samples taken from normal areas consisted of normal urothelium with chronic inflammatory infiltration in a few of these cases. In one case (instillation was performed 2 h before the cystoscopy) no normal samples were taken.

Immunohistochemical analysis showed a weak reaction of AU A1 with the basal layer of the normal urothelium (Fig. 3A), while all the tumors showed a more intense reaction. The intensity of the staining and the number of the positive cells increased with the grading of the tumors. Grade I tumors reacted only on the lower one-third of the epithelium at a depth of 1–2 cells, while Grade II tumors reacted more strongly on the lower and the middle one-third of the epithelium (Fig. 3, B and C). Grade III tumors showed intense reaction of 70–100% of the cells (Fig. 3D). In situ carcinoma was also positive. Immunostaining of 11.4.1. was negative in all cases.

Radioactivity targeted on the tumors and normal tissues, at various time points after AU A1 administration, is shown in Fig. 4. The uptake by the tumors, in all cases was higher than that of normal tissues of the same patients. The mean uptake of both antibodies on tumor and nontumor samples is shown in Table 1. It must be emphasized that in 5 of 9 cases at 2 h, 4 of 8 at 24 h, and in all 3 cases at 48 h, there was no measurable uptake in the nontumor samples. When 11.4.1. was administered no activity was found in tumor samples in 2 of 4 cases, while in the remaining 2 cases the uptake was more than 30-fold lower than the respective values for specific antibody. There was no radioactivity in the blood of the patients at any of the time points they were counted.

Statistical analysis of the results shows that there is significant difference between the uptake of AU A1 in tumor and
The correlation between tumor uptake of AUA1 and the grade of the tumors is shown in Table 2. The uptake increased with the grade of the tumors. There is a significant difference between the uptake by the normal tissue and the Grade II tumors at both 2 h \( (P < 0.05) \) and 24 h \( (P < 0.001) \) after the instillation.

**DISCUSSION**

This pilot study shows that intravesical administration of AUA1 monoclonal antibody results in selective accumulation of the antibody in the tumor with very low or no uptake by normal urothelium. This difference in tumor and normal uptake is favorable for immunotherapy and in fact represents one of the best achieved by using monoclonal antibodies up to date \( (9, 18) \). The uptake of AUA1 in tumors was significantly different than that of normal samples at 2 and 48 h, while there was no significant difference in the uptake of nonspecific antibody at any time point. The uptake of 11.4.1. was significantly lower than AUA1 and well below the uptake of nonspecific antibodies after i.v. administration reported for other tumors \( (18) \). The correlation of the uptake of the specific antibody with the grade of the tumors represents an advantage of this approach since the frequency of recurrence and invasion increases with the grade of the tumors. Grade II and especially Grade III tumors need additional treatment apart from the surgical excision.
AUA1 or 11.4.1 (150–200 μg) labeled with 0.3–0.5 mCi of 111In were administered intravesically and remained in the bladder for 1 h. Cystoscopy was performed at 2, 24 and 48 h after the instillation. Tumor and nontumor samples were taken and counted for radioactivity. The presence of tumor in the samples was determined after histological examination.

### Table 1 Mean uptake of tumor* and nontumor tissues

<table>
<thead>
<tr>
<th>Time after instillation (h)</th>
<th>2</th>
<th>24</th>
<th>48</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antibody AUA1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor</td>
<td>0.32 ± 0.50 (n = 10)</td>
<td>0.22 ± 0.30 (n = 8)</td>
<td>0 (n = 3)</td>
</tr>
<tr>
<td>Nontumor</td>
<td>0.075 ± 0.075 (n = 2)</td>
<td>0.025 ± 0.025 (n = 2)</td>
<td>0.15 ± 0.26 (n = 4)</td>
</tr>
</tbody>
</table>

* Expressed as 10^3 × percentage of injected dose/g of tissue.

**Table 2 Correlation of the grade of the tumors with the mean uptake of AUA1**

AUA1 labeled with 111In was administered to 19 patients (a total of 22 instillations). Tumor was found in 16 cases. The grade of the tumors was determined after histological examination of the samples.

<table>
<thead>
<tr>
<th>Time after instillation (h)</th>
<th>2</th>
<th>24</th>
<th>48</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade I</td>
<td>0.2 (n = 1)</td>
<td>0.36 ± 0.49 (n = 4)</td>
<td>0.40 ± 0.13 (n = 2)</td>
</tr>
<tr>
<td>Grade II</td>
<td>4.9 ± 2.8 (n = 4)</td>
<td>1.55 ± 0.25 (n = 2)</td>
<td>7.35 (n = 1)</td>
</tr>
<tr>
<td>Grade III</td>
<td>17 (n = 1)</td>
<td>0.10 (n = 1)</td>
<td></td>
</tr>
</tbody>
</table>

* Expressed as 10^3 × percentage of injected dose/g of tissue.

### REFERENCES


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