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Biodistribution and Tumour Localization of Radiolabelled Monoclonal Antibody During Continuous Infusion in Nude Mice with Human Tumour Xenografts*

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Abstract—*The distribution in athymic nude mice of radiolabelled monoclonal antibody 791T/36 has been assessed during continuous infusion from subcutaneously implanted Alzet Osmotic Mini-pumps. During prolonged administration (up to 15 days) blood levels continued to rise. At 15 days, distribution of radiolabel was virtually identical to that seen after a single parenteral dose. Blood levels were in good agreement to those expected from whole body levels indicating satisfactory entry of antibody into the vascular compartment. Gel filtration chromatography of osmotic mini-pump contents and circulating radiolabel showed that the antibody had retained its structural integrity. In mice with human tumour xenografts examined after a 5-day infusion of a mixture of ^{131}I-791T/36 antibody and ^{125}I-control IgG2b, blood levels of both radiolabels were comparable to those expected from whole body levels and there was effective tumour localization of the antibody to 2.5 times that of control IgG.*

These studies have demonstrated that prolonged administration of monoclonal antibody is feasible, that antibody enters the vascular compartment satisfactorily and that it can then localize in tumour deposits.

INTRODUCTION

MONOCLONAL antibodies directed against human tumour-associated antigens are being evaluated as therapeutic agents, either as unmodified antibody [1] or conjugated to cytotoxic agents or toxins [2–10]. Intermittent parenteral administration has frequently been used, but obviously improved therapeutic responses might be seen if antibodies or conjugates were administered continuously to give stable or increasing levels of available material. Mini-pumps, working osmotically, are available for continuous infusion of materials into small rodents and have been extensively used to administer conventional drugs, hormones etc. in experimental studies but have not been frequently used for administration of antibodies, particularly monoclonal antibody against human tumour-associated antigens. In the present study the use of these mini-pumps to continuously deliver monoclonal antibody in mice has been examined. This has involved measurement of circulating and tumour levels of antibody and characterization of circulating radiolabel in mice with appropriate human tumour xenografts.

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MATERIALS AND METHODS

Antibody and control IgG2b

Monoclonal antibody 791T/36 (mouse IgG2b) was purified from hybridoma ascitic fluid and control IgG2b from normal mouse serum as previously described [11]. They were radiolabelled with ^{131}I and ^{125}I respectively (specific activity approx. 30 MBq/mg) using an iodogen technique [11]. Preparations were labelled and diluted in sterile phosphate-buffered saline pH 7.2 (PBS).

Osmotic mini-pumps

Osmotic mini-pumps (Alzet Mini-Osmotic Pump, Model 2002 Alzet Research, Palo Alta, California, U.S.A.) were obtained from Scientific Marketing Associates, London. The overall dimensions of these pumps when assembled were 3.0 × 0.7 cm. They were filled asceptically accord-

ing to manufacturer's instructions with ^{131}I-antibody or a mixture of ^{131}I-antibody and ^{125}I-control IgG2b and implanted subcutaneously into untreated or tumour xenograft-bearing nude mice. In tumour-bearing mice the mini-pumps were implanted contralaterally to the tumours. The batches of pumps used had nominal pump rates of 0.465 μl/hr and 0.480 μl/hr with mean filling volumes for both 238 μl. Thus these pumps would be expected to deliver their contents over a period of approx. 20 days.

Athymic nude mice and tumour xenografts

Athymic nude mice were obtained from Olac U.K. Ltd (Oxon, U.K.). For distribution studies in xenografted mice, animals had established subcutaneous growths of human osteogenic sarcoma 791T [11].

Determination of antibody blood half life and intravascular space of 791T/36 antibody

Mice were weighed and immediately injected intravenously under ether anaesthesia with ^{131}I-labelled 791T/36 antibody (350KBq in 0.2 ml saline). Blood samples were taken from the tail vein into 10 μl microcapillary pipettes (Drummond Microcaps, Drummond, U.S.A.) after 0.1, 0.25, 0.5 and 1 hr and then at intervals up to 72 hr after injection to a total of 10 collections/mouse. Blood samples were counted for ^{131}I and counts plotted against time in a semi-logarithmic plot.

The clearance curves consisted of 2 exponential components. The data in both the first (α) and second (β) components were extrapolated using the method of Sterling [12] to give respectively (i) the count rate in the blood at time zero if no equilibration had taken place from which the intravascular blood volume was calculated from the known initially injected count rate, (ii) the hypothetical blood count rate at time zero after equilibration with the extravascular compartment. From this and the extrapolated data from the α phase relative volumes of the intra- and extravascular fluid spaces could be calculated.

Determination of whole body half times of antibody

Groups of mice were injected subcutaneously and intravenously with 0.2 ml ^{131}I labelled 791T/36 antibody containing about 10^5 cpm ^{131}I. Radioactivity in mice was counted immediately and then at daily intervals in a 7.5 × 7.5 cm well gamma scintillation crystal detector (John Caunt Scientific, Oxford, U.K.). Count rates were corrected for physical decay and background activity and expressed as a percentage of the initial count.

Determination of blood and tissue levels of antibody

To determine long-term blood levels of 791T/36 antibody 2 mice received implantation of osmotic pumps containing ^{131}I labelled antibody. Blood samples were taken periodically over a 15-day interval. Mice were then killed and dissected and organs weighed and counted for radioactivity. Serum was prepared from the blood at termination and examined by Sephacryl S300 gel filtration. In addition, remaining contents of mini-pumps were washed out with PBS, added to normal mouse serum and similarly chromatographed.

To determine tumour localization of 791T/36 antibody, 3 mice with 791T xenografts were implanted with pumps containing a mixture of ^{131}I-791T/36 and ^{125}I-control IgG2b (100 μg/ml of each). They were killed after 5 days, dissected and radioactivity counted in weighed samples of blood, tumour, organs and residual carcass.

Tissue levels of radiolabels were expressed as tissue : blood ratio

$$\frac{\text{count rate/g tissue}}{\text{count rate/g blood}}$$

Gel filtration

Gel filtration chromatography of serum containing radiolabelled preparations was carried out on Sephacryl S300 (column dimensions 1.5 × 90 cm; flow rate 15 ml/hr elution in PBS). Absorption of UV light at 280 nm was monitored continuously and 1 ml fractions collected for radioactivity counts.

RESULTS

Blood half time and survival of 791T/36 antibody

Figure 1 shows blood kinetics of ^{131}I-791T/36 antibody after intravenous injection. The mean half time of the initial α phase was 7.8 hr, that of the β phase being 69.7 hr. Extrapolation of the α phase gave initial blood count rates/ml which were a mean of 11.2% of the body weight in g. Extrapolation of the β phase gave a mean estimated intravascular blood vol. of 42% of the total distribution space of the antibody.

Whole body survival and blood levels of antibody after bolus injection

Groups of 3 mice were injected intravenously or subcutaneously with ^{131}I-791T/36 antibody, and mice individually counted for ^{131}I immediately and at intervals over 8 days (Fig. 2). The mean half time of ^{131}I was 72.9 hr and 77.0 hr respectively for intravenous and subcutaneous injection. Three days after injection, 100 μl blood samples were taken from each mouse and counted for ^{131}I. The expected count rate in blood was calculated for each mouse from the total whole body count, a blood vol.

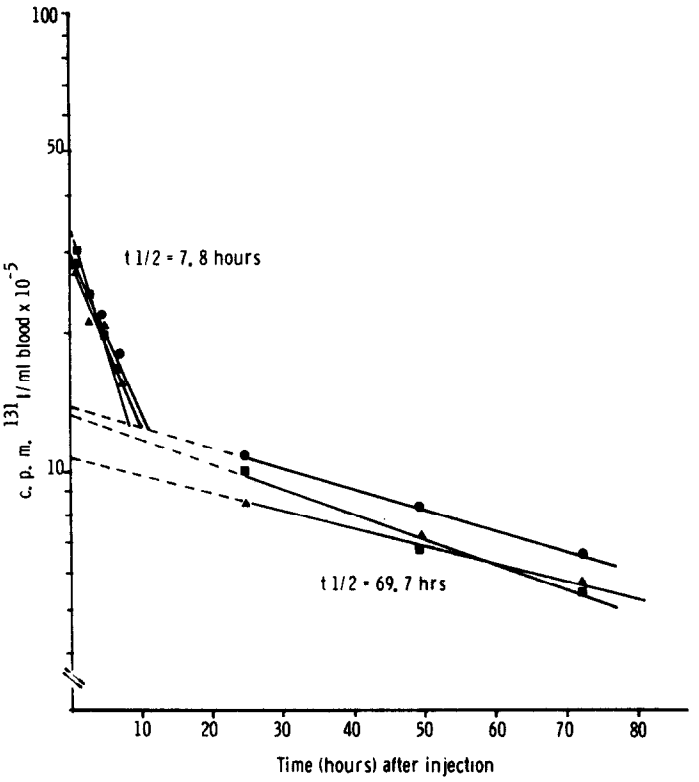

Fig. 1. Blood levels of ^{131}I-791*T*/36 *monoclonal antibody in mice following intravenous injection. Extrapolation of the* α *phase was used to calculate initial blood level of* ^{131}I *at time zero, from which total intravascular volume was calculated as* 11.2% *of body weight. Extrapolation of the* β *phase was used to calculate the proportion of antibody in the intravascular compartment as* 42%.

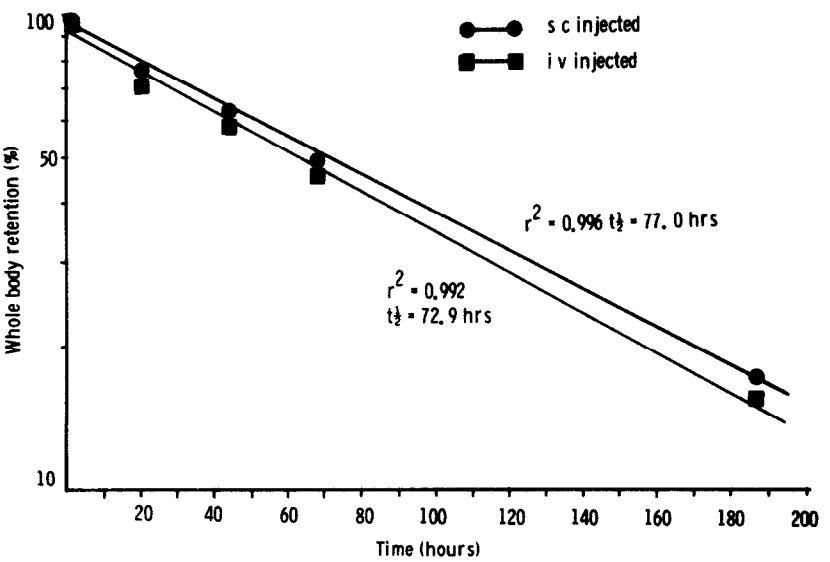

Fig. 2. Whole body survival of ^{131}I-791*T*/36 *monoclonal antibody following subcutaneous or intravenous injection. Mean of* 4 *mice/group.*

(ml) of 11.2% of the body weight (g) and relative intravascular compartment of 42%. The results (Table 1) showed good agreement between expected and observed ^{131}I count rate in blood after both intravenous injection and subcutaneous injection.

Blood levels of 791*T*/36 *antibody during continuous subcutaneous administration with mini-osmotic pumps*

Figure 3 shows mean blood level data from 2 mice implanted with mini-osmotic pumps containing ^{131}I-791T/36 antibody at 166 μg/ml. ^{131}I was detected in blood after one day, the earliest time of examination, and continued to rise over the 15 day observation period to a mean count rate equivalent to that of 630 ng/ml of antibody.

At 15 days these mice were killed and dissected. Figure 4 shows the biodistribution of the radiolabel. The lung had the highest amount of ^{131}I, the

Table 1. Blood levels of ^{131}I-791T/36 monoclonal antibody in mice after injection or continual infusion

Method of administration	Preparation	Time (days)*	Count rate (cpm/ml) in blood of: ^{131}I-791T/36 Observed†	Expected‡	O/E	^{125}I-IgG2b Observed	Expected	O/E
Intravenously	^{131}I-791T/36	3	2920	3490	1.19	–	–	–
			1613	1710	1.06	–	–	–
			3024	3620	1.19	–	–	–
Subcutaneously	^{131}I-791T/36	3	3063	3070	1.00	–	–	–
			3037	3740	1.23	–	–	–
			2868	3010	1.05	–	–	–
Mini-osmotic			74000	114000	1.54	–	–	–
pump	^{131}I-791T/36	15	81000	123000	1.51	–	–	–
Mini-osmotic	^{131}I-791T/36	5	5452	4058	0.74	18306	16382	0.89
pump	^{125}I-IgG2b		6344	6234	0.98	17651	17710	0.98
			7583	6834	0.90	21110	17788	0.84

*Time after injection or mini-osmotic pump implantation.
†Observed from counting aliquots of blood: values are shown for individual mice.
‡Expected from whole body count rate assuming 42% of antibody to be in the intravascular compartment and a blood vol. (ml) of 11.2% of the body weight (g).

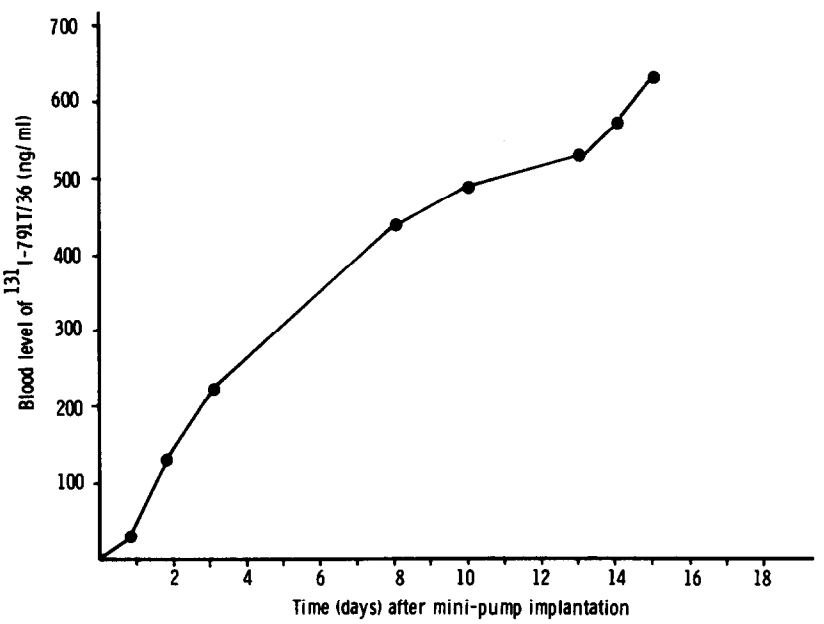

Fig. 3. Blood levels of ^{131}I-791T/36 antibody during continuous infusion from subcutaneously implanted mini-pumps. Mean of 2 mice. Blood level is shown as antibody concentration calculated from ^{131}I count rates and initital specific activity of the labelled material.

intestine the lowest. From the concentration of antibody in the pumps and the nominal pumping rate it was calculated that these 2 mice had both received a total of 27.5 μg of antibody over the 15 day period. Total count rates in dissected mice showed radiolabel equivalent to 2.89 and 3.04 μg of antibody corresponding to 10.5 and 11.5% survival of the radiolabel respectively. Taking the blood vol. (ml) as 11.2% of the body weight (g) and intravascular compartment as 42% of the total, if the antibody was distributed in these mice in the same manner as followed bolus injection, their blood would be expected to contain 0.37 and 0.41 μg of antibody/ml. Observed values from the ^{131}I count rate in the blood (Table 1) were 0.56 and 0.62 μg/ml respectively.

Characteristic of circulating radiolabel

Serum from the mice which had had mini-osmotic pumps infusion of ^{131}I-791T/36 for 15 days was examined by gel filtration chromatography. A single peak of radioactivity was observed coincident with the second serum protein peak (Fig. 5A). Remaining pump contents, after washing out with PBS and

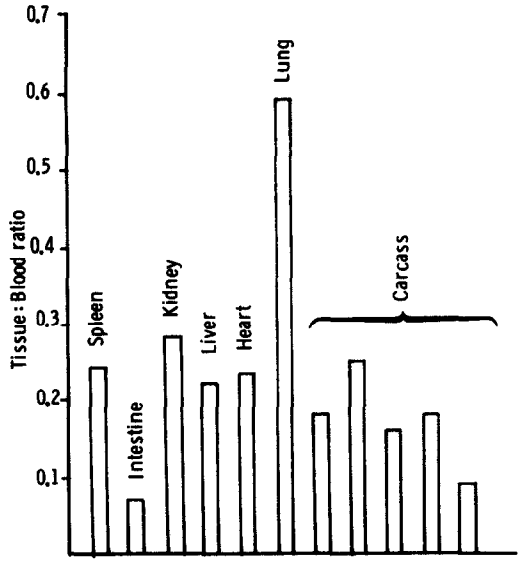

Fig. 4. Tissue distribution of ^{131}I-791*T*/36 *antibody after 15 days continuous infusion with mini-pumps. Mean of 2 mice.*

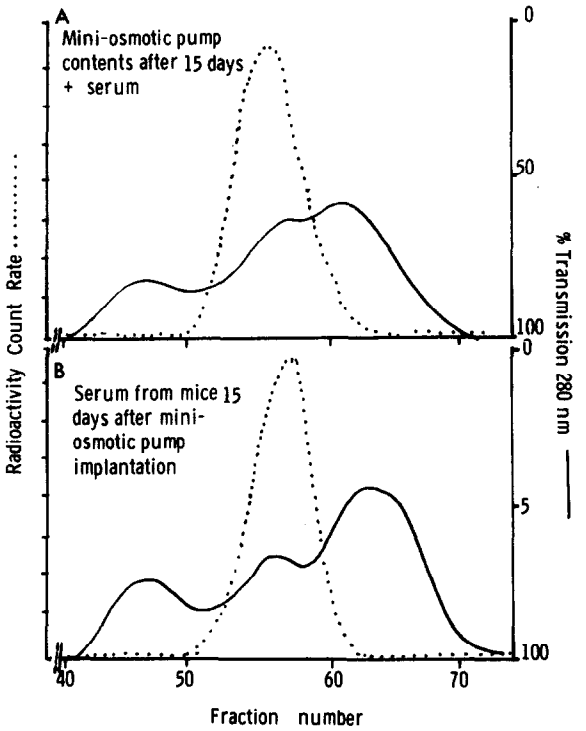

Fig. 5. Sephacryl S300 gel filtration profiles of ^{131}I-791*T*/36 *A. recovered from mini-pumps after 15 days subcutaneously in mice B. in serum of these mice.*

addition to normal mouse serum, showed a virtually identical profile (Fig. 5B).

Distribution of ^{131}I-791*T*/36 *and* ^{125}I *control IgG2b in xenograft-bearing mice*

Three mice with established 791T osteosarcoma xenografts were implanted subcutaneously with mini-osmotic pumps containing a mixture of ^{131}I-791T/36 and ^{125}I control IgG2b (100 μg/ml of each) and killed after 5 days. Distribution of the

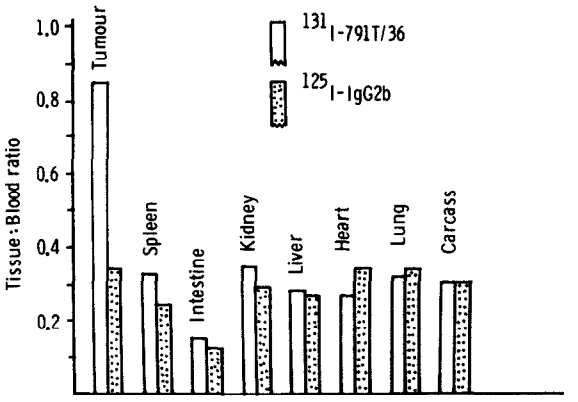

Fig. 6. Tissue distribution in 791T xenograft bearing mice of ^{131}I-791*T*/36 *and* ^{125}I *control IgG2b after 5-day continuous administration. Mean of 3 mice.*

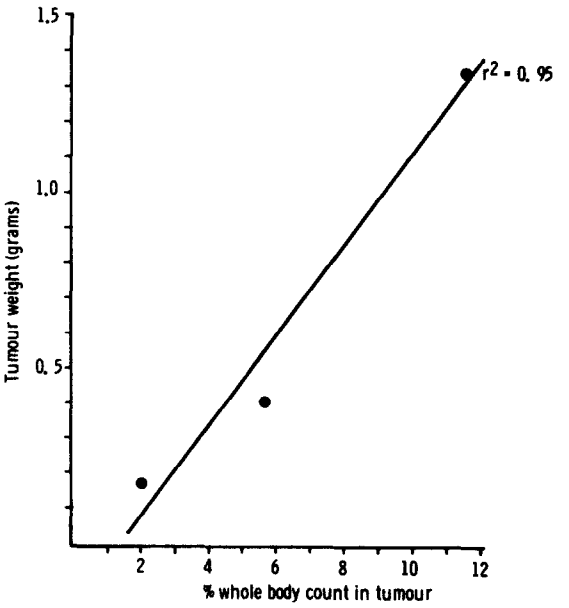

Fig. 7. Correlation between 791T xenograft tumour weights and the proportion of the whole body count of ^{131}I *localized in them after 5 days infusion.*

radiolabels is shown in Fig. 6. Tissue levels of ^{131}I and ^{125}I, expressed as a tissue : blood ratio, were similar in all normal organs, but in tumour ^{131}I levels were 2.5 times those of ^{125}I. When ^{131}I count in the tumours was normalized to the whole body count of radiolabel, it was evident that there was a correlation between uptake of radiolabel and tumour weight (Fig. 7).

From the antibody and control IgG content of the mini-osmotic pumps and the nominal pump rate it was calculated that the mice had received infusions over the 5-day period of 2.76 and 3.36 μg of ^{131}I-791T/36 and ^{125}I-control IgG2b respectively. Count rates in dissected organs and carcass showed means of 29.3 and 37.5% whole body retention of the 2 radiolabels. The count rate of ^{131}I in tumour tissue corresponded to 0.08, 0.12 and 0.14 μg of antibody/g of tumour in the 3 mice. The observed count rate of the 2 radiolabels in the blood

agreed closely with those expected from the total body count rates (Table 1).

DISCUSSION

The 791T/36 monoclonal antibody has previously been extensively investigated for localization in human tumour xenografts, and these studies have included a determination of the specificity, rate and intra-tumour site of deposition of the antibody [11, 13]. Therefore this antibody was chosen for the present study to evaluate the technical feasibility and tumour localization potential following continuous administration.

Initially an examination of the blood and whole body levels of radiolabel following simple intravenous injection was undertaken. After intravenous injection, the blood levels showed the typical biphasic decline. The first extravasation phase had a half time of 7.8 hr, the second, representing catabolism, a half time of about 70 hr. This part of the study was carried out to enable calculation of the blood volume in relation to weight (11.2%) and the intravascular proportion of the total distribution volume (42%).

Following a single subcutaneous injection the whole body half time of radiolabel (77 hr) was similar to that of the blood half time following intravenous administration. Observed blood levels of radiolabel 3 days after subcutaneous injection of antibody were in good agreement with those expected from the whole body count rate and distribution pattern of intravenously injected antibody. Thus these date show that subcutaneously injected antibody does enter the circulation and the circulating level in relation to whole body levels is virtually identical to that seen after the extravasation phase following intravenous injection. Thus the subcutaneous site should be suitable for a continuous administration of antibody.

During continuous infusion of ^{131}I labelled 791T/36 antibody, blood levels of radiolabel rose over the 15-day observation period. At 15 days, when the test was terminated, the level of circulating radiolabel was in good agreement with that expected from the whole body count, showing that during this prolonged administration antibody was present in the circulation in keeping with the intravascular pattern expected from a single subcutaneous dose. The pattern of distribution in other organs was virtually identical to that previously reported after a single intravenous injection of antibody [11, 13]. Gel filtration studies showed that antibody remaining in the mini-pumps and in the circulation was still as monomeric IgG, no aggregation or lower molecular weight degradation products were seen. This blood pattern is similar to that previously reported following a single administration of radiolabelled 791T/36 antibody [11, 13].

For mice with 791T xenografts and receiving ^{131}I-791T/36 and ^{125}I-IgG2b from osmotic minipumps, tumour levels of antibody were 2.5 times higher than control IgG after 5 days of administration. Again blood levels of radiolabels were comparable to those expected from the whole body level of radiolabels, indicating distribution after slow subcutaneous release to be similar to that after simple single injection. A full kinetic analysis of tumour levels of radiolabelled antibody during continuous infusion has not been attempted in the present study, and obviously from theoretical considerations alone this would be complex, depending on absolute rate of antibody infusion, rate of tumour localization and clearance of antibody and rate of blood and whole body clearance.

Although more prolonged and possibly higher absolute tumour levels of antibody should be achieved following continuous administration it does not follow, of course, that increased discrimination between tumour and blood and other organs will result since there is continuous infusion into, rather than decline from, the circulation. Thus in the present study, tumour levels of radiolabel, expressed as a proportion of the whole body count rate, averaged 9%/g (Fig. 7). Analysis of earlier data following a single injection show an increase over the first 4 days up to 50% of the whole body count/g of 791T tumour [11] but it should be emphasized that this was not due to an increase in absolute levels of antibody in tumour (at least after the first 2 days) but to a decline in whole body and blood levels of antibody which were faster than that in the tumour.

In the practical context it is unlikely that this technique would add to the visualization of tumours by external imaging after infusion of radiolabelled antibody since here tumour detection is improved by rapid clearance of blood and normal tissue radioactivity while following continuous infusion blood levels are continuously increasing. It is in therapy of tumours either by antibody alone or drug or toxin conjugates that this approach may yield enhanced effects. Continuous infusion of monoclonal antibody-based therapeutic agents has however, as yet, been little evaluated, although Herlyn *et al.* [1] have shown suppression of colorectal carcinoma xenografts with the 17-1A monoclonal administered in this way. One can envisage that this approach may be particularly suitable for antibody–drug conjugates with relatively short *in vivo* survival times since it may lead to more efficient maintenance of tumour levels of conjugates than could be achieved by frequently repeated injections. The 791T/36 antibody has already been conjugated to a number of drugs and toxins including methotrexate [5], daunomycin [4], vindesine [10] and ricin toxin A chain. *In vivo* anti-tumour effects have

been seen with some of these conjugates but it is apparent from biodistribution studies that a limitation to their therapeutic effectiveness is their *in vivo* survival [14] (Byers *et al.* unpublished; Pimm *et al.* unpublished) and the feasibility of improving these effects using continual infusion devices is currently being explored.

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