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Monoclonal Antibodies in the Treatment of Central Nervous System Malignancies

John T. Kemshead, Varnavas Papanastassiou, Hugh B. Coakham and Barry L. Pizer

INTRODUCTION

MONOCLONAL ANTIBODIES (Mabs) clearly do not cross the blood–brain barrier when administered systemically. Consequently, their use as targeting agents within the central nervous system (CNS) is limited to situations where they are either given topically or when the "barrier" is disrupted. In the latter instance, accumulation of isotope is low, approximately 10^{-3}% of the injected dose-gram of tumour [1]. This poor level of uptake is not restricted to CNS malignancies, as similar results have been noted in other solid tumours such as ovarian, colon and small cell lung carcinoma [2]. Improved binding to primary CNS tumours has been shown in animal models by intra-arterial administration of radiolabelled Mabs, but this has not proved to be the case in clinical investigations [3]. Therefore, until this problem has been overcome, Mabs will only have a role in the therapy of CNS tumours when local administration is possible.

For primary intracerebral tumours, Mabs have been given by either direct injection into a tumour resection cavity or, when appropriate, instillation into a cystic lesion [4]. Where tumours have spread throughout the cerebrospinal fluid (CSF) pathways, Mabs have been administered into the intrathecal space following either intraventricular or lumbar injection [5]. With respect to the targeted agent, either radioisotope or biological response modifiers (e.g. targeted lymphocyte activated killer cells), have been explored in the CNS compartment [6]. Here we describe the use of targeted radiation therapy, as this has formed the main focus of our studies over the last 6 years.

INTRATHECAL INJECTION OF Mabs INTO PATIENTS WITH LEPTOMENINGEAL SPREAD OF MALIGNANCY

In our group, over 40 patients have received targeted radiation therapy for leptomeningeal spread of malignancy. All had relapsed after maximal conventional therapy and include patients with primitive neuroectodermal tumours (PNET), CNS leukaemia, gliomatosis, melanoma and carcinomatous meningitis. Of these, the majority have received intraventricular injections via an Ommaya reservoir, as it has been demonstrated that Mab distribution throughout the CNS pathways is improved using this route as compared to lumbar injection [7].

Entry criteria of our studies dictate that the patient's tumour must bind the Mab chosen as the targeting agent and free-flow of CSF has to be demonstrated by myelography. Furthermore, patients undergo a full clinical assessment including blood and CSF chemistry and either cranial computed tomography (CT) or magnetic resonance imaging (MRI). Patients are excluded from the study if parenchymal disease is identified.

Correspondence to J.T. Kemshead.
The authors are at The Imperial Cancer Research Fund Paediatric & Neuro-Oncology Group; and H.B. Coakham is also at the Department of Neurosurgery, Frenchay Hospital, Bristol BS16 1LE, U.K.
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Prior to treatment, individuals are given a regimen to block ^{131}I uptake into the thyroid and in anticipation of a meningitic reaction, associated with administering protein into the CSF, all patients are placed on low-dose dexamethasone (1 mg twice daily). This is tailed off over a 3-week period following therapy.

Care naturally has to be taken to prepare Mabs for CNS administration. Purity of material is checked extensively using a variety of biochemical investigations. In addition, reagents are screened for sterility and pyrogenecity at each stage of their preparation. Mabs are labelled with ^{131}I to a specific activity of 185–555 MBq/mg protein. Prior to infusion, radiolabelled Mabs are checked to ensure that they are biologically active, not aggregated and contain less than 10% free iodine [8].

RESULTS OF THERAPY

Approximately two thirds of patients receiving intrathecal targeted therapy fall into two groups, those with either PNET or CNS leukaemia. Of the former, 19 patients have been treated as part of a phase I/II study, with 15 fully evaluable for response. Patients aged 6–62 years received a single injection of Mab conjugated to between 962–2220 MBq ^{131}I. 6 responses have been observed: 3 complete (clearance of tumour cells from the CSF) for 3, 8 and 10 months, 2 partial, for 2 and 7 months and 1 case of static disease for 18 months.

Meningeal irritation was observed in 13 of 19 patients, but in no case was this severe and all symptoms resolved within 4 days. 1 patient with a compromised CSF flow had an episode of raised intracranial pressure 3 days after therapy that was controlled by aspiration from his Ommaya reservoir. A further individual developed an encephalopathy on day 4, which lasted for 3 days followed by complete recovery.

Delayed toxicity is manifest by myelosuppression, which in the current context, is almost certainly dose-limiting. Of 11 evaluable individuals, 4 had WHO grade 3/4 bone marrow toxicity. This occurred following administration of between 1.48–2.22 GBq radiolabelled Mab and was reversible in all cases. Myelosuppression occurs through the radiolabelled Mab clearing from the CSF into the blood compartment. Peak blood levels of 20–30% of the injected dose are found within 36–48 h of administration. Myelotoxicity is brought about by the ^{131}I-Mab circulating in the blood rather than the Mabs binding to haemopoietic progenitor cells. Doses to bone marrow of 150–210 cGy have been calculated in the patients where grade 3/4 toxicity was observed.

Similar results have been obtained in patients with relapsed null and common acute lymphoblastic leukaemia (cALL). 7 patients aged 3–16 years with no systemic disease have been treated in second or subsequent CNS relapse [9]. Patient preparation was similar to that for the PNET group, except that myleography was omitted and in 5 out of 6 cases, systemic continuation therapy was discontinued for 2 weeks before and 4

weeks after intrathecal conjugate administration. This was to enable a proper evaluation of bone marrow toxicity. Patients with null ALL received a CD19 Mab, HD37 and those with cALL a CD10 reagent, WCMH. Amounts of ^{131}I varied from 629–14.80 MBq conjugated to 3–8 mg of Mab. Transient responses, lasting 4–8 weeks, were observed in 5 out of 6 patients. CSF leukaemic counts fell from 1300–4000 to zero in 3 out of 5 responding individuals. Toxicity in this group was less than that seen in patients with PNET. The only individual in whom grade 3/4 myelosuppression was noted was 1 where systemic chemotherapy was continued throughout the period of Mab treatment. This was despite the theoretical increased risk of Mab accumulating in bone marrow due to targeting CD10 and CD19 antigens present upon haemopoietic cells.

These clinical investigations into CNS leukaemia followed extensive studies into the "specificity" of targeted radiation therapy using a variety of cell lines and a soft agar cloning system. The model system employed was designed to mirror the clinical situation as far as possible. In every case, greater killing was observed with specific reagents as compared with control Mabs. The model has also served to demonstrate that use of a cocktail of two different specific Mabs is more efficacious than individual reagents.

These *in vitro* studies show the direction for improving responses in both patient groups. We are proposing to repeat targeted Mab therapy for patients with both medulloblastoma and CNS leukaemia and, where appropriate, use a cocktail of Mabs. 1 patient has been treated with a total of four injections of a HD37/WCMH cocktail. Following an initial injection of 1.70 GBq he received, at weekly intervals, 3 × 1.11 GBq. Whilst his CNS disease responded to therapy, he developed systemic leukaemia 14 weeks from beginning Mab treatment and thus was no longer evaluable for response. As with other studies, a systemic anti-mouse Ig response was noted in this patient. This resulted in an increasingly rapid clearance of Mab from the blood after each injection. However, such rapid changes in clearance kinetics were not observed within the CSF compartment. These differential changes in blood and CSF pharmacokinetics have also been observed in 1 patient with PNET receiving repeated therapy. This points to a benefit of using murine Mabs, as their rapid clearance from the blood compartment in the face of static CSF pharmacokinetics will reduce the whole body and bone marrow doses with respect to repeated administration.

Whilst total body, bone marrow and organ dosimetry has been calculated in individuals receiving intrathecally targeted radiation therapy, tumour dosimetry is not possible due to the nature of the disease. The *in vitro* system described above may, therefore, also be useful in developing dosimetric models to aid in our understanding of targeting to both leptomeningeal and diffuse disease in the CSF.

DIRECT INJECTION OF Mabs INTO THE PRIMARY TUMOUR SITE

Primary brain tumours are highly appropriate for this type of therapy as, in the majority of cases, they are only locally invasive and gross metastatic disease is uncommon. Our preliminary studies on the direct injection of Mabs into tumours have centred on patients with grade III/IV malignant glioma. For this patient group, the outlook remains bleak, with an average median survival of 45 weeks (range 22–61) [10]. Two groups of patients have been studied, those following resection of recurrent disease and those with cystic gliomas. Unlike the treatment of leptomeningeal disease, this approach allows the estimation of radiation doses to the tumour as well as to normal tissues such as brain and bone marrow.

Patients have been selected for the study either after debulking surgery for a recurrent tumour or if they were failing initial therapy in the case of cystic tumours. All patients had received external beam radiotherapy as part of their initial treatment. Mabs were administered via an Ommaya reservoir inserted into either the resection cavity or the cystic component of the tumour. Prior to therapy, patients were placed on steroid and anticonvulsant cover for 3 days prior to and 3 weeks after administration of radiolabel. For the same time period they also received a thyroid blocking regimen.

RESULTS OF THERAPY

6 patients received 777–2280 MBq of ^{131}I conjugated to a Mab recognising the human neural cell adhesion molecule (NCAM). 3 suffered no acute side-effects, 2 with cystic lesions and 1 with recurrent disease. 1 patient with a cystic lesion had a minor focal fit during Mab administration that did not require treatment. The other 2 with recurrent disease developed problems due to raised intracranial pressure. In 1 patient, this occurred 3 weeks from therapy after cessation of steroid cover. He responded well to repeated steroid therapy. The other individual already receiving a maximum dose of steroids required intravenous mannitol and hydrocortisone to control his symptoms, followed by debulking of necrotic tumour 3 weeks from therapy. In contrast to the patients receiving intrathecally injected Mabs, no bone marrow toxicity was observed.

The number of patients entered into this study, along with their relatively short follow-up, makes any conclusions regarding the efficacy of treatment difficult. However, in the case of patients with cystic lesions, a reduction in the frequency of cyst aspiration was observed. 2 patients who required weekly aspiration prior to Mab therapy remained stable for 6 and 8 months, respectively. The third individual has CT evidence of reduced intracranial pressure and is currently stable 3 months from therapy.

Our interest in this approach to targeting radiation therapy lies in the favourable pharmacokinetics observed in the patients to date. Blood peak activities have varied from 1.4–3.4% of the injected dose, approximately 10-fold less than those seen in individuals receiving intrathecally administered conjugates. Tumour clearance curves have been variable with estimated biological half-lives varying from 2.9 to 29 days (mean 11, median 8.2).

Based on this data, dosimetric calculations have been made using a modification of the MIRD scheme for the various radiation components [11]. Doses to brain and whole body were taken as the sum of β and γ activity from circulating blood plus a contribution from γ-radiation from isotope contained within the tumour. In the case of red bone marrow, an additional component (due to its high vascularity) from activity in blood within the marrow itself was added. The largest part of the tumour dose is due to β-particles from the radioisotope contained within the tumour, along with a small γ-element from the same source.

The low blood peak activity results in small whole body and red bone marrow doses. These varied from between 0.08 and 0.32 Gy (mean and median 0.21 Gy) and between 0.05 and 0.18 Gy (mean 0.09 Gy, median 0.065 Gy) respectively. These values correspond to an average of 0.14 Gy per Bq administered for whole body and 58×10^{-3} Gy per Bq for red bone marrow. In addition to residence time, tumour doses depend on the degree

of binding of the radioimmunoconjugate to either the cavity or cyst wall. Bearing in mind that the maximal β-particle range for ^{131}I is 0.9 mm and the dimensions of the cysts/cavities in these patients, any unbound isotope contributes little to tumour dose. Due to the difficulties in directly measuring antibody binding, tumour doses have been calculated as a range, the lower and upper limits representing 0% and 100% binding, respectively. These were high, ranging between 63 and 500 Gy (mean 226, median 128 Gy) for 0% binding and between 612 and 4630 Gy (mean 2125, median 1750 Gy) for 100% binding. These have been calculated assuming no parenchymal diffusion and are, therefore, delivered to a shell of cells approximately 1 mm thick around the tumour cavity/cyst.

The above data indicates that, for the first time in our studies, bone marrow toxicity will almost certainly not be dose limiting. At the current level of conjugate administration, we are also well below doses that would be expected to be toxic to normal brain (mean 6.4 range 4.7–7.9 Gy), although patients have already received relatively high doses of external beam radiotherapy. Due to the long residence time of the radiolabelled Mab in the tumour, it is not practical to increase markedly the dose of conjugate as patients would have to remain in isolation rooms for an excessive time. We are, therefore, proposing to repeat Mab therapy at monthly intervals. Careful pharmacokinetic studies will be necessary to estimate if systemically generated anti-mouse Ig responses will enter the tumour site and effect radiolabelled Mab residence times.

CONCLUSIONS

Both studies on diffuse disease and direct injection of Mabs into the tumour have concentrated on the use of ^{131}I as the targeting agent. The use of this is a compromise as the β-particle emissions have been suggested to be too long to be optimal for targeting single cell disease and too short to damage solid tumour away from the site of Mab binding.

Whilst either short range β or α-emitters are theoretically better isotopes to target single cell disease within the CSF, difficulties exist in both linking them to Mabs as well as obtaining them with suitable radiochemical purity. In contrast, we are proposing to change to ^{90}Y for our intrathecal programme. This isotope is a pure β-emitter, with maximal path length of 5 mm and higher linear energy transfer than ^{131}I. As a consequence, one would rely less on the need for diffusion of the radiolabel to kill tumour cells invading the brain parenchyma. At the same time, patients would not have to be confined to isolation rooms.

Difficulties, however, remain to be overcome as we initially proposed using a macrocycle to hold the ^{90}Y in a stable configuration with Mab. Whilst this is ideal, the chemistry of linkage to the Mab is relatively complex and the linkers used between the Mab and the macrocycle have proved to be highly immunogenic. An alternative approach is to use ^{90}Y-labelled diethylenetriaminepentacetic acid (DTPA) conjugated Mabs. This type of linkage of isotope to the Mab is far less stable than when macrocycles have been used and unacceptable bone marrow toxicity has been reported. In our studies, this may not be a problem as peak blood levels of isotope are low. One further factor in deciding on choice of isotope is cost, ^{90}Y being markedly more expensive than ^{131}I.

It is our belief that, based on the above, Mab targeting has a role to play in the management of CNS malignancy. Whether this will ultimately be through the selected delivery of isotopes, drugs or toxins is currently unclear. What is perhaps more apparent is that targeting alone will not offer a single curative modality, but, like so many other approaches to therapy, it will find a niche when used in conjunction with conventional approaches.

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