A comparison of targetting of neuroblastoma with mIBG and anti L1-CAM antibody mAb chCE7: therapeutic efficacy in a neuroblastoma xenograft model and imaging of neuroblastoma patients

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Abstract. Iodine-131 labelled anti L1-CAM antibody mAb chCE7 was compared with the effective neuroblastoma-seeking agent ^{131}I-labelled metaiodobenzylguanidine (MIBG) with regard to (a) its therapeutic efficacy in treating nude mice with neuroblastoma xenografts and (b) its tumour targetting ability in neuroblastoma patients. The SK-N-SH tumour cells used in the mouse experiments show good MIBG uptake and provide a relatively low number of 6,300 binding sites/cell for mAb chCE7. Tumours were treated with single injections of ^{131}I-MIBG (110 MBq) and with ^{131}I-labelled mAb chCE7 (17 MBq) and both agents showed antitumour activity. After therapy with ^{131}I-chCE7, the subcutaneous tumours nearly disappeared; treatment with ^{131}I-MIBG was somewhat less effective, resulting in a 70% reduction in tumour volume. A calculated tumour regrowth delay of 9 days occurred with a radioactivity dose of 17 MBq of an irrelevant control antibody mAb 35, which does not bind to SK-N-SH cells, compared with a regrowth delay of 34 days with ^{131}I-mAb chCE7 and of 24 days with ^{131}I-MIBG. General toxicity appeared to be mild, as assessed by a transient, approximate 10% maximum decrease in body weight during the treatments. The superior growth inhibition achieved by ^{131}I-chCE7 compared with ^{131}I-MIBG can be explained by its prolonged retention in the tumours, due to slower normal tissue and plasma clearance. Cross-reaction of mAb chCE7 with L1-CAM present in normal human tissues was investigated by direct binding of radioiodinated mAb to frozen tissue sections. Results showed a strong reaction with normal human brain tissue and weak but detectable binding to normal adult kidney sections. Seven patients with recurrent neuroblastoma were sequentially imaged with ^{131}I-MIBG and ^{131}I-chCE7. The results underlined the heterogeneity of neuroblastoma and showed the two imaging modalities to be complementary. ^{131}I-chCE7 scintigraphy may have clinical utility in detecting metastases which do not accumulate ^{131}I-MIBG, and the antibody may hold potential for radioimmunotherapy, either by itself or in combination with ^{131}I-MIBG.

Keywords: Neuroblastoma – Anti L1-CAM – Antibody chCE7 – Metaiodobenzylguanidine – Radionuclide therapy – Radioimmunoscintigraphy

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Introduction

Neuroblastomas are tumours which arise during the development of the sympathetic nervous system. They can often be cured in stages I–II, but patients with more advanced disease (stages III and IV) and recurrent tumours have a poor prognosis. Such relapses may appear as solid tumours, as bone marrow infiltrations, or as metastases in the bone or other distant sites, and current radiological methods cannot always determine the full extent of metastatic disease. Imaging with iodine-123 metaiodobenzylguanidine (MIBG), a catecholamine analogue

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which is specifically taken up in tumour cells originating from the neural crest, is a very sensitive and specific modality for the detection of neuroblastoma, because most neuroblastomas take up MIBG [1]. Because of its highly selective tumour uptake and retention, ^{131}I-MIBG has been used successfully as a systemic treatment and has been introduced as a first-line treatment before surgery and chemotherapy [2, 3]. However, tumour heterogeneity is a major obstacle limiting its efficacy, because during the course of the disease metastases can arise which do not take up MIBG. Targetting of neuroblastoma-specific antigens with radiolabelled monoclonal antibodies may be a means to destroy tumour cells which escape MIBG therapy. Several monoclonal antibodies against neuroblastoma have been tested for diagnosis and therapy. These include antibodies against the G_{D2} ganglioside [4, 5] and against the cell adhesion protein N-CAM [6]. For both anti-G_{D2} and anti-N-CAM-directed antibodies, the targetting of tumour lesions negative for MIBG has been reported in a few cases [7]. Sequential imaging studies of relapsed patients with MIBG and anti-neuroblastoma antibodies after MIBG therapy, however, are rare.

MAb chCE7 is a high-affinity (K_d ~10^{-10} *M*) chimeric monoclonal antibody [8] which is internalized into neuroblastoma cells [9, 10]. The original murine mAb CE7, from which the chimeric mAb was derived, showed no binding to normal tissues and blood cells with the exception of a weak reaction with adrenal medulla and sympathetic ganglia [11]. Recently, the target antigen for mAb chCE7 was isolated and partially cloned from SK-N-BE2(c) neuroblastoma cells and identified as a form of the cellular adhesion molecule L1 (L1-CAM) [12]. Expression of L1-CAM in neuroblastomas of different grade has been documented [13] and the murine mAb CE7 reacted with all of 15 tested neuroblastoma biopsy specimens [11]. L1-CAM mRNA expression was found to be absent from normal tissues such as colon, heart, liver, lung, muscle, spleen and testis, whereas L1-CAM mRNA was detected in brain and kidney [12]. Western blot analysis of brain and kidney tissues using mAb chCE7 indicated that L1-CAM protein is strongly expressed in human brain and shows very weak but detectable expression in human kidney [12]. In view of the application of radioiodinated mAb chCE7 for radioimmunodiagnosis and -therapy, in this study the binding of ^{131}I-labelled mAb chCE7 to tissue sections of human brain and kidney is described, with the aim of further characterizing the binding of mAb chCE7 to these non-target tissues.

First clinical results with ^{131}I-mAb chCE7 imaging in neuroblastoma patients with extensive bone marrow metastases showed that chCE7 is taken up rapidly and strongly by bone marrow infiltrations [14]. In the present study a number of patients with recurrent neuroblastoma were sequentially imaged with MIBG and mAb chCE7 in order to find out more about L1-CAM expression in tumour relapses. In addition, the therapeutic efficacy of ^{131}I-chCE7 and ^{131}I-MIBG was assessed in nude mice bearing neuroblastoma xenografts. Results show a high therapeutic efficacy in the animal model and exemplify the heterogeneity of neuroblastoma encountered in neuroblastoma patients. Sequential imaging with MIBG and mAb chCE7 can identify clinical situations where MIBG-negative metastases can be targetted by mAb chCE7.

Materials and methods

Materials

Antibody. MAb chCE7 is a high-affinity, internalizing chimeric mAb of the IgG1 subtype, which recognizes the cellular adhesion molecule L1 (L1-CAM) [8, 9, 12]. It was produced and purified from transfectant mouse SP2/O cells, as described previously [8]. MAb 35, a murine anti-CEA antibody [15], was used as a control in the therapy experiments.

Tissues. Frozen sections of normal human kidney and brain tissue were obtained from autopsy material (Institute for Pathology, University Hospitals at Basel, Switzerland) with a maximal post-mortem delay of 15 h and were stored at –80°C.

Cell lines. SK-N-SH and SK-N-BE2(c) neuroblastoma cells were from R. Mairs (University of Glasgow, UK), and LN-229 glioma cells were from E. Van Meir (University Hospital CHUV, Lausanne, Switzerland). The human renal carcinoma cell line Foehn was obtained from the Institute of Pathology, University of Zurich, Switzerland, and the human neuroblastoma cell line SK-N-AS was originally obtained from Prof. K. Blaser (SIAF, Davos, Switzerland). Additional human neuroblastoma cells (LAN-1, LAN-5, CHP-212, SK-N-M17, SK-N-DZ, SK-N-FI, SK-N-LE) and other tumour cell lines [ACN, SAV (neuroepithelioma), SK-N-PNET (primitive neuroectodermal tumour)] were kindly provided by N. Gross (University Hospital CHUV, Lausanne, Switzerland).

Radiopharmaceuticals. ^{131}I-MIBG (specific activity 37–185 MBq/mg) for clinical imaging studies and ^{131}I-MIBG (1.3–1.7 GBq/mg) for animal studies was purchased from Amersham (Amersham Buchler GmbH, Braunschweig, Germany).

^{125}I-MIBG (specific activity 810 MBq/mg; >99% purity) was prepared according to the Cu(I) catalysed isotopic exchange method described by Franceschini et al. [16].

^{125}I for protein iodination was from Amersham (Amersham Switzerland).

Chemicals. Chemicals were from Fluka (Buchs, Switzerland) unless otherwise stated.

Methods

Binding of mAb chCE7 to human tumour cells. Analysis by flow cytometry (FACS) was performed with duplicate samples of $2 \cdot 10^6$ cells in a buffer consisting of 2.7 m*M* KCl, 1.2 m*M* KH_2PO_4, 138 m*M* NaCl, 8.1 m*M* Na_2HPO_4 and 5.6 m*M* D-glucose (PBSG). Cells were incubated for 2 h at ambient temperature with 50 µg

mAb chCE7 in 1 ml PBSG including 1% bovine serum albumin (BSA). Control samples without antibody were incubated in parallel. After two washes with PBSG (centrifugation at 300 *g* for 5 min at 4°C), the cells were incubated with a 1/16 dilution of goat anti-human IgG-FITC conjugate (Sigma, Buchs, Switzerland) in PBSG/BSA for 1 h at ambient temperature in the dark. Cells were washed three times with 2 ml of PBSG at 4°C and then analysed in a flow cytometer.

For Scatchard analysis, mAb chCE7 was radioiodinated with ^{125}I to a specific activity of 4 µCi/µg as described previously [9]. Saturation binding of ^{125}I-labelled mAb chCE7 was measured by incubating duplicate samples of $0.2 \cdot 10^6$ SK-N-SH cells with increasing concentrations of ^{125}I-chCE7 (25–250 ng) for 2 h at 37°C. Non-specific binding in the presence of 6 µg unlabelled chCE7 was determined in parallel and subtracted, and the data were analysed by the Scatchard method.

Uptake and retention of ^{125}I-MIBG in SK-N-SH cells was measured as described previously [17]. In brief, SK-N-SH cells grown in DMEM medium supplemented with 10% fetal calf serum (approximately 125,000 cells/cm^2) were incubated with non-saturating concentrations of 10^{-8} *M* ^{125}I-MIBG with or without 4 µ*M* imipramine. After 2 h, the cell-associated radioactivity was determined, and in parallel cultures the medium was replaced by ^{125}I-MIBG free medium to assess the radioactivity retained by the cells after a 4-h "washout" period.

Antibody labelling with ^{131}I. MAb chCE7 was labelled with ^{131}I (Nordion) to a specific activity of 5 mCi/mg using the Iodogen method as described previously [9]. Quality controls of labelled antibody included thin-layer chromatography to assess radiochemical purity and tests for osmolarity, sterility and pyrogenicity. Immunoreactivity of labelled preparations was measured using a binding assay with SK-N-AS neuroblastoma cells [9]; data were evaluated according to the method of Lindmo et al. [18] and immunoreactivity was >50%.

Binding of ^{131}I-labelled mAb chCE7 to tissue sections. Frozen tissue sections (25 µm) were from post-mortem tissue of human adult brain and human adult kidney and were kept at –80°C prior to antibody staining. No fixation procedure was used. Sections were warmed up at ambient temperature for 30 min and were presaturated with 0.1 *M* NaCl, 0.05 *M* Na phosphate buffer pH 7.3 (PBS) containing 2% BSA for 60 min at 37°C. Sections were then incubated for 4 h on ice in PBS/0.5% BSA containing 100,000 cpm/ml of ^{131}I-chCE7. Parallel sections were incubated with 10 µg mAb chCE7/ml. After incubation, the sections were washed three times in ice-cold PBS/0.2% BSA and were dried for 20 min at ambient temperature. They were then subjected to electronic autoradiography in a "Packard" Instant Imager. After imaging, sections were fixed in PBS containing 4% formaldehyde and were stained with haematoxylin-eosin.

Analysis of anti-tumour activity in nude mice with neuroblastoma xenografts. Experiments were performed in accordance with the national regulations for animal experimentation and protocols were approved by the animal welfare committee of the Netherlands Cancer Institute. Female athymic BALB/c *nu/nu* mice were obtained from the specific pathogen-free breeding unit of the Netherlands Cancer Institute's Animal Department. Before and during all experiments, mice were handled in laminar flow hoods and kept in filtertop cages. Pathogen-free food and drinking water were available ad libitum.

Tumour inductions and passaging of tumours using the SK-N-SH human neuroblastoma cell line were as described previously [17]. Four weeks after implantation, tumours reached on average 170 mg (range 20–440 mg). Tumour volume doubling times averaged 4 days. Therapy with radiopharmaceuticals was started when mice were 10–13 weeks old (19–27 g body weight). To prevent radioiodide uptake by the thyroid, mice were injected daily with an excess of KI solution (i.p., 1 mg/20 g body weight) from 1 day prior until day 8 after the administration of the radiopharmaceuticals. Groups of five mice received intravenous injections into the tail vein of 110±7 MBq of ^{131}I-MIBG or 17.8±1.0 MBq of ^{131}I-mAb chCE7. Control groups of five tumour-bearing mice received saline or 17.3±0.8 MBq of ^{131}I-mAb 35. Tumour size was measured every other day with a Vernier caliper in three dimensions and tumour volumes were calculated as $(L \times W^2/2)$. Body weights were measured daily. Tumour regrowth rates were estimated from the slope of semi-logarithmic plots of the tumour volume against time curves using unweighted linear regression analysis. By interpolation, the individual regrowth time required to reach four times the pretreatment tumour size was determined. Tumour growth delay was defined as the difference in time that the control and the treated group required to reach the end point.

Patient selection and imaging studies. Comparative whole-body scintigraphy in seven neuroblastoma patients was performed with ^{131}I- or ^{123}I-MIBG and ^{131}I-mAb chCE7. Six patients with recurrent neuroblastoma stage IV and one patient with neuroblastoma stage III were sequentially imaged with MIBG and mAb chCE7. Some patients were selected because of discrepancies between imaging and clinical parameters or because of suspicious findings (e.g. symptoms, results of bone marrow aspiration).

Either 18.5 MBq ^{131}I-MIBG (patients 2, 3, 6, 7) or 185 MBq ^{123}I-MIBG (patients 1, 4, 5) was injected, preceded or followed by 20–37 MBq ^{131}I-chCE7, i.e. 1 week before or after ^{131}I-MIBG, or 1 day after ^{123}I-MIBG.

Multiple 10-min acquisition spot views of the entire body in a 256×256 matrix were taken, using a dual-head gamma camera (ADAC Vertex or Dual Genesys) with high-energy (^{131}I) or low-energy/high-resolution (^{123}I) collimators 24 and 48 h after administration of the tracers. The uptake of the tracers was scored qualitatively: 0=negative, 1=weakly positive (<liver activity), 2=positive (equal to liver activity), 3=intense uptake (>liver activity).

Results

Reaction of mAb chCE7 with human tumour cell lines and with normal human tissues

Binding of the murine CE7 mAb from which chimeric mAb chCE7 was derived had previously been tested on nine different human neuroblastoma cell lines and all of them were found to react with the mAb [11]. An additional 13 human neuroblastoma cell lines as well as cell lines originating from glioma, primitive neuroectodermal tumour (PNET), neuroglioma and a renal carcinoma cell line were analysed by FACS. It was found that all the neuroblastoma cell lines bind mAb chCE7, although large differences in L1-CAM expression were evident (Fig. 1). A glioma cell line (LN-229) and a PNET cell line (SK-N-PNET) did not bind mAb chCE7. Taken together, the previously reported results with the murine [11] and the present results with the chimeric mAb CE7

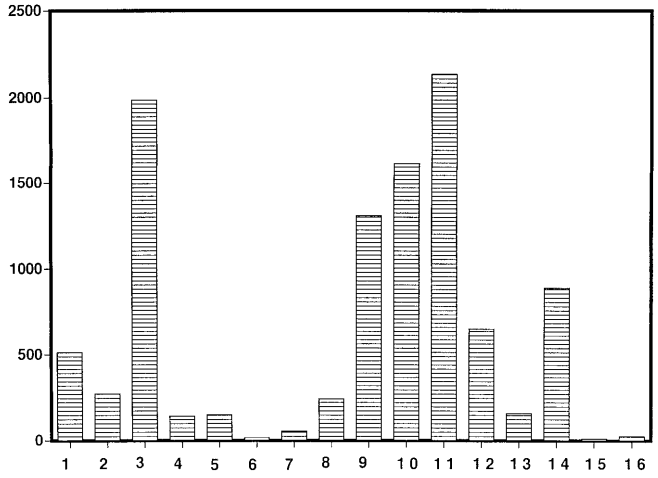

Fig. 1. Binding of mAb chCE7 to neuroblastoma and other human tumour cells: FACS analysis. Fluorescence intensity is depicted in arbitrary units on the y-axis. *1*, ACN; *2*, CHP-212; *3*, Foehn; *4*, LAN-1; *5*, LAN-5; *6*, LN-229; *7*, SAV; *8*, SK-N-AS; *9*, SK-N-BE; *10*, SK-N-BE2c(M); *11*, SK-N-BE2(c)(G); *12*, SK-N-M17; *13*, SK-N-D7; *14*, SK-N-Fl; *15*, SK-N-LE; *16*, SK-N-PNET. Neuroblastoma cell lines: 2, 4, 5, 8–15 [10, 11: SK-N-BE2(c) cells obtained from different sources]; neuroepithelioma cells: 1, 7; glioma cell line: 6; PNET cell line: 16; renal carcinoma cell line: 3

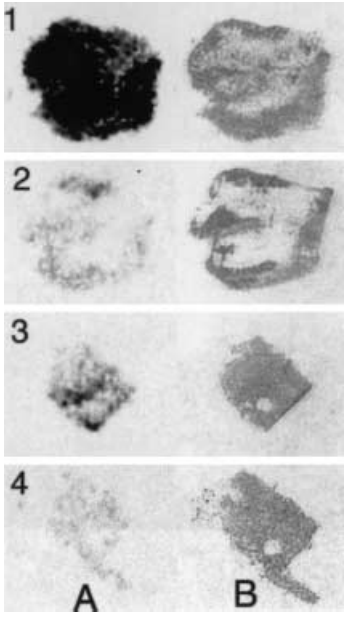

Fig. 2A, B. Binding of ^{131}I-labelled mAb chCE7 to human tissue sections. **A** Autoradiography; **B** haematoxylin-eosin staining. Frozen, unfixed sections of human brain (*1, 2*) and human kidney (*3, 4*) were incubated with ^{131}I-chCE7, autoradiographed and subsequently stained, as described in the Methods section. *2, 4*: Non-specific binding in the presence of 10 µg/ml of mAb chCE7

underline the prevalence of L1-CAM expression in neuroblastoma cells.

A number of normal human tissues, including fetal brain tissue, were tested for binding of the murine CE7 mAb using the immunoperoxidase technique, and the only tissues that were found to react weakly were adrenal medulla and lumbar sympathetic ganglion [11]. Subsequent to the identification of the protein recognized by mAb chCE7 as L1-CAM, normal human tissues were analysed for L1-CAM expression with both northern and western blot analysis. Human brain and kidney tissue was found to express L1-CAM mRNA and protein [12]. Because of the importance for applications in radioimmunotherapy of awareness of cross-reactions with these normal human tissues, the direct binding of ^{131}I-labelled mAb chCE7 to sections of adult human brain and kidney was analysed (Fig. 2). Strong binding to brain sections was found, whereas binding to sections of normal adult kidney was weak (Fig. 2). The results confirm that with the exception of brain tissue, mAb chCE7 binding is absent from normal tissues, or very weak in the case of kidney tissue.

In vitro targetting efficiencies of ^{125}I-MIBG and ^{125}I-chCE7 antibody in monolayer cultures of human SK-N-SH neuroblastoma cells

In order to compare the efficacy of MIBG and mAb chCE7 in treating experimental tumours, xenografts of SK-N-SH human neuroblastoma in nude mice were chosen. This animal model has been shown to be clinically relevant and has been applied in several therapeutic or pharmacokinetic studies with radiolabelled MIBG [17, 19]. Both uptake of ^{125}I-MIBG into SK-N-SH cells and binding of ^{125}I-chCE7 to SK-N-SH cells were assessed, and good MIBG uptake and retention as well as a (relatively small) number of binding sites for mAb chCE7 were found. Scatchard analysis of saturation binding data of ^{125}I-chCE7 to SK-N-SH cells indicated a B_{max} of 6,300 (n=2) and a K_d of ~10^{-10} M. ^{125}I-MIBG uptake was found to be 32.7%±9.6% (n=15) of added radioactivity, and after 4 h 57.6%±7.4% (n=15) of radioactivity was retained intracellularly. Compared with the human neuroblastoma cell line SK-N-AS, which has been used in biodistribution studies [9, 20] and shows approximately 40,000 binding sites for mAb chCE7, and with the human neuroblastoma cell line SK-N-BE2(c), which has been used for the isolation of the target antigen for mAb chCE7 [12] and shows approximately 200,000 binding sites per cell, SK-N-SH cells express fewer binding sites for mAb chCE7. The xenograft model was selected for the therapy study because SK-N-SH cells take up MIBG well and is certainly not biassed in favour of high antibody uptake.

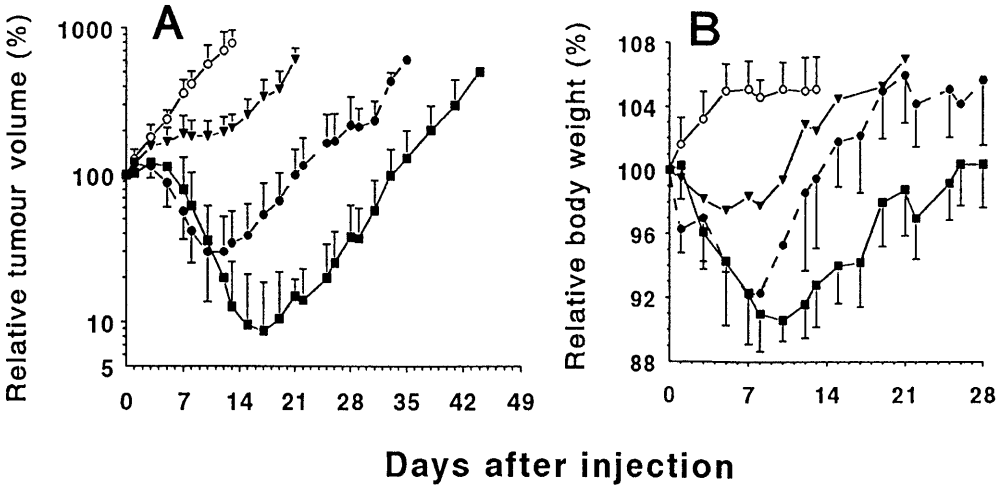

Fig. 3. Tumour growth inhibition (**A**) and body weight changes (**B**) of nude mice bearing neuroblastoma (SK-N-SH) xenografts after i.v. therapy with ^{131}I-labelled radiopharmaceuticals. Animals received single injections of saline (*open circles*), 17.3±0.8 MBq irrelevant control antibody mAb35 (*triangles*), 110±7 MBq MIBG (*closed circles*) or 17.8±1.0 MBq mAb chCE7 (*closed squares*). Tumour sizes and body weights (corrected for the tumour weight) are expressed as a mean percentage of the pretreatment value (±SD, *n*=5–6)

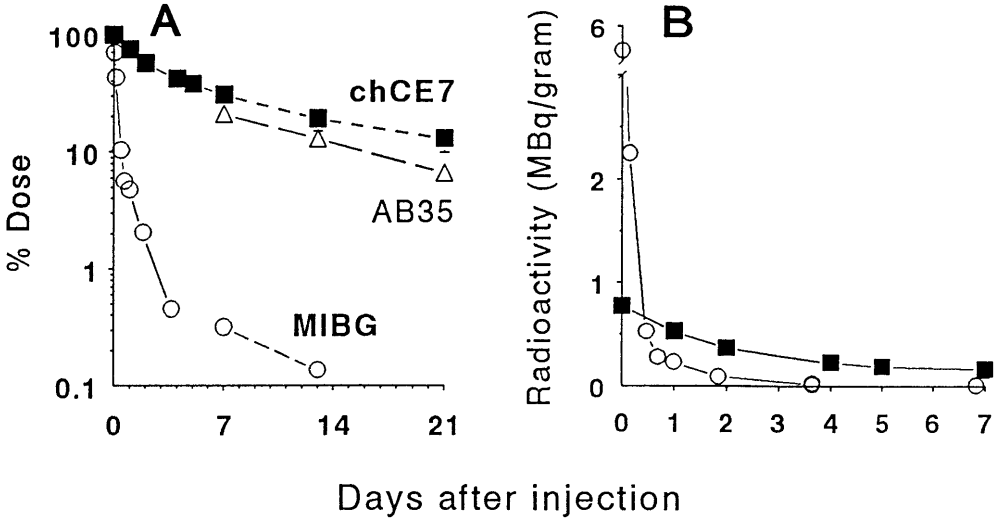

Fig. 4A, B. Whole-body clearance of radiopharmaceuticals in tumour-bearing nude mice. **A** Whole-body radioactivity as % of injected dose. **B** Whole-body radioactivity in MBq/g. *Dotted lines* represent tumour-bearing mice undergoing i.v. therapy with 18 MBq ^{131}I-chCE7 antibody (*squares*), 17 MBq of ^{131}I-mAb 35 control antibody (*triangles*) or 110 MBq ^{131}I-MIBG (*circles*). Data from previously performed tracer experiments were used to construct the *solid lines*

Therapeutic efficacy of ^{131}I-mAb chCE7 and ^{131}I-MIBG in nude mice bearing SK-N-SH neuroblastoma xenografts

In a pilot experiment with nude mice bearing SK-N-SH neuroblastoma xenografts, tumour uptake of ^{131}I-chCE7 and of ^{131}I-MIBG was determined 24 h post injection and was found to be 10.9%±1.3% ID/g (*n*=4) for ^{131}I-chCE7 and about fourfold less for ^{131}I-MIBG. For ^{131}I-MIBG the maximum tumour loading was 2.4%±0.5% ID/g (*n*=5), reached 1 h post injection. Since the ^{131}I-mAb is retained for much longer at the tumour site than the rapidly clearing ^{131}I-MIBG, a far higher radiation exposure is achieved with the radiolabelled mAb. Because of this large difference in radiation exposure, the dose of ^{131}I-MIBG chosen for the therapy experiment was more than six times that of ^{131}I-chCE7. Groups of five mice with a median tumour size of 90 mg were treated by single injections of ^{131}I-MIBG, ^{131}I-chCE7 and ^{131}I-mAb 35 (as a control mAb), and the resulting tumour growth curves are shown in Fig. 3A. Results showed rapid growth of untreated controls, some growth delay with the non-specific ^{131}I-mAb 35 and significant antitumour effects of both ^{131}I-MIBG and ^{131}I-mAb chCE7. After therapy with ^{131}I-chCE7 the subcutaneous tumours nearly disappeared, whereas therapy with ^{131}I-MIBG was somewhat less effective, resulting in a maximum tumour volume reduction of 70%. The ^{131}I-chCE7 therapy retarded tumour growth by an average of 34 days, which was clearly superior to the tumour growth delay of 24 days achieved with ^{131}I-MIBG. The greater efficacy of the treatment with the ^{131}I-labelled antineuroblastoma antibody compared with the treatment with ^{131}I-MIBG can be explained by the very different whole-body radioactivity levels obtained with the radiopharmaceuticals. The whole-body clearance rates of the ^{131}I-labelled antineuroblastoma mAb chCE7 and the irrelevant ^{131}I-labelled control mAb 35 were similar, whereas the clearance of the low-molecular-weight ^{131}I-MIBG was much more rapid (Fig. 4A). ^{131}I-MIBG levels were about 100-fold lower than radioactivity levels of the antibodies and below the detection level at day 21 (Fig. 4B).

Assessment of general toxicity was based on the reduction in the body weight of mice during the post-treatment period, and indicated that both targetted radio-

Table 1. Summary of clinical status and comparison of imaging results

Patient no./ age	Diagnosis	Localizations	MIBG result[a]	chCE7 result[a]
1. Male, 5 years	NB, IV	Bone marrow BMA+, MIBG+	0–1	1
2. Male, 1 year	NB, IV	Primary tumour:		
		R abdomen	1	2
		Bone (orbitae)	2	0
		Bone marrow	1	0
3. Female, 11 months	NB, III	Primary tumour:		
		Thorax	3	3
4. Female, 4 years	NB, IV	Bone (MIBG only), 2 lesions confirmed by surgery with probe; later progressive	2	0
5. Female, 2 years	NB, IV	Recurrence:		
		Abdomen	2	0
		Bone metastases:		
		– Skull	1	2
		– R humerus	2	1
		– R femur (cent.)	2	0
		– R femur (peri.)	0	2
		– L femur	1	1
		– R tibia	2	2
		– L knee	0	3
		– Pelvis	0	2
		– Skull (new)	0	3
6. Female, 4 years	NB, IV	Primary tumour:		
		R adrenal	3	3
		Bone metastases:		
		– Orbita	2	0
		– Thoracic spine	2	0
		– R femur	2	1
		– L femur	1	1
		– L tibia	1	1
7. Male, 3 years	NB, IV	Primary tumour:		
		R adrenal	3	1
		Bone marrow	2	1

L, Left; R, right
[a]Tracer uptake was scored qualitatively: 0=negative, 1=weakly positive (<liver activity), 2=positive (equal to liver activity), 3=intense uptake (>liver activity)

therapies were well tolerated (Fig. 3B). Like ^{131}I-MIBG, ^{131}I-chCE7 caused mild toxicity with a maximal weight loss of approximately 10%, but the animals recovered much more slowly than those treated by ^{131}I-MIBG. This somewhat increased toxicity induced by the radiolabelled antibody could be explained by the fact that the whole-body radiation exposure during radioimmunotherapy with ^{131}I-chCE7 was 1.7 times higher than that with ^{131}I-MIBG.

Sequential imaging of neuroblastoma patients with MIBG and mAb chCE7

Clinical status and imaging results of the seven neuroblastoma patients who were sequentially imaged with $^{131/123}$I-MIBG and ^{131}I-mAb chCE7 are summarized in Table 1. All of the patients suffered from recurrent neuroblastoma after preoperative ^{131}I-MIBG therapy and surgery. Some of the patients presented diagnostic dilemmas, showing discrepancies between MIBG scintigraphy and other clinical parameters. Abnormal radionuclide uptake sites on the ^{131}I-chCE7 images were clearly evident 24 h post injection, with low levels of activity observed in the liver and spleen. Primary tumour sites present in four patients were detected by both MIBG and chCE7. Figure 5 illustrates imaging of a solid tumour mass in the thorax close to the spine after preoperative treatment with ^{131}I-MIBG, an example of both MIBG and mAb chCE7 being avidly taken up by the tumour. Altogether 24 lesions were detected in the seven patients: 19 (or possibly 20) were shown by $^{131/123}$I-MIBG and 17 were visualized by ^{131}I-chCE7. For 13 tumour sites the results of both imaging techniques were concordant, although the level (degree) of uptake varied: 6×chCE7=MIBG, 3×chCE7>MIBG, 4×chCE7<MIBG. Discordant findings were obtained in four patients

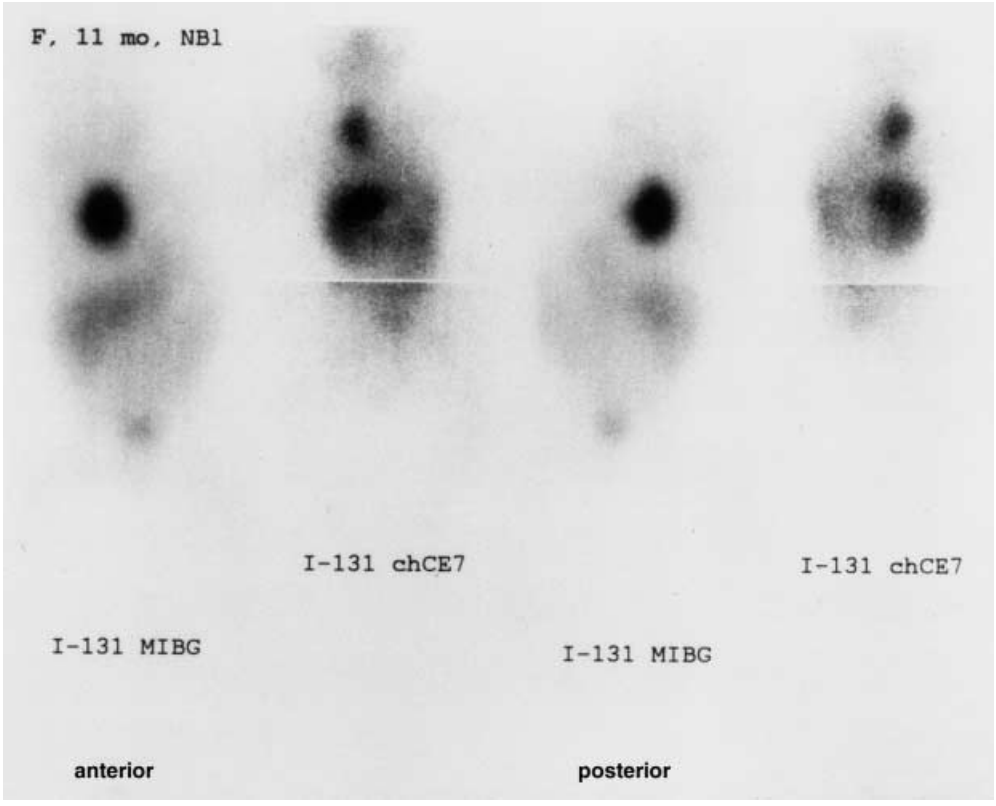

Fig. 5. Comparative scintigraphy using ^{131}I-MIBG and ^{131}I-mAb chCE7 in an 11-month-old girl (patient 3) presenting with a primary neuroblastoma (stage III) in the right median thorax. Tumour is equally well shown with both tracers, although the normal uptake in the liver is more prominent using the antibody

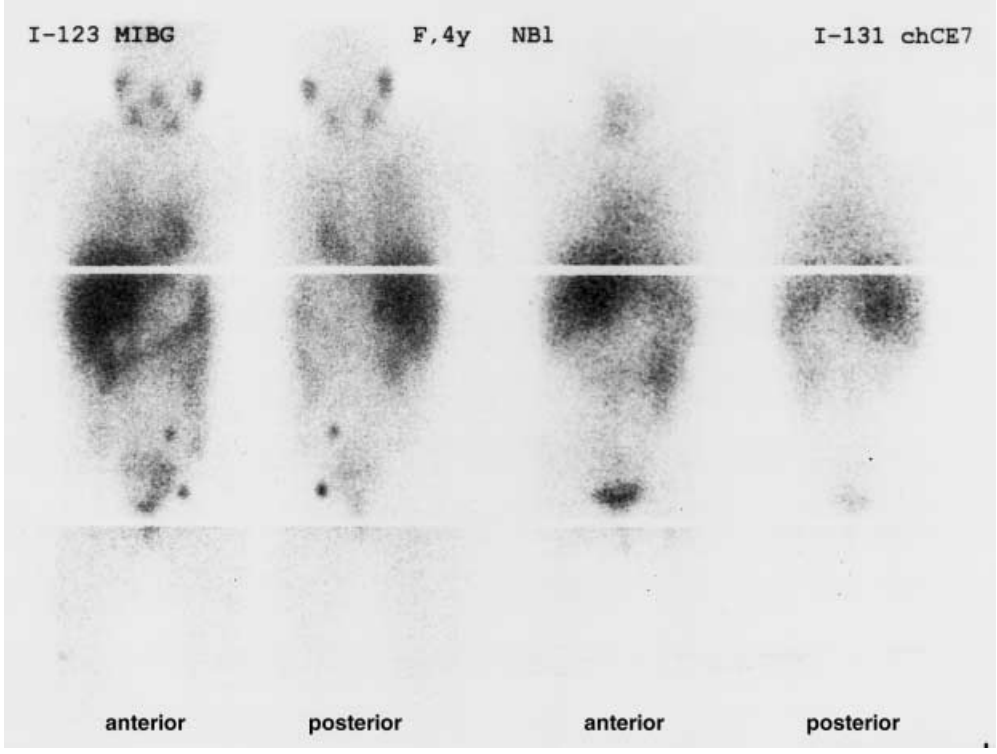

Fig. 6. A 4-year-old girl (patient 4) who had been successfully treated with ^{131}I-MIBG and surgical resection of an abdominal neuroblastoma (stage III). During follow-up, while all other parameters were negative, ^{123}I-MIBG scintigraphy revealed two spots in the groin and/or pelvis (*left*), not shown on the ^{131}I-mAb chCE7 scintigram (*right*). Two lesions in the pelvic bone were histologically confirmed after probe-guided surgery. Fifteen months later the patient developed diffuse bone marrow metastases

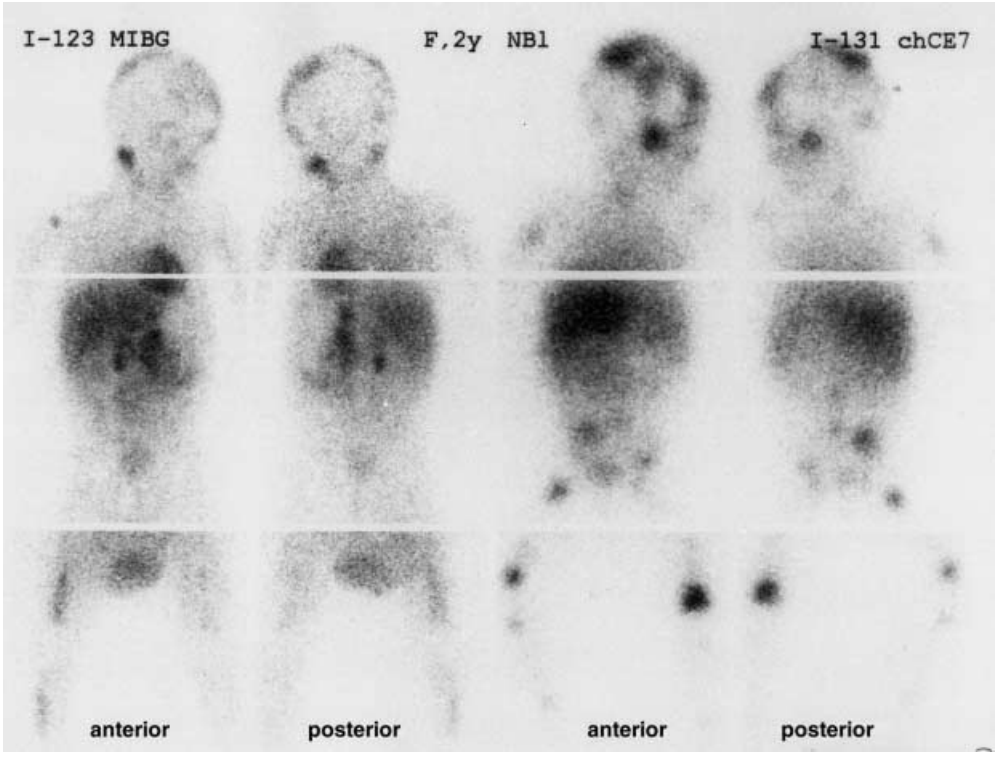

Fig. 7. Comparative scintigraphy illustrating the complementary findings of ^{123}I-MIBG and ^{131}I-mAb chCE7 in a 2-year-old girl with recurrent neuroblastoma in the abdomen and the right femur (patient 5). ^{123}I-MIBG scintigraphy shows pathological concentration in the abdominal recurrence, right orbit, right humerus, right femur and right tibia (faint uptake in the skull and left femur). ^{131}I-mAb chCE7 scintigraphy reveals pathological concentration in the skull, the right orbit, right humerus, pelvis, right femur at either end of the ^{123}I-MIBG uptake, right tibia, and, most intensely, above the left knee, but not in the abdominal mass or the central right femur shaft

(11 tumour sites): 4×chCE7-positive/MIBG-negative (one patient) and 7×chCE7-negative/MIBG-positive. Figure 6 shows imaging with MIBG of two bone lesion which were negative for mAb chCE7. Both lesions were confirmed by probe-guided surgery and were later found to be progressive. Figure 7 shows heterogeneous tumour recurrences in patient no. 5. Initially the ^{123}I-MIBG scintigram (not shown) had been negative, but 3 weeks after the first cycle of chemotherapy the patient presented a new swelling of the skull and a painful left knee. At this time combined ^{123}I-MIBG/^{131}I-mAb chCE7 scintigraphy was performed. MIBG uptake is apparent in the abdominal region where the primary tumour was located, as well as in a number of bone metastases. A number of (new) bone metastases in the skull, pelvis, tibia and knee are only detected by mAb chCE7.

Discussion

Neuroblastoma is a tumour of the sympathetic nervous system which occurs predominantly in young children and is often detected when at an advanced stage (III or IV). Whereas localized disease without distant metastases (stage I or II) is treated by surgery and has a good prognosis, the 5-year survival rate in patients with advanced disease is limited despite maximal therapy. This may partly be due to unfavourable genetic factors, toxicity and the induction of resistance to chemotherapy, but the treatment is also complicated by the fact that neuroblastoma often presents as a heterogeneous tumour. ^{131}I-MIBG scintigraphy, with its high sensitivity and specificity, has an important role in the diagnosis, staging and follow up of neuroblastoma. Higher doses of this radiopharmaceutical are used for treatment. The reported response to ^{131}I-MIBG therapy is comparable with that to combination chemotherapy, but ^{131}I-MIBG therapy is associated with considerably less toxicity in these young children. Nevertheless, an initial good response may be followed by recurrence or progression of the disease.

The targetting mechanism of MIBG is based on an active uptake-1 mechanism at the cell membrane and storage in neurosecretory granules in the cytoplasm, cellular properties characteristic for a certain degree of differentiation of tumour cells. Radiolabelled monoclonal antibodies bind to specific cell surface proteins which are also expressed in immature tumour cells where uptake and storage sites for MIBG may not be expressed. In addition, in some necrobiotic areas of tumour, where cellular uptake functions are impaired by lack of blood/oxygen supply, cells may still have clonogenic potential and radiolabelled antibody may bind and destroy metabolically inactive tumour cells. Thus the different characteristics of antibody targetting may enable the detection of recurrence or progression of disease in an early phase as well as targetting to metabolically inactive sites of tumour. Combination of therapies using different targetting mechanisms therefore seems a logical next step, and in view of the advantages of targetted radionuclide therapy, an alternative form of cell-specific radiation therapy is the most attractive option for clinical use in patients with advanced stage neuroblastoma.

The chimeric monoclonal antibody chCE7 is directed against the L1-CAM protein, which is overexpressed in neuroblastoma, and may therefore be a useful targetting vehicle. The preclinical studies evaluating radioiodinated and radiocopper-labelled high affinity mAb chCE7 in nude mice bearing human neuroblastoma xenografts showed high tumour uptake and favourably low levels in normal tissues [9, 20]. During the course of these studies it was found that mAb chCE7 is internalized into its target cells [10], which may represent an advantage for intracellular delivery of cytotoxic agents or short-range radiation. An additional advantage of antibodies as targetting vehicles is the possibility of labelling them with metallic radionuclides such as copper-67, yttrium-90, rhenium-186 or lutetium-177, beta particle emitters with, for therapeutic applications, superior radiation characteristics to ^{131}I, whereas MIBG labelling is so far restricted to the radiohalogens.

For clinical application, cross-reactivity with normal human tissues is an important factor in evaluating an mAb for in vivo targetting, because the aim is to limit toxicity of radiation delivered by "tumour-specific" antibodies to normal tissues. The CE7 monoclonal antibody was originally generated by immunizing mice with human neuroblastoma cells. Its cross-reaction with normal human tissues was reported early on, the findings indicating that only adrenal medulla and sympathetic ganglia reacted weakly with the antibody [11]. Following the identification of its target antigen as L1-CAM and the results obtained on L1-CAM mRNA expression in normal human tissues [12], direct binding of ^{131}I-mAb chCE7 to sections of human brain and human kidney, tissues which were found to express L1-CAM, was specifically investigated. Figure 2 shows strong binding of ^{131}I-mAb chCE7 to a section of human brain and weak but detectable binding to normal kidney. Because systemically applied radiolabelled antibodies do not cross the blood-brain barrier, the reaction with normal brain does not impair the usefulness of the antibody in targetting tumours outside of the brain. Concerning the weak reaction of the antibody with normal kidney tissue, the whole-body scintigrams in this study do not show kidney uptake, indicating that binding is below the detection level. However, when considering the use of mAb chCE7 in passive immunotherapy, a therapy in which high amounts of unlabelled monoclonal antibodies are applied over long periods, possible effects on normal kidney tissue should be kept in mind.

The therapeutic effects shown in the present study when nude mice with SK-N-SH neuroblastoma xenografts were treated with single injections of mAb chCE7 underline the potential of mAb chCE7 for targetted radioimmunotherapy of neuroblastoma. In this animal model the therapeutic efficacy of ^{131}I-labelled mAb chCE7 compared favourably with that of ^{131}I-MIBG (Fig. 3). This was probably due to the very different pharmacokinetics of the two radiopharmaceuticals. Clearly an intact monoclonal antibody maximizes tumour uptake and retention of radioactivity, owing to its longer half-life in the blood and consequent availability for tumour binding. These results demonstrating therapeutic efficacy of mAb chCE7 in the animal model prompted us to initiate a small study comparing imaging with MIBG and mAb chCE7 in patients with recurrent neuroblastoma.

In the described pilot study of comparative whole-body scintigraphy using ^{131}I-MIBG and ^{131}I-labelled chCE7 antibody in seven patients with recurrent progressive neuroblastoma, discrepancies between the two types of imaging were seen. In most of the patients additional information was gained by performing scintigraphy with ^{131}I-chCE7 and in some, imaging with MIBG and mAb chCE7 yielded strikingly complementary findings. Figure 7 demonstrates a clinical example in which it seemed that the longer existing (more differentiated?) lesions took up ^{123}I-MIBG, whereas the latest, rapidly progressing (immature?) lesions strongly took up ^{131}I-mAb chCE7. These results are encouraging, but require confirmation in a larger number of patients. In addition to the published sensitivity and specificity of $^{123/131}$I-MIBG imaging, the contribution of radioimmunoscintigraphy in the diagnosis, staging and follow-up of neuroblastoma needs to be established. Combined use of ^{131}I-MIBG and ^{131}I-mAb chCE7 may not only lead to improved detection of the disease, but also indicate which is the optimal therapy for the individual patient: ^{131}I-MIBG therapy, radioimmunotherapy, combined ^{131}I-MIBG/^{131}I-mAb chCE7 therapy or no radionuclide therapy at all.

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