Intravesical Administration of Radiolabelled Tumour-associated Monoclonal Antibody in Bladder Cancer

Konstantinos N. Syrigos, Magsood Khawaja, Thomas Krausz, Gordon Williams and Agamemnon A. Epenetos

From the Departments of Clinical Oncology (K.N. Syrigos), Urology (M. Khawaja, G. Williams), Histopathology (T. Krausz), Imperial College of Science, Technology and Medicine, Hammersmith Hospital Campus, and 'Antisoma' Ltd. (A.A. Epenetos), London, England

Correspondence to: Professor Konstantinos N. Syrigos, Kifisias 40 & Arkadias, 115 26 Athens, Greece. Tel: +30 1 77 19 975. Fax: +30 1 77 78 838

Acta Oncologica Vol. 38, No. 3, pp. 379–382, 1999

Considerable progress has been made over the past decade in the use of tumour-associated monoclonal antibodies (mAbs) as carriers of cytotoxic agents in the management of several malignancies. In the present study we investigated the tumour localization and biodistribution of the mAb HMFG1 to bladder cancer following intravesical administration. HMFG1, which has been raised against the polymorphic epithelial mucin (PEM), was labelled with ^{125}I and administered intravesically 2 h and 24 h before cystoscopy. During cystoscopy, biopsies were taken from the normal bladder and the malignant lesion. Tissue samples were tested for HMFG1 immunostaining and for radioactivity with the use of a γ-counter. The mean ratio of tumour to normal uptake for ^{125}I-HMFG1 was 5.81 ± 1.23, at 2 h and 2.17 ± 0.42 at 24 h. No radioactivity was detected in the blood. We conclude that HMFG1 could be used intravesically for the successful delivery of a cytotoxic agent.

Received 6 April 1998
Accepted 18 August 1998

Although the management of cancer by exploiting properties distinguishing neoplastic and normal cells has always been an attractive concept, it was the development of hybridoma technology and the resulting tumour-associated monoclonal antibodies (mAbs) (1, 2) that offered new prospects for this strategy. Twenty years later, some of the applications of mAbs in oncology are now part of the everyday diagnosis and treatment (i.e. immunohistochemistry, radioimmunodetection), while others are concerned with intensive investigation.

Bladder carcinoma is a common human malignancy. As the fifth most common cancer in men in Europe and North America, it accounts for 2% of all human malignancies, and 7% of all urinary-tract malignancies (3). It usually originates from transitional epithelium and is commonly papillary and multicentric. Most of the cases can be controlled by transurethral resection. However, recurrences occur in 80% of cases and 10% progress to a higher grade carcinoma with a poorer prognosis. Intravesical administration of several immunomodifiers (BCG) and chemotherapeutic agents (thiotepa, epirubicin, mitomycin C, doxorubicin) has been used in addition to endoscopic resection as a means of reducing recurrences (4, 5), although absorption of the drugs from the bladder leads to significant systemic toxicity, which limits the dose that can be administered safely. Furthermore, age-adjusted death rate has remained unchanged over the past 50 years, at approximately 7 per 100000 (6), indicating that the effectiveness of the combination of traditional therapeutic schemes has reached a plateau. Therefore, the need for novel therapeutic strategies is apparent. Bladder cancer, although exhibiting heterogeneity between patients and between different tumours arising in the same patient (7) has, nevertheless, some characteristics that make it suitable for studies using mAbs: at least 75% of bladder cancers present as superficial disease, many of them multifocal, requiring treatment of the bladder as a whole (8) and, when instilled intravesically, the antibody is in direct contact with the tumour.

The mouse monoclonal antibody HMFG1 was raised against the high molecular weight glycoprotein (MW > 400000), polymorphic epithelial mucin (PEM) (9). The expression of PEM in bladder cancer has already been investigated and it has been shown that the cells that most

 ISSN 0284-186X

express it are mainly the cells of the upper third of the tumour papillae (10), making this molecule a suitable candidate for targeting via the intravesical route of administration.

In the present study we investigated the tumour uptake and the biodistribution of the mAb HMFG1, after intravesical administration, in patients with bladder carcinoma. The aim was to assess HMFG1 as a possible candidate for guided cytotoxic therapy of superficial bladder cancer.

MATERIAL AND METHODS

Patients

Six patients undergoing cystoscopy for known or suspected bladder cancer entered the study. All patients were admitted to the Urology Department of the Imperial College of Science, Technology and Medicine, Hammersmith Hospital and gave written consent before entering the study. None of the participants had received any previous treatment, systematic or intravesical, for their bladder cancer.

HMFG1

This is a class IgG_1 mouse immunoglobulin, which recognizes a polypeptide epitope of the PEM core protein (11). It reacts strongly with a wide range of human carcinomas, including bladder cancer (10, 12).

Radiolabelling

HMFG1 was radiolabelled with Iodine-125 (IMS 30, Amersham International, Amersham, Bucks.), with the application of the Iodo-Gen method (13) and with a labelling efficiency of 80–95%. Free ^{125}I was removed by using a Sephadex G50 column (Pharmacia, Uppsala, Sweden). Immunoreactivity of radiolabelled HMFG1 before and after administration into the bladder was estimated with the application of ELISA and competition RIA, using plates coated with peptide containing the sequence Pro-Asp-Thr-Arg-Pro (PDTRP). In vivo stability of the radioconjugate was tested by sodium-dodecyl-sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and fast protein liquid chromatography (FPLC).

Instillation of the radiolabelled mAb

We diluted 180–250 μg HMFG1, labelled with 0.3–0.5 mCi of ^{125}I in 50 ml 0.9 g/100 ml NaCl solution and this was administered intravesically through a catheter. It was kept in the bladder for 1 h, during which time the patients were encouraged to rotate position. The above method of intravesical administration of mAbs has been shown to achieve an even distribution of the mAb within the bladder (14, 15). The bladder was then emptied and washed twice with normal saline, before removing the catheter. Cystoscopy was performed 2 h (3 patients) and 24 h (3 patients) after the administration of the antibody.

Tissue and blood samples

During cystoscopy, samples from the tumour and macroscopically normal areas were randomly taken, fixed in Methacarn, weighed and counted in a gamma counter for radioactivity. They were then processed and embedded in paraffin. Sections were cut and examined with haematoxylin-eosin and an avidin-biotin immunolocalization method, using 'naked' HMFG1.

Blood samples were obtained before as well as at 5, 15, 30 min and 1, 2, 12 and 24 h after the instillation. All samples were counted for radioactivity.

Statistical analysis

We statistically analysed our data using Student's *t*-test.

RESULTS

Tumour was found in 4 out of 6 cases studied (2 cases at 2 h and 2 cases at 24 h after instillation). All tumours were superficial carcinomas (Grade I: 2; Grade III: 2), while none of the patients had multifocal lesions. All tissue samples taken from normal areas consisted of normal urothelium with chronic inflammatory infiltration.

Immunohistochemical analysis

Immunohistochemical analysis showed a weak reaction of HMFG1 in all normal samples, mainly located at the superficial layer cells. All tumours reacted strongly with HMFG1. Antibody localization was more intense in the upper third of the neoplastic epithelium. A positive correlation was found between the intensity of HMFG1 localization and the advanced grade of the disease. The reaction was located both on the cytoplasm and the cell membrane of the malignant cells (see Fig. 1).

Radiolabelling efficiency and immunoreactivity

Radiochemical purity, as determined by the proportion of ^{125}I bound to antibody, was 80–95%. Radiolabelling of HMFG1 did not result in significant immunoreactivity loss, while FPLC demonstrated similar profiles for ^{125}I-HMFG1 before and after bladder instillation.

Tissue distribution

Tumour and normal tissue mean uptake at 2 and 24 h after the instillation of the radiolabelled HMFG1 is shown in Table 1. It can be seen that there is a significant difference between the uptake of the antibody by the normal and malignant urothelium at both time points ($p < 0.05$).

No radioactivity (above the background) was found in any of the blood samples at any of the time points at which they were sampled.

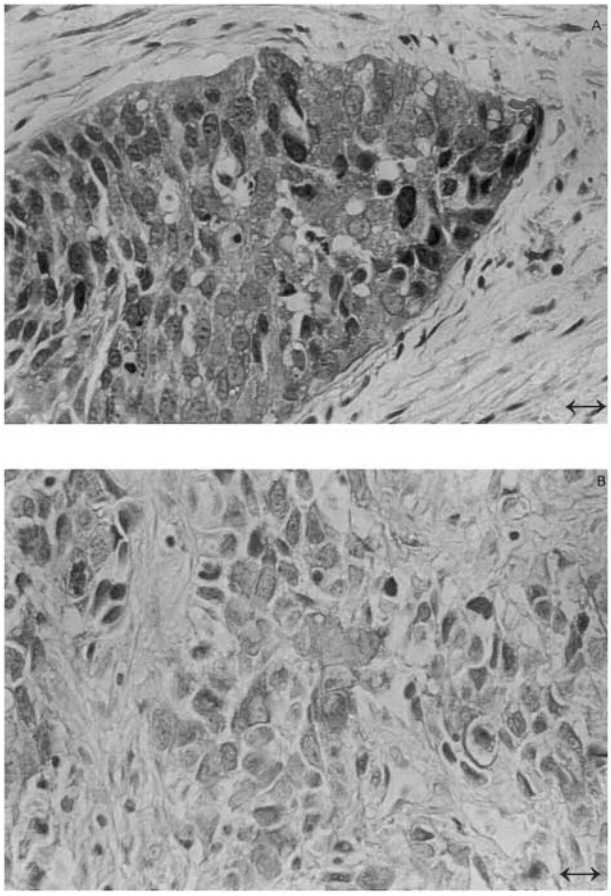

Fig. 1. HMFG1 localization in bladder cancer samples. The reaction was located on both the cytoplasm and the cell membrane of the malignant cells (A), was more intense in the upper third of the neoplastic epithelium and correlated positively with the advanced grade of the disease (A, B) (avidin-biotin indirect immunoperoxidase staining; bar: 150 μm).

DISCUSSION

Over the past two decades several efforts have been made to support the therapeutic use of monoclonal antibodies against various malignancies. Most therapeutic strategies have employed the systemic administration of tumour-associated mAbs, used either alone ('naked' mAbs), or conjugated to a cytotoxic agent (radionuclides, conventional chemotherapeutic drugs, toxins). Unfortunately their application has been associated with poor tumour uptake ($< 0.01\%$ of the injected dose), the binding of the mAb to normal tissues and the development of human antimouse antibodies (HAMA) (16).

Table 1

Mean uptake of the ^{125}I-HMFG1 by normal and malignant urothelium samples, after intravesical administration

Samples	Time after instillation	
	2 h	24 h
Normal	0.79 ± 0.14*	0.49 ± 0.13*
Tumour	5.81 ± 1.23*·	2.17 ± 0.42*

* Expressed as % i.d/g $\times 10^3$
· $p < 0.05$

To address the above problems several innovative approaches are being developed, including the use of other routes of administration, in addition to the systemic one, for the regional delivery of the mAbs (i.e. intraperitonealy for ovarian carcinoma, intrathecally for meningeal carcinomatosis). Intravesical treatment of superficial bladder cancer with conventional chemotherapeutic agents or with BCG is a long-established practice and this tumour seemed appropriate for local application of mAbs-conjugates. Intravesical administration of mAbs that can recognize bladder tumour-associated antigens has several theoretical advantages over systemic use. The antibody is in direct contact with the tumour cells, rather than being diluted in the circulation, and cross-reactivity with other normal tissues is of lesser importance. According to this and previous studies (14, 15, 17), intravesically administered mAbs are not absorbed systemically. Therefore a human antimouse antibody (HAMA) response is unlikely, allowing repeated antibody administrations.

Our study demonstrated that intravesical administration of HMFG1 results in selective accumulation of the antibody in the tumour with very low uptake by normal urothelium. The difference between normal and tumour uptake is favourable for immunotherapy and, up until now, represents one of the best results achieved by using monoclonal antibodies. The uptake of HMFG1 by the tumours was significantly increased at 2 h, compared with the 24 h uptake. This loss of tumour localization over time may be caused by the shedding of the superficial cells to which the antibody is bound.

According to our findings, HMFG1 targets mostly the upper third of the tumour cells, making it a suitable carrier of a cytotoxic agent. As the lower two-thirds of the tumour expresses PEM to a lesser extent, repeated administrations should be used to achieve sufficient tumour targeting.

Our data indicate that conjugation of mAbs to conventional cytotoxic drugs is unlikely to be of any therapeutic value, because of the insufficient amount of drug that would reach the tumour. In fact, intravesical administration of doxorubicin and mitomycin C conjugated to mAbs resulted in failure to administer a cytotoxic dose to the tumour, while the toxicity was not significantly reduced (18). However, other drug delivery systems, with the potential of amplifying the tumour: non tumour uptake ratio might be more beneficial. These include the radiotherapy and the antibody-dependent enzyme prodrug therapy (ADEPT) where a non-toxic prodrug is activated to the active cytotoxic agent at the tumour site, by an enzyme-mAb conjugate, localized on the surface of the malignant cells. In fact, in vitro and preclinical studies of ADEPT on

bladder cancer have already been completed, with encouraging results (19). Finally, although our findings suggest that the above strategies could be used in the management of superficial bladder cancer, as the number of patients studied at each time point is relatively small, it is difficult to draw any definite conclusions regarding the kinetic properties of the substance used.

In addition to their therapeutic use, our data indicate that radiolabelled HMFG1 could be used for immunoscintigraphy of bladder cancer, after intravesical administration. Unfortunately, the small number of patients that we included in our study made it difficult to correlate the uptake with the size and the grade of the tumours. Nevertheless, a previous study using the anti-MUC-1 antibody NCRC48 demonstrated successful imaging of the malignant lesion in some of the patients studied (17). It is clear that further studies are needed in order accurately to evaluate the potential diagnostic role of the intravesical administration of radiolabelled mAbs. Other issues that need to be addressed, following our study, include the possible advantage of antibody fragments and the depth of antibody penetration.

In conclusion, we investigated the tumour localization and biodistribution of the mAb HMFG1 to bladder cancer following intravesical administration and we confirmed the selective targeting of bladder cancer. Although further studies are needed, our study indicates that the above strategy has the potential for therapeutic interventions.

REFERENCES

1. Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 1975; 256: 495–7.
2. Kohler G, Milstein C. Derivation of specific antibody-producing tissue culture and tumor lines by cell fusion. Eur J Immunol 1976; 6: 511–9.
3. Silverberg E, Lubera JA. Cancer statistics, 1989. CA Cancer J Clin 1989; 39: 3–20.
4. Tolley DA, Hargreave TB, Smith PH, et al. Effect of intravesical mitomycin C on recurrence of newly diagnosed superficial bladder cancer: interim report from the Medical Research Council Subgroup on Superficial Bladder Cancer (Urological Cancer Working Party). Br Med J Clin Res Ed 1988; 296: 1759–61.
5. Tolley DA, Parmar MK, Grigor KM, et al. The effect of intravesical mitomycin C on recurrence of newly diagnosed superficial bladder cancer: a further report with 7 years of follow up. J Urol 1996; 155: 1233–8.
6. Silverberg E, Boring CC, Squires TS. Cancer statistics, 1990. CA Cancer J Clin 1990; 40: 9–26.
7. Raghavan D, Shipley WU, Garnick MB, Russell PJ, Richie JP. Biology and management of bladder cancer. N Engl J Med 1990; 322: 1129–38.
8. Heney NM, Nocks BN, Daly JJ, et al. Ta and T1 bladder cancer: location, recurrence and progression. Br J Urol 1982; 54: 152–7.
9. Taylor-Papadimitriou J, Peterson JA, Arklie J, Burchell J, Ceriani RL, Bodmer WF. Monoclonal antibodies to epithelium-specific components of the human milk fat globule membrane: production and reaction with cells in culture. Int J Cancer 1981; 28: 17–21.
10. Conn IG, Crocker J, Emtage LA, Wallace DMA. HMFG2 as a prognostic indicator in superficial bladder cancer. J Clin Pathol 1991; 41: 1191–5.
11. Arklie J, Taylor-Papadimitriou J, Bodmer W, Egan M, Millis R. Differentiation antigens expressed by epithelial cells in the lactating breast are also detectable in breast cancers. Int J Cancer 1981; 28: 23–9.
12. Burchell J, Durbin H, Taylor-Papadimitriou J. Complexity of expression of antigenic determinants, recognized by monoclonal antibodies HMFG-1 and HMFG-2, in normal and malignant human mammary epithelial cells. J Immunol 1983; 131: 508–13.
13. Fraker PJ, Speck JC Jr. Protein and cell membrane iodinations with a sparingly soluble chloroamide, 1,3,4,6-tetrachloro-3a,6a-diphrenylglycoluril. Biochem Biophys Res Commun 1978; 80: 849–57.
14. Syrigos KN, Epenetos AA. Radioimmunotherapy of ovarian cancer. Hybridoma 1995; 14: 121–4.
15. Kunkler RB, Bishop MC, Green DJ, Pimm MV, Price MR, Frier M. Targeting of bladder cancer with monoclonal antibody NCRC48—a possible approach for intravesical therapy. Br J Urol 1995; 76: 81–6.
16. Bamias A, Bowles MJ, Krausz T, Williams G, Epenetos AA. Intravesical administration of indium-111-labelled HMFG2 monoclonal antibody in superficial bladder carcinomas. Int J Cancer 1993; 54: 899–903.
17. Bamias A, Keane P, Krausz T, Williams G, Epenetos AA. Intravesical administration of radiolabeled antitumor monoclonal antibody in bladder carcinoma. Cancer Res 1991; 51: 724–8.
18. Li L, Zhang Y, Lin Q, Li C, He M. Clinical application of monoclonal antibody-drug conjugate to immunotargeting chemotherapy of bladder cancer. Chin Med J Engl 1995; 108: 764–8.
19. Syrigos KN, Rowlinson-Busza G, Epenetos AA. In vitro cytotoxicity following specific activation of amygdalin by β-glucosidase conjugated to a bladder cancer-associated monoclonal antibody. Int J Cancer (in press).