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Targeted diagnosis of bladder and ureteral carcinoma using radiolabelled BDI-1

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Abstract The aim of this study is to investigate the possibility of radioimmunoimaging (RII) by radiolabelled anti-bladder carcinoma monoclonal antibody BDI-1 applied to diagnosis of bladder cancer and ureteral cancer. BDI-1 was labelled with ^{131}I and ^{99m}Tc. The immunoreactivity, pharmacokinetics and biodistribution in mice were studied. RII was performed in 46 patients.The results showed that ^{131}I, ^{99m}Tc-BDI-1 have satisfactory immunoreactivity and excellent tumor-locating properties. The blood clearance half-life $T_{1/2}\alpha$ and $T_{1/2}\beta$ were 35 h in the first phase and 151 h in the second phase, respectively. Thirty-nine patients were studied by an intravesical administration method; the sensitivity was 90.5%. Seven patients were studied by an intravenous administration method. The RII results of three cases with primary or recurrent bladder cancer and three cases with ureteral cancer were confirmed histologically. RII was negative in one patient with suspected lung metastasis that was shown on radiography. The investigation revealed that RII can be used as an auxiliary method for the detection of bladder cancer and may be valuable for the diagnosis of ureteral cancer.

Key words Radioimmunoimaging · Monoclonal antibody · Bladder cancer · Technetium-99 m Iodine-131

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Introduction

Since radioimmunoimaging (RII) of ^{131}I-labelled anti-carcinoembryonic antigen (CEA) monoclonal antibody was used in 1978 by Goldenberg et al [5], the diagnosis and treatment of malignant diseases using radiolabelled monoclonal antibodies (McAbs) have been widely studied. At present, radiolabelled monoclonal antibodies have been used successfully to image a wide variety of malignant tumors including melanoma, ovarian epithelial cancer, CEA- and human chorionic gonadotrophin (HCG)-producing tumors [6, 12]. Encouraging results have been obtained, although some problems still need to be overcome as this technique approaches routine clinical use [8, 15].

Bladder and ureteral cancers are common urological malignancies and occupy first position in urological cancers in China [4]. Traditional diagnosis is established by cystoscopy and biopsies of bladder mucosa. Additional examinations such as ultrasonography, radiography and computed axial tomography (CT) may also contribute to the diagnosis. These methods have variable sensitivity but all are non-specific and for flat, in situ carcinoma, small solid lesions, bladder cancer metastatic focis and ureteral cancer, qualitative diagnosis is relatively difficult. By contrast, monoclonal antibodies can localise tumour tissues with high specificity by r–ray photoscanning.

Recently there have been several approaches to the diagnosis and treatment of bladder cancer using radiolabelled monoclonal antibodies [1, 10, 11, 18]. BDI-1 is an anti-bladder carcinoma cell line BIU-87 monoclonal antibody prepared by the Urology Institute of Beijing Medical University [14]. In the present report, BDI-1 was labelled with ^{99m}Tc and ^{131}I. Studies including in vitro immunoreactive analysis, biodistribution and RII in an animal model and biodistribution and metabolism in humans have been done. The ability of radiolabelled BDI-1 to localise specifically in bladder and ureteral carcinoma in 46 patients has been evaluated. Through

the specific binding of the monoclonal antibody to human bladder tumour, which is proved by in vivo tumour imaging, the treatment of human bladder carcinoma with radio-labelled BDI-1 might be possible in the future without damaging normal tissues.

Materials and methods

Preparation of monoclonal antibody

The BDI-1 monoclonal antibody was obtained from a hybridoma produced by fusing spleen cells from mice immunised against the bladder carcinoma cell line BIU-87 with SP 2/0 mouse myeloma. It was purified from mouse ascitic fluid by protein A sepharose CL-4B affinity chromatography. The monoclonal antibody was characterised by indirect immunofluoresence assay and ABC-ELISA immunohistochemical staining that demonstrated that BDI-1 has a strong binding reaction with bladder transitional cell carcinoma tissue and BIU-87 and E-J bladder carcinoma cell lines but there was no reaction with normal bladder tissue and other normal human tissue [14].

Radiolabelling and immunoreactive analysis

Radiolabelling BDI-1 with ^{131}I and ^{99m}Tc was performed by the chloramine-T method [9] and 2-ME direct reducing method [17]. Immunoreactivity of radiolabelled BDI-1 was analysed by Lindmo's method [13].

Biodistribution and RII of nude mice

Balb/c nude mice bearing human bladder cancer BIU-87 xenografts with a diameter of 0.5–1.0 cm were used in the experiment. 6.29 MBq/16 μg ^{131}I-BDI-1 was injected into tail veins. Imaging was performed from 1 to 7 days post i.v. Two mice were killed and radioactivity in tumour and normal tussues was measured 2, 3, 5 and 7 days after injection. T/NT (dose% per gram of tumour/dose% per gram of normal tissues) was calculated. As a control experiment, nude mice bearing irrelevant tumour (Lovo) were administered with the same dose of ^{131}I-BDI-1 and nude mice bearing BIU-87 carcinoma xenografts were administered ^{131}I-NMIgG; T/NT was measured on the fifth day after injection. 14.4 MBq/0.2 ml ^{99m}Tc-BDI-1 was administered intravenously and RII was performed 4, 16 and 22 h after injection. Radioactivity in tumour and normal tissues was measured and T/NT was calculated after imaging.

Clinical trial

Safety testing

Before radiolabelled BDI-1 was used clinically, sterility, pyrogenicity and acute toxicity were tested according to [3]. The clinical programme was approved by the Ethics Committee of the First Hospital of Beijing Medical University.

Patients

A total of 46 patients (40 males and 6 females) aged 45–77 years (mean 65 years) were studied; these included 42 patients with bladder cancer, 3 patients with ureteral cancer and one patient with suspected lung metastasis of bladder cancer. Four patients with normal bladder and one case with bladder inflammatory focus were selected as controls.

Lugol's solution was administered orally 3 days prior to and 7 days after intravenous injection of ^{131}I-BDI-1 to block the thyroid ^{131}I uptake. A skin test using 10 μg/0.1 ml unlabelled BDI-1 was performed before administration and only patients with a negative result entered the study.

Intravesical administration of radiolabelled BDI-1 and RII

18.5–74 MBq ^{131}I-BDI-1 or 148–222 MBq ^{99m}Tc-BDI-1 was dissolved in 50 ml NS and infused into the bladder through a catheter. The labelled antibody solution remained 0.5–1.5 h in the bladder and was subsequently washed out three times with 100 ml PBS. Then 100 ml PBS was infused and RII was performed immediately. Criteria of positive imaging was T/NT greater than 1.3.

Intravenous administration of radiolabelled BDI-1 and RII

After intravenous injection of 5 mg dexamethasone, 111-185 MBq/300 mg ^{131}I-BDI-1 dissolved in 250 ml NS was administered by intravenous instillation within 20 min. RII of the whole body and tumour site was performed 1, 2, 3, 5 and 7 days after injection and blood pool imaging using ^{99m}Tc-RBC was performed the third day after injection. Dual radionuclide subtraction method [7] was used to obtain superior quality images. Blood (1 ml) and urine (1 ml) samples were collected at different time intervals for assessment of McAb pharmacokinetics.

For determining the metabolic form in urine, 2 ml of 20% TCA (trichloroacetic acid) and 100 μg (in 100 μl) BSA were added and then centrifuged. The radioactivity in the suspension (free ^{131}I) and precipitate (^{131}I-protein) was measured.

Results

The immunoreactivity of radiolabelled BDI-1

The immunoreactive fractions of ^{131}I-BDI-1 and ^{99m}Tc-BDI-1 were 64% and 81%, respectively and the association constants were 5.27×10^8 l/mol and 1.22×10^9 l/mol respectively. The immunoreactivity of ^{99m}Tc-BDI-1 was higher than that of ^{131}I-BDI-1.

RII and T/NT of nude mice

Both ^{131}I-BDI-1 and ^{99m}Tc-BDI-1 provided good scintigraphic images of transplanted BIU-87 tumours. Tissue distribution studies confirmed the result of scintigraphic images (Figs. 1 and 2, Tables 1 and 2). T/NT increased with time. T/NT is a main factor affecting the quality of RII. It has been reported that larger or superficial tumours can be detected when T/NT is 1.4–1.9 [2] and for the detection of smaller or deeper tumours, T/NT greater than 5 is necessary [16]. T/NT of ^{131}I-BDI-1 and ^{99m}Tc-BDI-1 were greater than 5 at 168 h and 22 h, respectively for all tissues. It indicated that ^{131}I-BDI-1 and ^{99m}Tc-BDI-1 may be of potential use for RII of bladder cancer and its metastases. T/NT of irrelevant tumour (Lovo) and ^{131}I-NMIgG control groups was much lower than for the experimental group.

Biodistribution and metabolism of ^{131}I-BDI-1 in patients

The uptake of radioactivity in heart, liver and spleen was higher in the first 2 days after injection similarly to blood

Table 1 T/NT in nude mice after intravenous injection of ^{131}I-BDI-1 and ^{131}I-NMIgG

Tissues	Experimental group				Control group 1	Control group 2
	48 h	72 h	120 h	168 h	120 h	120 h
Blood	0.91	0.99	1.38	5.48	0.26	0.10
Muscle	8.55	8.43	12.31	27.70	2.39	1.46
Liver	4.40	6.34	6.09	13.50	1.17	0.70
Heart	3.57	3.92	6.60	11.68	1.00	0.50
Spleen	4.43	6.70	6.28	11.20	1.53	1.16
Lung	2.19	1.98	3.67	6.05	0.65	0.36
Kidney	5.97	5.76	6.91	13.50	1.11	0.63
Bone	4.81	10.04	14.00	37.57	4.28	1.85
Stomach	8.43	6.04	5.20	15.94	0.54	1.21
Colon	22.70	23.50	19.60	75.14	4.84	2.17
Small intestine	13.70	11.58	15.51	35.07	2.65	1.96
Bladder wall	3.92	4.04	5.24	5.11	0.86	0.48

Experimental group: nude mice bearing bladder cancer xenografts i.v. ^{131}I-BDI-1. Control group 1: nude mice bearing bladder cancer xenografts i.v. ^{131}I-NMIgG. Control group 2: nude mice bearing colon cancer xenografts i.v. ^{131}I-BDI-1

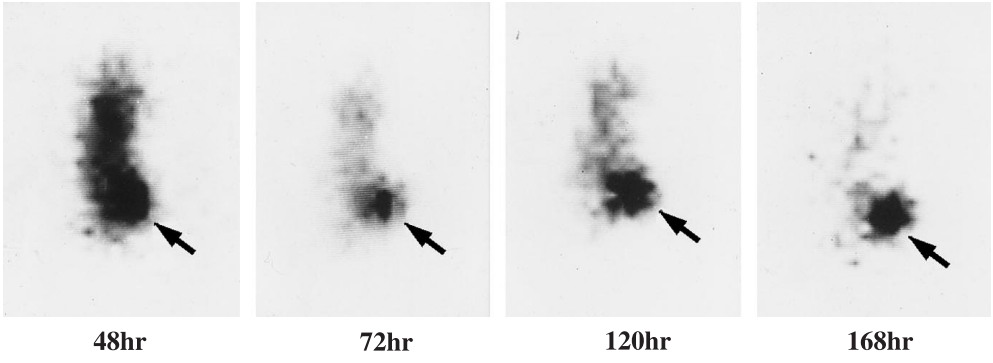


Fig. 1 Radioimmunoscintigraphy of nude mice at 48, 72, 120, 168 h after injection of ^{131}I-BDI-1

Table 2 Uptake dose (%ID/g) and T/NT in nude mice bearing bladder carcinoma xenografts after intravenous injection of ^{99m}Tc-BDI-1

Tissues	4 h		16 h		22 h	
	%ID/g	T/NT	%ID/g	T/NT	%ID/g	T/NT
Tumour	1.45		4.98		20.70	
Blood	1.31	1.11	4.04	1.32	6.90	3.00
Liver	0.66	2.20	1.34	3.72	3.50	5.90
Heart	0.78	1.86	1.77	2.81	3.30	6.30
Spleen	0.41	3.54	1.34	1.72	2.00	10.30
Bladder wall	0.25	5.80	1.41	3.53	2.40	8.60
Lung	0.97	1.49	1.81	2.75	3.80	5.40
Stomach	0.11	13.18	0.28	17.79	1.30	15.90
Colon	0.14	10.36	0.93	12.77	1.30	15.90
Small intestine	0.15	9.66	0.46	10.83	1.00	20.70
Kidney	0.88	1.65	2.16	2.31	7.20	2.90
Muscle	0.13	11.15	0.33	15.09	1.00	20.70
Bone	0.18	8.06	0.54	9.22	2.20	9.40

pool images. Tumour images were visualised the third day after injection and became increasingly distinct with time as the background radioactivity decreased. The blood clearance curve (Fig. 3) of ^{131}I-BDI-1 could be divided into the first fast clearance phrase (before 72 h post-i.v.) and subsequent slow clearance phrase (after 72 h post-i.v.) with $T_{1/2}\alpha$ and $T_{1/2}\beta$ of 35 h and 151 h, respectively. The urine clearance curve (Fig. 4) showed

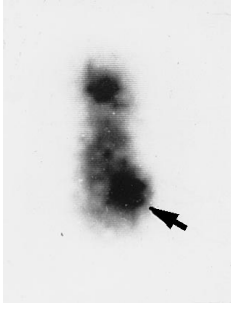


Fig. 2 Radioimmunoscintigraphy of nude mice at 16 h after injection of ^{99m}Tc-BDI-1

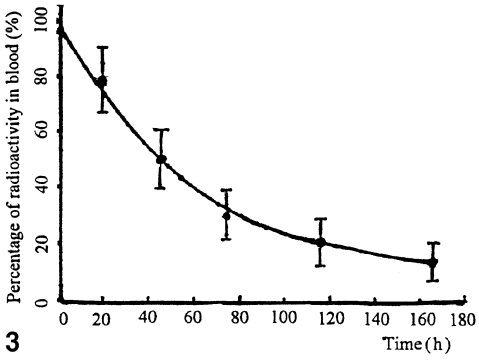
that urine clearance was fast 22 h after injection and slowed later, with a urine clearance rate of 32% and 15% in the first and second days after injection, respectively; 83% of total radioactivity was cleared 8 days after injection. In urine, 89%–93% of radioactivity was free ^{131}I.

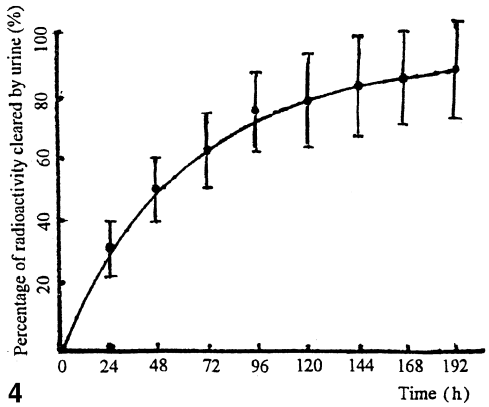
RII result by intravesical administration method (Figs. 5, 6, 7)

Sixteen patients with ^{131}I-BDI-1 and twenty three patients with ^{99m}Tc-BDI-1 who had histologically proved

Fig. 3 Blood clearance curve of ^{131}I-BDI-1

Fig. 4 Urine clearance curve of ^{131}I-BDI-1





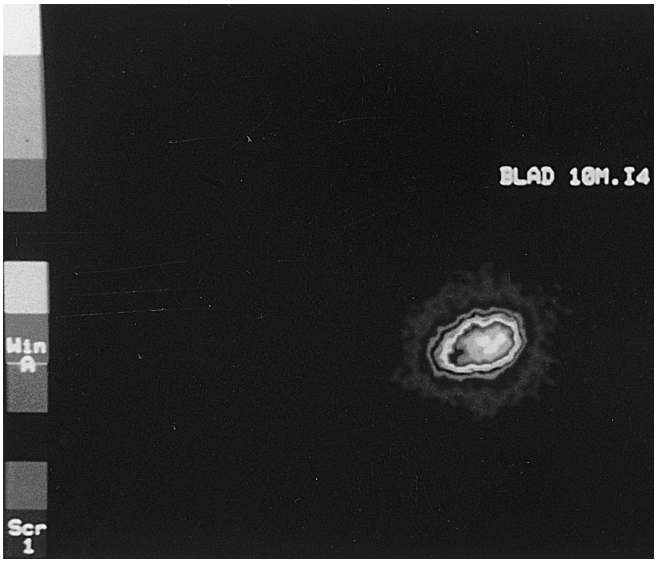


Fig. 5 Planar anterior image of bladder after intravesical administration of ^{131}I-BDI-1. Uptake is seen in the three tumours (∅ 0.5 cm)

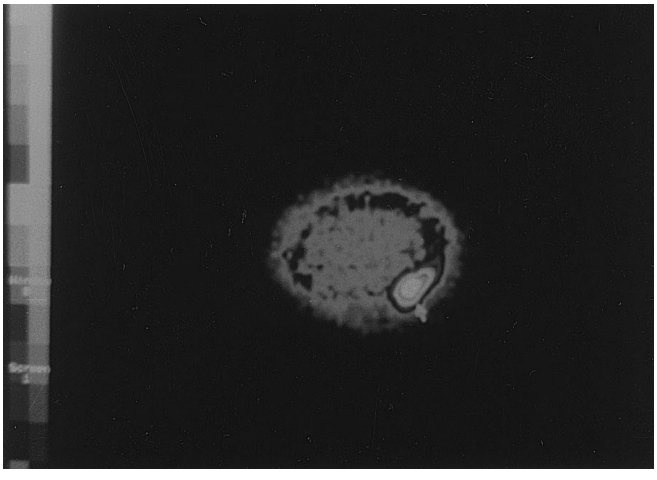


Fig. 6 Planar anterior imaging of bladder after intravesical administration of ^{99m}Tc-BDI-1. Increased activity is seen in the tumour (in the left inferior wall)

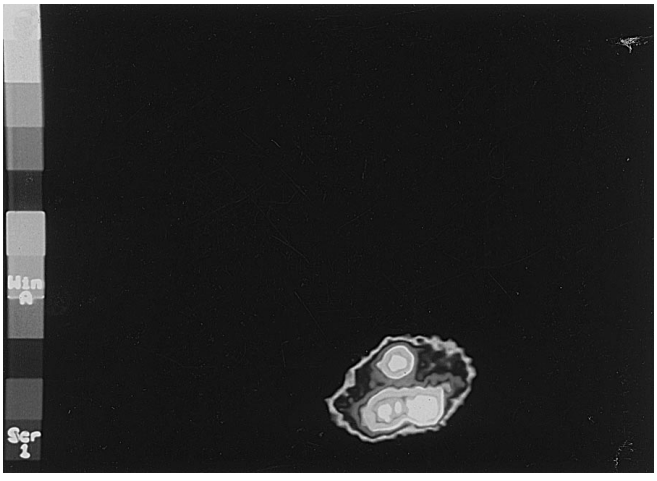


Fig. 7 Planar anterior imaging of bladder after intravesical administration of ^{99m}Tc-BDI-1. Increased activity is seen in the three tumours

59 foci of bladder transitional cell carcinoma, two foci of squamous cell carcinoma and two foci of mucosal carcinoma were studied. These three types of cancer could all be imaged. RII correctly detected 57 of 59 foci and three RII findings were false-positive. The sensitivity was 90.5% (57/59). RII was negative in the four normal bladders and one inflammatory focus. RII showed negative in one bladder cancer patient after chemotherapy subsequently proved by cystoscopy. The minimum tumour detected by RII had a diameter of 0.5 cm.

RII result by intravenous administration method (Fig. 8)

Seven patients, including three with primary or recurrent bladder cancer, three with ureteral cancer and one with suspected lung metastasis of bladder cancer were studied. RII showed a positive result for the three patients with bladder cancer. Two cases with ureteral cancer were positive and then histologically proved to be transitional cell carcinoma after surgery. One case with ureteral tu-

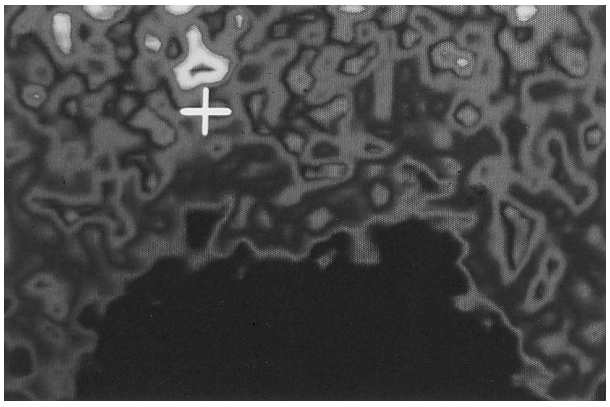


Fig. 8 Planar anterior imaging of ureteral cancer. The image was obtained by dual radionuclide subtraction method using ^{131}I-BDI-1 and ^{99m}Tc-RBC. The tumour was localised by comparing with the ^{131}I-BDI-1 image which shows the right dropsical ureter

mor was negative and then proved to be benign by urine cytological and histological examination. In one patient, radiography revealed a 4.2 × 2.5 cm focus in the right upper lung. RII showed a negative result after 5 weeks of chemotherapy, but there was no histological confirmation.

Discussion

The results of in vitro immunoreactive analysis and T/NT values in nude mice bearing human bladder carcinoma xenografts demonstrated that ^{131}I, ^{99m}Tc-BDI-1 have satisfactory immunoreactivity and specificity and may have potential use in clinical detection of bladder cancer and its metastasis. Two radionuclides, ^{131}I and ^{99m}Tc labelled BDI-1 were used in our study and all obtained satisfactory radioimmunoscintigrams. ^{99m}Tc has many favourable nuclear properties compared with ^{131}I, such as low radiation dose to patients, suitable energy for scintigraphic imaging and is widely used in nuclear medicine. Furthermore, ^{99m}Tc-BDI-1 has higher immunoreactivity and is more stable in vivo than ^{131}I-BDI-1. The disadvantage of ^{99m}Tc-BDI-1 is that its half-life (6 h) is too short for RII as the metabolic rate of McAb in normal tissues is relatively low; about 24 h is usually needed to obtain clear images. Using an intravesical administration method can overcome this shortfall because the whole process only takes about 1–2 h. Therefore we considered that for RII by intravesical method ^{99m}Tc-BDI-1 is preferable and for the intravenous method ^{131}I-BDI-1 is to be preferred.

The routine methods for detecting bladder cancer include cystoscopy, biopsy of bladder mucosa, ultrasonography, CT and cytological diagnosis. Most bladder cancer can be confirmed by these methods. The advantages of RII are that it is easily performed and less invasive. It is most useful to patients who have difficulty coping with cystoscopy examination. There were two such patients in our study. In one patient, the urethra was too narrow to allow access for the cystoscopye and in another patient the bladder volume was too small and the bladder mucosa was seriously swollen so the cystoscopy examination was failure; RII was a success in both cases. Therefore, we considered that RII can serve as an auxiliary method for detecting bladder cancer and can be used selectively. The selective accumulation of radioactivity in tumour tissues in both nude mice and patients also justifies the development of radioimmunoconjugates for radioimmunotherapy.

Until now there has been a lack of effective methods for qualitative diagnosis of ureteral cancer. Routine methods for the detection of ureteral cancer, including ultrasonography, intravenous pyalography and CT only use morphological criteria. Cytological diagnosis can give the qualitative information but the positive rate is low. RII can give information both in morphology and in property of a tumour and it is a noninvasive method. The primary result that RII qualitatively detected three ureteral tumours in the study showed that RII may be valuable for the diagnosis of ureteral cancer. The diagnostic value of RII for the detection of metastatic foci needs further investigation.

BDI-1 is an anti-bladder transitional cell carcinoma monoclonal antibody. But in our study, we found that bladder squamous cell carcinoma and bladder mucosal carcinoma also gave positive results by RII. We conclude therefore that BDI-1 has cross-immunoreactivity to both those pathological types of cancer.

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