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A Phase I/II Study of the Intralesional Injection of Ricin–Monoclonal Antibody Conjugates in Patients with Hepatic Metastases

J.R. Zalcberg, G. Pietersz, B. Toohey, J. Laird, R. Huggins, A.S. Zimet, O. Hennessy, A. McKenzie and I.F.C. McKenzie

A phase I/II study of the intralesional administration of ricin-labelled monoclonal antibodies was conducted in patients with hepatic metastases of gastrointestinal origin. The anti-carcinoembryonic antigen (CEA) antibody I-1 was conjugated to blocked ricin via a disulphide bridge. After a test dose of antibody, patients were injected with ricin–antibody conjugates under computed tomography (CT) guidance on two occasions 1 week apart. Patients with stable or responding disease would receive a third course. The dose of ricin relative to surface area was increased in a predefined manner in cohorts of 3 patients. A total of 27 patients with hepatic metastases were entered into this study. All patients had metastatic colorectal cancer (26 patients) or adenocarcinoma of unknown primary with elevated CEA levels (1 patient). The presence of malignancy was documented cytologically in 9 of 11 patients tested. Minor responses were seen in 7 patients. However, no major objective responses or changes in the growth rate of injected lesions were observed. Toxicity was generally mild, the most common being hepatic capsular pain 24–48 h after each injection. 6 patients experienced rigors. One patient had anaphylaxis. Human anti-mouse and anti-ricin antibody responses were observed. Although substantial amounts of ricin conjugated to monoclonal antibodies were delivered into single lesions, this therapeutic approach was unsuccessful. Future studies of ricin-labelled antibodies should incorporate the systemic administration of immunoconjugates.
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INTRODUCTION

MONOCLONAL ANTIBODIES have had a major impact on many aspects of cancer research and diagnosis. However, therapeutically, there are only a limited number of examples in which this technology has improved the results of conventional therapy. For the last decade, the effective use of monoclonal antibodies as carriers of drugs, [1, 2] isotopes [3] or toxins [4] has been the ultimate goal of numerous studies. This endpoint has been difficult to achieve and the potential difficulties that need to be overcome before this approach to therapy can be effectively exploited have been extensively discussed [5]. In particular, there has been considerable discussion over the relative tumour specificity of antibodies in comparison to normal tissues. However, in view of the fact that conventional drugs have no specific affinity for tumours, other than that afforded by the unique vascular supply of primary and secondary cancers, limited antibody-related tumour specificity may be able to provide some biological advantage.

A more important limitation of the use of monoclonal antibodies as carriers of various toxic agents may be that in most animal experiments and human trials, where antibody conjugates have been administered systemically, less than 0.1% of the injected dose of antibody actually reaches its target [6]. This finding may have important implications for normal tissue toxicity, although it is not clear that this figure differs markedly when compared to the localisation potential of standard cytotoxic drugs.

To overcome the potential limitation of reduced antibody localisation in tumour tissue, we initiated a study in animal models using a ricin-labelled antibody conjugate [7]. In view of

Correspondence to J.R. Zalcberg.
J.R. Zalcberg, J. Laird and A.S. Zimet are at the Medical Oncology Department, Heidelberg Repatriation Hospital, Heidelberg West, Victoria; G. Pietersz, B. Toohey and I.F.C. McKenzie are at the Austin Research Institute, Heidelberg, Victoria; R. Higgins is at the Department of Statistics, Latrobe University, Victoria; O. Hennessy and A. McKenzie are at the Department of Radiology, Heidelberg Repatriation Hospital, Victoria, Australia.
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the complete disappearance of tumour xenografts in this model, a clinical trial of intralesional therapy using a ricin-labelled monoclonal antibody was commenced. The aims of this study were to determine whether the local injection of such conjugates could inhibit the growth of liver metastases from colorectal cancer, and to determine the toxicity of ricin-labelled monoclonal antibodies administered in this manner.

We hypothesised that, compared to its systemic administration, there may be a greater cytotoxic effect if the entire ricin–antibody conjugate were to be injected directly into the tumour. Since we have found that whole ricin conjugates were more active than immunoconjugates involving the ricin alpha chain [8], and that blocked ricin substantially reduced non-specific toxicity without altering cytotoxic activity, this novel compound was used in this phase I/II study.

PATIENTS AND METHODS

Patients

To be eligible for this study, patients were required to have histologically-proven adenocarcinoma arising in the gastrointestinal tract with metastases to the liver, measurable by computed tomography (CT) scan, nuclear scintigraphy or ultrasound. Patients were required to have an estimated life expectancy of more than 6 weeks and an ECOG performance status of 2 or less. Normal haematological values, renal function, clotting profile and bilirubin were required, although normal liver enzyme levels (alkaline phosphatase and gamma glutamyl transferase) were not required.

Exclusion criteria included pregnancy and acute complications of disease, such as bleeding or infection (patients were eligible when these had resolved). A minimum of 4 weeks should have elapsed since the last course of chemotherapy (6 weeks in the case of mitomycin C or nitrosoureas). Patients were only entered into this study after detailed discussion allowing written, informed consent.

Intralesional therapy

Eligible patients received a test dose (0.05 μg) of antibody 1 h prior to intralesional therapy. Test doses were administered after patients received phenergan 25 mg intravenously (i.v.) and dexamethasone 10 mg i.v. If no local or system reactions were observed, patients were imaged by CT scan, the index hepatic lesion identified under CT guidance and a fine needle introduced. CT was used to confirm that the needle was properly sited and, after aspirating the tumour (not done in all cases), the ricin-labelled monoclonal antibody was injected whilst moving the needle within the area of the tumour.

Following therapy, the patients were closely monitored as in-patients for 24–48 h and then discharged. Blood samples were taken before and after each injection of ricin conjugate for liver function tests, ricin kinetics, human anti-mouse (HAMA) and human anti-ricin (HARA) antibodies. In patients with multiple hepatic metastases, a single lesion was identified as the index lesion in each case. This lesion was the only tumour deposit injected. For the purposes of measuring tumour response (defined using standard WHO criteria), a second untreated lesion was identified as the control.

Treatment plan

Patients were injected on two occasions, 1 week apart. The liver was imaged approximately 1 month following the two injections, and in those patients with stable disease, a third, and on one occasion a fourth injection was administered. Patients were regularly followed up and repeat CT scans performed at 8- and 12-weekly intervals.

Table 1 relates to the amount of ricin in the conjugate. Doses were escalated after a minimum of 3 patients were treated at each dose level.

At the time this trial was started, there was little published or anecdotal experience of administering native ricin conjugated to monoclonal antibodies to patients. Thus, a cautious dose escalation program was used in this trial. The recommended dose of native ricin for phase II studies is 27 $\mu g/m^2$ [9].

Antibody

The anti-carcinoembryonic antigen (CEA) antibody I-1 was used in this trial [10]. The antibody was conjugated to blocked ricin via disulphide bridges as described previously [8], and filtered through a 0.22-μ filter. Later in the trial, the antibody was also passed through an endotoxin column. Different batches were used throughout the study. The specificity of this particular antibody has been previously documented [10]; in that study, 34/50 colonic tumours reacted with the antibody.

Human anti-mouse, human anti-ricin antibodies and ricin kinetic studies

Blood samples were obtained from patients before and at various time points after the injection of ricin–antibody conjugates. Serum was used in standard antibody and pharmacokinetics assays based on enzyme immunoassay techniques developed in our laboratory [11].

Statistics

To analyse response categories for each tumour, the proportional increase in the area of each tumour at time t compared to the initial area of the same tumour was computed and analysed using simple linear regression analysis. The differences between the proportional increases at each time point for each individual were computed and the growth in index (treated) tumour deposits compared to the growth of control (untreated) lesions. Control and index lesions were not necessarily the same size.

RESULTS

A total of 27 patients were entered into this study. Patient demographics are illustrated in Table 2. All patients had liver metastases secondary to colorectal cancer or adenocarcinoma of unknown primary where, because of significant elevation in CEA levels, the primary was presumed to be of gastro-intestinal origin. Accurate localisation of the fine needle (Figure 1) was confirmed in every case. Following injection, the distribution of the conjugate within the index lesion was confirmed in 1

Table 1. Ricin dose

Ricin dose ($\mu g/m^2$)	Number of patients
2	3
4	3
6	3
10	3
15	3
25	3
28	3
35	3

Table 2. Patient demographics

	No. of patients
Total number of patients	27
Male/female ratio	23/4
Median age (years)	65
Range	42–77
Tumours types	
Colon cancer	12
Rectal cancer	8
Gastric cancer	4
Adenocarcinoma of unknown primary	3
Prior chemotherapy	12

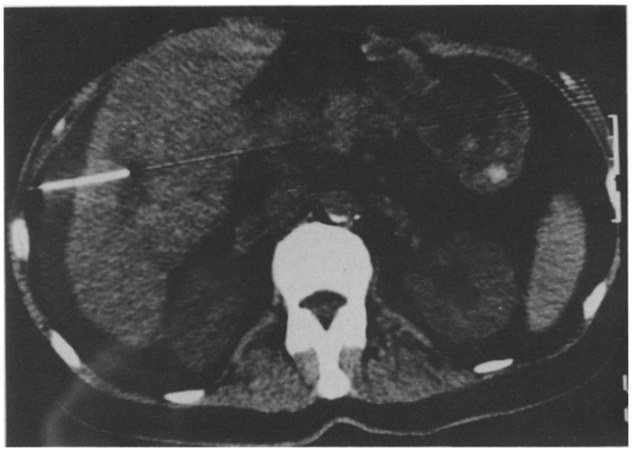

Figure 1. Computed tomography scan of the liver illustrating accurate needle localisation in the index lesion.

patient in whom radio-opaque contrast was added to the syringe containing the conjugate (Figure 2).

In 11 patients, cytological material was obtained for analysis, and in 9 of these cases the presence of malignant cells was confirmed (data not shown). This practice was only commenced late in the trial, but in every case, the clinical details and imaging parameters were entirely consistent with liver metastases, such that all patients were considered to have metastatic disease

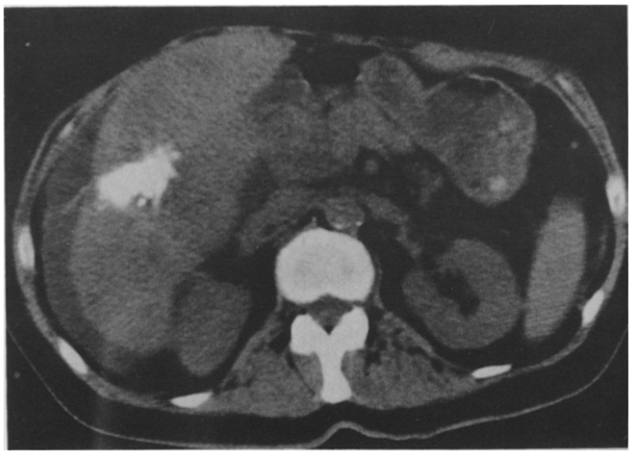

Figure 2. Localisation of the conjugate in the target lesion was demonstrated by adding a small volume of radio-opaque contrast to the injected material.

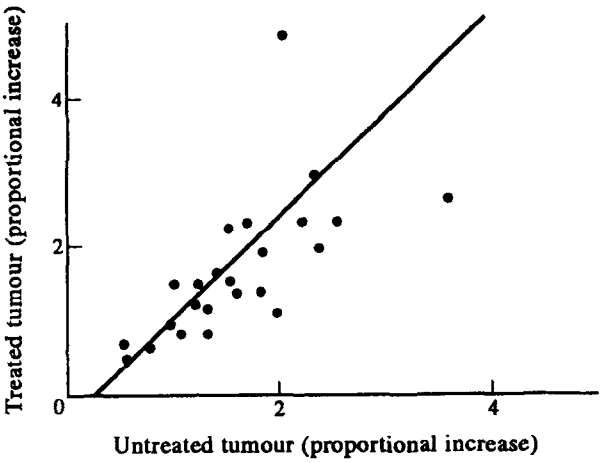

Figure 3. Relationship between the proportional growth of the index (injected) lesion and a control (untreated) tumour deposit.

independent of their involvement in this study. Sampling errors were thought to account for the negative cytology in 2 patients. In some of these 9 patients, sufficient material was obtained to confirm the binding of the monoclonal antibody I-1 to tumour cells in these patients.

Minor responses were observed in 7 patients. These included improvements in liver pain and/or falls in CEA levels and/or improvements in liver function tests. However, no significant decrease in tumour size (greater than 50%) was observed in any patient. This conclusion was confirmed by retrospectively reviewing all CT scans with a single radiologist who identified a control lesion in addition to the known index lesion in the pretreatment scans. The proportional increase in the size of the index lesion was plotted against control lesions (Figure 3) measured over the entire time period that patients were followed (generally until death). No difference was observed in the growth rate of the injected (index) lesion and control tumours (Figure 3).

Toxicity was generally mild (Table 3), the most common problem being hepatic capsular pain for the first 24–48 h after each injection. In 2 cases, this was so severe that the patients refused to have further treatment. 6 patients experienced rigors within the first 24 h following treatment. They were treated with paracetamol. Subsequently, all batches of the conjugate were passed through an endotoxin column (see Patients and Methods), and no further such episodes were noted. One patient had

Table 3. Toxicity

	Number of patients	WHO grade
Nausea	8	1
	3	2
Vomiting	0	
Anaphylaxis	1	4
Pain	10	1
	2	2
Fever	0	
Rigors	6	1–2
Diarrhoea	1	1

Worst grade of toxicity (based on World Health Organization, WHO criteria).

significant anaphylaxis occurring minutes after her fourth injection. This responded to intravenous decadron and adrenaline was not required. No other patient received more than three injections.

Human anti-mouse (HAMA) and anti-ricin antibodies (HARA) were tested in 8 patients. In 3 cases, both HAMA and HARA antibodies were detected as illustrated for a particular patient in Figure 4. HAMA responses seemed to be delayed in their appearance whilst HARA responses tended to diminish with time (data not shown).

DISCUSSION

This phase I/II study was established to determine whether the intralesional delivery of ricin-monoclonal antibody conjugates to patients with metastatic liver disease would lead to a tumour response. Although the immediate application of this approach to routine medical practice would probably be limited to the treatment of isolated lesions, such as brain or pancreatic tumours that are commonly incurable by surgery or radiotherapy, the study was designed to test whether the apparent lack of clinical effectiveness of immunoconjugates in solid tumours was their relatively poor localisation in target tissues. The intralesional injection of immunoconjugates provided an opportunity to overcome this limitation. That the same limitation probably also exists for standard cytotoxic drugs is now more generally appreciated. However, we had observed substantial tumour shrinkage in animal experiments using the intralesional approach [7] and felt that formal clinical testing of this alternative delivery mechanism was required.

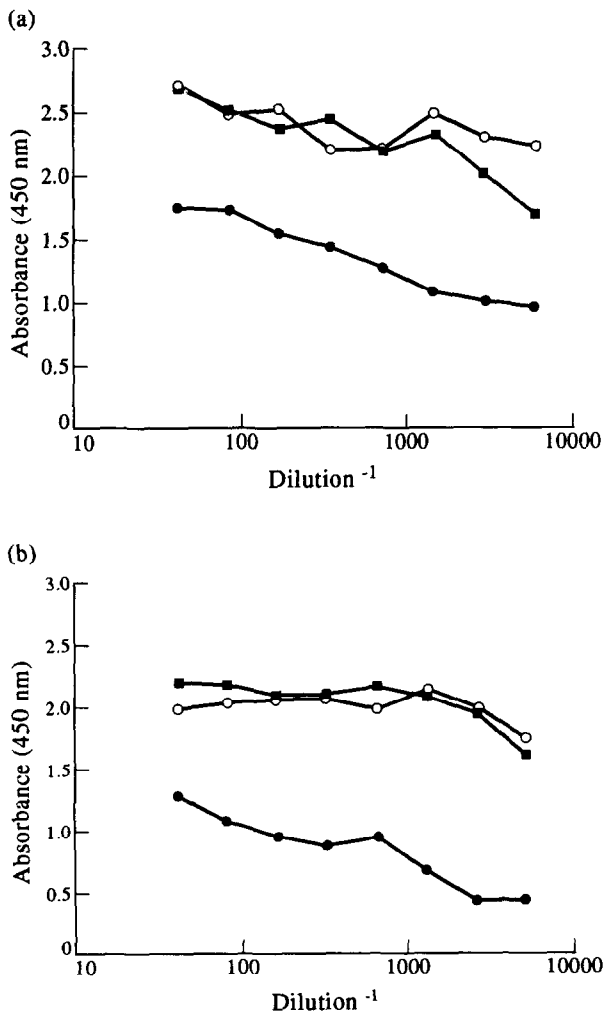

Figure 4. Human anti-mouse (a) and human anti-ricin (b) antibodies in 1 patient. In each case, serum from this patient (○ — ○) at a particular time point was compared to positive (■ — ■) and negative (● — ●) controls.

No significant tumour responses were observed in this study (Figure 3). Although the data have been represented as the proportional increase in the size of index lesions compared to controls within the 1 patient, graphs of actual tumour measurements or changes in tumour size with time against changes in liver function tests all failed to confirm significant anti-tumour activity (data not shown). Whilst the change in tumour size relative to controls may mask anti-tumour activity, if the conjugates were able to stimulate a generalised immune response affecting both lesions, the data overall do not suggest that significant tumour responses occurred.

As with prior studies of immunoconjugates, the explanations for the failure of such therapy are likely to be multifactorial. However, this study indicates that the failure of immunoconjugates to localise within target lesions is unlikely to be the explanation. In comparing the lack of effectiveness of this approach to the animal experiments [7], one possible explanation relates to the larger tumour volumes in the clinical situation relative to the dose of conjugate injected. A second explanation may relate to the fact that CEA, the target antigen, was not internalised. Coupled with the fact that relatively high levels of CEA in the external milieu of the cell may have "neutralised" the immunoconjugates, this limitation may have interfered with the efficacy of the conjugates. Finally, tumour heterogeneity may have interfered with the adequate binding of antibodies to tumour cells.

The generally accepted criteria for discontinuing phase I/II studies is the development of dose-limiting toxicity (DLT) or substantial anti-tumour effects. In this case, the trial was stopped prematurely without having demonstrated either of these effects. This decision was based on two grounds. Firstly, the manufacture of sufficient quantities of the conjugate proved difficult in the setting of a research laboratory. The manufacturing process not only required large amounts of antibody, which had to be conjugated and the end product prepared for use in humans, but it was difficult to prepare this reagent in volumes small enough to inject into patients (1–2 ml; larger volumes were difficult to inject into tumours).

Secondly, from the clinician's viewpoint, the failure to demonstrate any initial benefits made patient accrual difficult, as we were only able to inject a single lesion despite the fact that most patients had much more widespread disease. This created an ethical dilemma that seriously limited patient accrual.

This study is the first clinical report of the intralesional administration of an immunoconjugate. However, intralesional therapy with cytokines or other cellular toxins has some distinct advantages and has been used successfully in patients with hepatoma [12], squamous cell carcinoma [13] and Kaposi's sarcoma [14]. Whilst the results of this study were negative, we have demonstrated that native ricin conjugated to monoclonal antibodies can be relatively safely administered to patients with advanced cancer, particularly if care is taken to avoid acute allergic reactions. Locoregional therapy with immunotoxins will continue to be pursued by our group, although the design of such studies has been substantially improved by this experience.

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Predictors of Residual Mass Histology Following Chemotherapy for Metastatic Non-seminomatous Testicular Cancer: a Quantitative Overview of 996 Resections

E.W. Steyerberg, H.J. Keizer, G. Stoter and J.D.F. Habbema

Following chemotherapy for metastatic non-seminomatous testicular cancer, surgical resection may demonstrate that residual masses contain purely benign tissue (necrosis), or potentially malignant tissues (histologically viable cancer cells or mature teratoma). The morbidity, mortality and costs of resection demand that resection is based on empirical data rather than on subjective judgements. We reviewed 996 resections from 19 studies to quantify predictors of the histology at resection. Predictors were analysed for each study and combined in a pooled odds ratio (OR). Predictors of necrosis were: (1) a teratoma-negative primary tumour (OR = 5.1); (2) normal tumour markers before chemotherapy [α-fetoprotein (AFP): OR = 2.8; human chorionic gonadotrophin (HCG): OR = 1.9; both AFP and HCG: OR = 5.7]; (3) a smaller postchemotherapy abdominal mass (e.g. ≤20 mm: OR = 3.7); (4) a large shrinkage (≥90%: OR = 3.1); (5) lung resections versus abdominal resections (OR = 1.7). Cancer was found in only 4% of residual retroperitoneal masses ≤20 mm. Further research may combine the primary tumour histology, marker level and mass size to improve clinical guidelines, which define subgroups of patients for whom the benefits of resection do not outweigh the risks.

Keywords: histology, meta-analysis, resection, residual mass, testicular cancer
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INTRODUCTION

SURGICAL RESECTION is widely accepted as the treatment of choice in the presence of residual masses following chemotherapy for metastatic testicular non-seminomatous germ cell tumours (NSGCT) [1, 2]. Resection provides the histological diagnosis of the residual mass, which may be purely benign with necrotic and/or fibrotic remnants only (necrosis), may contain mature teratoma elements (mature teratoma) or viable cancer cells/active malignancy (cancer). Resection of masses containing necrosis only is assumed to have no therapeutic benefit and is usually not followed by additional treatment. Resection of mature teratoma or cancer is considered to be beneficial as it prevents growth of (potentially) malignant cells [3]. Finally, the presence of viable cancer cells in the residual mass directs the decision to administer additional chemotherapy [4]. The prognosis after resection is generally favourable, with 5-year relapse-free survival over 85%