Eur J Cancer Clin Oncol, Vol. 23, No. 8, pp. 1225–1227, 1987.
Printed in Great Britain

0277–5379/87$3.00+0.00

Letter to the Editor

Demonstration of the Hepatic Uptake of Radiolabelled Immunotoxins Using Gamma Scintigraphy

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A RANGE of immunotoxins based on the combination of monoclonal antibodies and plant-derived toxins have recently been advocated as agents for the selective killing of tumours and metastatic deposits [1, 2].

We have recently monitored the biodistribution of an immunotoxin based on a conjugate of ricin toxin A chain and the monoclonal antibody 791T/36 by gamma scintigraphy. The 791T/36 antibody is known to recognize a 72,000 Dalton glycoprotein antigen associated with a range of human tumours [3].

The immunotoxin comprised immunoaffinity purified ricin toxin A chain (RTA) linked by a disulphide bridge to the intact monoclonal antibody 791T/36 (IgG2b). Radioiodination was conducted by reacting sulphydryl-protected RTA with [^{131}I]sodium iodide at 37–55 MBq/mg RTA using Iodogen at 0.1 mg/ml protein. After removing the excess unreacted iodide, the protecting group was removed by reacting the RTA with 50 mM dithiothreitol. The resulting [^{131}I]RTA-SH was then conjugated to monoclonal antibody 791T/36 using *N*-succinimidyl-2-(2-pyridyldithio)-propionate as previously described [3].

Nude mice (OLAC U.K. Ltd, Oxon, U.K.) were anaesthetized using ether and placed prone beneath an IGE Maxicamera II gamma camera fitted with a pin hole collimator. Each mouse was then injected i.v. with 0.4 MBq [^{131}I](RTA)-791T/36 conjugate and 60 × 60 s dynamic views were recorded by computer. Mice were periodically imaged up to 7 h after the injection of labelled immunotoxin. Additional nude mice were injected i.v. with 0.4 MBq [^{131}I](RTA) conjugated to normal immunoglobulin IgG2b and gamma camera views recorded as described previously.

Regions of interest (ROI) were drawn around the whole body, heart, liver and bladder as shown in Fig. 1. Time activity curves were created from the dynamic views recorded following injection of the conjugate. Count rates were obtained from the images of each mouse and expressed as a percentage of the whole body radioactivity.

The dynamic images recorded immediately after i.v. injection demonstrated activity circulating throughout the whole body, with increased concentrations within the heart and liver. In each case the heart activity was seen to clear rapidly within the first 5 min and the proportion of the administered dose within the liver rose to a maximum at between 10 and 15 minutes post injection. Typical time activity curves from ROIs defined over the heart, liver and urinary bladder are shown in Fig. 2. The proportion of the administered doses contained within ROIs defined over the thyroid, stomach and urinary bladder were subsequently observed to increase for up to 7 h post injection.

Quantification of the image data demonstrated

Accepted 9 April 1987.
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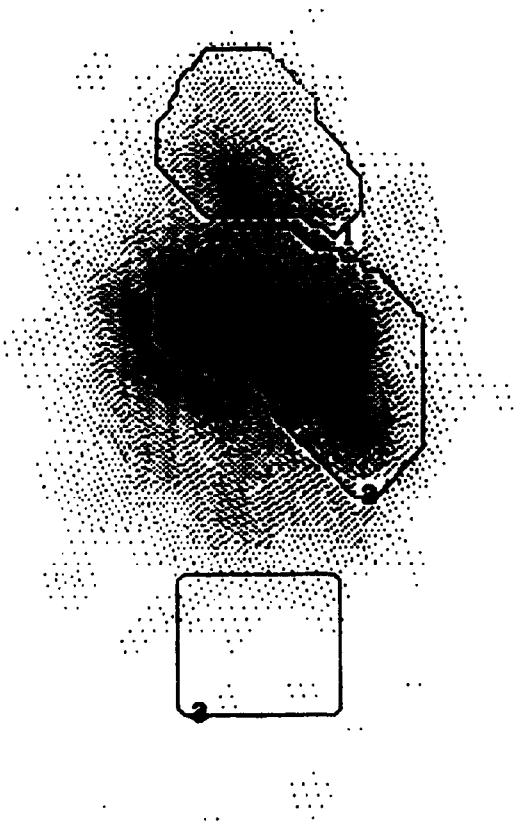

Fig. 1. Gamma camera image of a normal mouse recorded with 5 min *of injection with* [^{131}I](*RTA*)-791*T*/36 *immunotoxin. Radioactivity is visible mainly in the liver and heart. The ROIs were used to generate the time–activity curves in Fig.* 2.

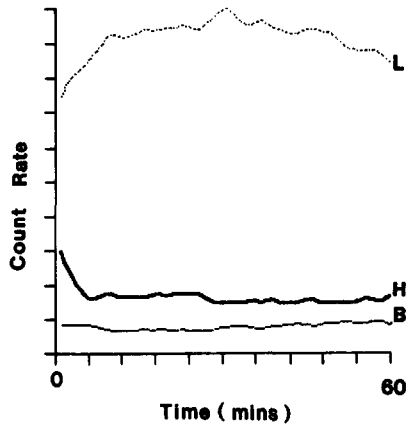

Fig. 2. Time–activity curves obtained by computer from the dynamic images. Radioactivity can be seen to clear rapidly from the heart H with rising levels in the liver L up to 30 min *after injection after which the level decreases.*

that whole body retention of radioactivity was generally greater than 50% up to 7 h post administration. Heart levels of activity were between 9 and 19% of the whole body radioactivity which reduced to less than 5% after 5 or 6 h. By far the greatest proportion of the administered dose accumulated in the liver (25–30%).

Levels of radioactivity measured from the images in the bladders of the mice injected with [^{131}I](RTA) conjugated to normal IgG were twice as high after 3 h as those measured from the images of the mice injected with the antibody conjugate. Otherwise no differences were observed between the distributions of the antibody and normal IgG linked immunotoxins.

In vitro studies have previously demonstrated that the monoclonal antibody 791T/36 is rapidly bound and internalized by tumour cells expressing the 791T/36 defined antigen and that 791T/36-RTA conjugates achieved cell toxicities of up to 5 orders of magnitude greater than those of antibody directly linked to conventional cytotoxic agents, thus comparing favourably with other RTA immunotoxins [3]. We consider experimental *in vivo* biodistribution studies an essential part of the strategy for the development of these immunoconjugates. In particular it is necessary to determine whether there is any significant *in vivo* clearance of the immunotoxins by the reticulo-endothelial system as previously described by Fodstad *et al.* [4]. The use of dynamic imaging with a gamma camera-computer system was considered the most appropriate method for monitoring the *in vivo* biodistribution of the 791T/36-RTA immunotoxin because the expected rapid pharmacokinetics of this material could not be conveniently monitored by dissection analysis following killing of the experimental animals.

The site of radiolabelling was considered an important aspect of the study as in this instance the distribution of the toxin moiety was required rather than the entire conjugate or the antibody alone. As the toxin A chain is a protein iodiation was possible prior to conjugation with the antibody. *In vitro* tests of radiolable stability showed that greater than 95% of radioactivity remained protein bound for a period of one month.

Rapid hepatic uptake of RTA–antibody conjugates would appear to be the main obstacle when administering immunoconjugates i.v. Clearly, further studies using gamma scintigraphy would be desirable to monitor the *in vivo* biodistribution of modified immunotoxins.

Acknowledgement—We would like to thank the Xoma Corporation, Berkeley, CA, for providing the radiolabelled immunotoxin.

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