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IMPROVING RADIOIMMONOTARGETING OF TUMORS

Variation in the amount of L6 MAb administered, combined with an immunoadsorption system (ECIA)

MICHAEL GARKAVIJ, JAN TENNVALL, SVEN-ERIK STRAND, KRISTINA NORRGREN, RUNE NILSSON, LARS LINDGREN and HANS OLOV SJÖGREN

Extracorporeal immunoadsorption (ECIA) is a new method for the selective removal of circulating radiolabeled monoclonal antibodies (MAb) from plasma to increase the uptake in tumor versus normal tissues (T/N-ratio). To ascertain whether the amount of MAb affects T/N ratios immediately and 24 h after ECIA, we used a rat model with two tumor sites—one intramuscular (im) and one below the subrenal capsule (SR). Extracorporeal immunoadsorption was done with an avidin-agarose column after injection of ^{125}I-labeled biotinylated L6 MAb. The animals received 10, 50 or 250 μg of L6 only (controls), or followed by ECIA. The efficacy of the procedure in removing plasma activity was 80–95%. For both tumor sites, the highest T/N-ratios were obtained with 10 μg L6. All T/N-ratios significantly improved for SR tumors by a factor ranging from 3.2 (lung) to 12.6 (bone marrow). The T/N-ratios were still elevated 24 h after ECIA. Injection of larger amounts of MAb, probably causing a higher degree of tumor saturation, will not necessarily improve the T/N ratio after ECIA.

Despite advances in experimental studies, the use of monoclonal antibodies (MAbs) in clinical practice still requires considerable improvement in the field of radioimmunotherapy. There have been only occasional clinical reports of the successful use of MAb targeting in tumor therapy (1). One of the most important parameters in radioimmunotherapy is the ratio of uptake of MAb in tumor tissue to that in normal tissue (T/N ratio). These ratios have usually been found to be too low for clinical purposes and several methods have been proposed and developed to increase target uptake or accelerate the excretion of unbound MAb. Examples of such methods are the application of secondary (anti-idiotypic) MAb (2), fragments of MAb (3), regional injections of radiolabeled antibodies (4) and the biotin and streptavidin system (5). In previous studies by our group, an increased tumor-to-normal tissue ratio was demonstrated after extracorporeal immunoadsorption (ECIA) in a theoretical compartment model (6), and later in animal models with anti-ovalbumin Ab (7), 96.5-anti-melanoma MAb (8) and L6-anti-pancarcinoma MAb (9). Tumor-to-normal tissue ratios were shown to increase 4- to 6-fold. Because promising therapeutic results have been obtained with L6 in patients with locally advanced breast carcinoma (10), we continued to investigate this MAb in an animal model, and analyzed the effect of different amounts of radiolabeled antibodies injected prior to ECIA.

The purpose of the present study was to ascertain whether the amount of radiolabeled L6 injected is a determinant of MAb biodistribution and target-to-nontarget ratios immediately after and 24 h after the start of (ECIA).

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From the Departments of Oncology (M. Garkavij, J. Tennvall), Radiation Physics (S.-E. Strand, K. Norrgren) and Tumor Immunology (R. Nilsson, L. Lindgren, H. O. Sjögren), Lund University, Lund, Sweden.
Correspondence to: Dr Michael Garkavij, Department of Oncology, University Hospital, S-221 85 Lund, Sweden.
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Material and Methods

Monoclonal antibody and radiolabeling

The monoclonal antibody was produced by Oncogen Corp. (Seattle, WA, USA) and kindly provided by I. and K.E. Hellström. It is an IgG 2a, murine anti-pancarcinoma MAb with an affinity constant of $4 \times 10^8\ M^{-1}$ (11). The MAb targets a non-shed cell surface antigen, which is highly expressed in lung, breast, ovarian and colon carcinomas (11). The molecular nature of L6 antigen has remained elusive (12). L6 was generated by immunizing mice with i.p. injections of lung adenocarcinoma cells. The L6 hybridoma was then injected into mice to produce ascites. An amount of 350 μg to 950 μg of L6 was labeled with 37 MBq ^{125}I (Amersham UK), using the chloramine-T method (13). Free iodine was separated from the MAb, in a Sephadex G25 column (PD10, Kabi Pharmacia, Sweden). The different Ab preparations had about the same specific radioactivity (30–50 KBq/μgL6). The radiolabeled MAbs were used within 3 days after labeling.

Biotinylation. The ^{125}I-labeled L6 was conjugated with biotin (N-hydroxysuccinimido-biotin, Sigma, USA) (14). The amount of biotin reagent per mg of L6 was optimized to gain maximal cell binding activity in vitro in combination with maximal avidin binding (data not presented). An amount of 31 μg of biotin reagent per mg of L6 MAb was found to be optimal.

Quality control. After radiolabeling and biotinylation, radioimmunoreactivity on H2981 tumor cells, as well as binding on avidin-agarose (Agarose-Avidin-D, Vector, Burlingame) were tested (8).

Animals and tumors. Male and female athymic rats (RNu/RNu) with a mean body weight of 230 ± 41 g were implanted intramuscularly (IM) and under the kidney capsule (SR) with 5×10^6 or 4×10^6 respectively of cultured tumor cells from the human lung adenocarcinoma cell line H2981, as previously described (9, 11). After 3 to 4 weeks, the xenografts reached a mean wieght of 0.6 ± 0.4 g. Tumors weighing less than 100 μg were excluded from the analysis. The animals were kept in cages with air filters and provided with standard food pellets and water ad libitum. The thyroid was not blocked for free iodine uptake. The study design was approved by the Swedish Ethics Committee for Animal Experimentation.

Experimental design and biodistribution studies. The set-up for the animal experiments is shown in Fig. 1. Nude rats were injected with 10, 50 or 250 μg of ^{125}I-labeled biotinylated L6 MAb by i.v. injection (v. femoralis) under ether anesthesia. Four to six rats were included in each group undergoing ECIA, as well as in the corresponding control groups. The animals were sacrificed and dissected either immediately or 24 h after the start of ECIA. The controls were sacrificed and dissected 0.5 or 6 h, 24 h, 48 h, 72 h and 96 h (for 50 μg L6 only) after i.v. injection of L6.

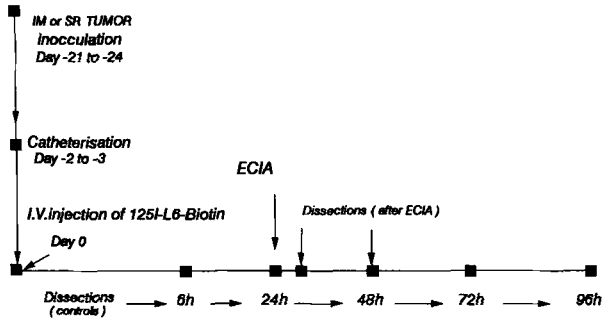

Fig. 1. Set-up of the animal experiments.

During the dissections several organs (right kidney, liver, lungs, bone marrow) and both the intramuscular (IM) and the subrenal capsule tumors (SR) in the left kidney were removed, weighed and measured for activity in an automatic well NaI counter (LKB, Sweden). Activity was expressed as percent of the injected dose per gram tissue (%/g) and was corrected for ^{125}I decay. Whole body retention was studied at 24-h intervals with a scintillation camera (GE400T, USA) equipped with a low energy parallel hole collimator. Four tumors were investigated histologically, using standard staining (hematoxylin-eosin) procedures to assess tumor morphology, i.e. the extent of cellular and stromal tumor components.

ECIA procedure. Before ECIA was done, the animals underwent arterial (a. carotis communis) and venous (v. jugularis) catheterization as previously described (7). After the catheters were connected to the ECIA system, blood was pumped through a plasma filter and then the separated plasma passed through the adsorption column with avidin-agarose (8). Approximately 3 plasma volumes were passed through the column during the 3-h adsorption procedure.

Statistical analysis. All the results are reported as means ± 1 SD. Two-sample analysis software (Statgraphics, STSC Inc., USA), which calculated sample statistics and a t-test assuming equal and unequal variances, were used to estimate the difference between the two means. Probabilities of more than 95% ($p < 0.05$) were considered significant.

Results

The efficacy of ^{125}I-labeling of L6 MAb ranged from 75% to 90%. Radioimmunoreactivity assay using H2981 tumor cells confirmed binding to the target cells exceeding 60% of the radiolabelled and biotinylated MAb. Binding of the biotinylated and radiolabelled L6 to avidin-agarose was always greater than 75%. As an indirect indication of free iodine in plasma, the activity uptake in the thyroid was estimated. The level of free iodine was neither correlated with ECIA nor with the amount of antibody administered.

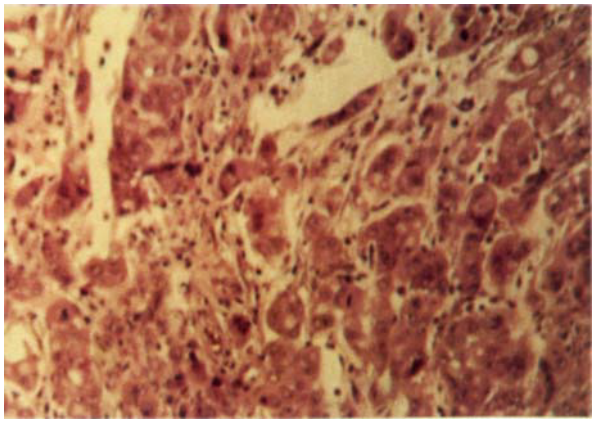

Fig. 2. Representative photomicroscopy (× 120, hematoxylin/eosin staining) of heterotransplanted subrenal capsule tumor. The tumor consisted predominantly of cellular elements with large proportions of mitotic cells. Vascularity of the tumor was sparse.

Both the IM and SR tumors consisted predominantly of cellular elements with large proportions of mitotic cells (Fig. 2).

Fig. 3 shows the whole body (WB) retention curves for animals that received 10, 50, or 250 μg of L6 only (controls) and for rats given the same amounts of MAb and then subjected to ECIA. There were no differences in WB retention between the three control subgroups. Immediately after ECIA, WB activity was reduced in the 10 μg, 50 μg and 250 μg subgroups by 43%, 39% and 38% respectively: the post-ECIA WB retention curves parallelled those of the controls (Fig. 3). In several cases scintigraphic visualization of tumors implanted in the well-vascularized region of the kidney (SR tumors) became possible after the ECIA procedure (Fig. 4a and b).

Based on pre- and post-absorption values, 80–95% of the circulating plasma activity was removed with ECIA (Fig. 5) The percentage of activity attached to L6 that was removed did not differ between the subgroups, and was thus not correlated to the amount of MAb injected. Notably, plasma activity was found to increase slightly during the 24 h after the start of ECIA.

Fig. 6 illustrates the biokinetics of ^{125}I-labeled biotinylated L6, at the three amounts analyzed, in IM and SR tumors with, and without ECIA. In animals subjected to ECIA, the most favorable uptake was seen immediately after the procedure in SR tumors exposed to 10 μg L6 (median 1.85%/g). Uptake was lower for 50 μg L6 (0.94%/g, $p < 0.21$) and for 250 μg L6 (0.60%/g, $p < 0.01$). Note that the tumor activity concentration (%/g) does not reflect the absolute amount of MAb in the tumor (μg/g) tissue. Activity showed a non-significant decrease at both tumor sites 24 h after the start of ECIA in the 10 μg and 50 μg subgroups, but remained at the same low level in the 250 μg subgroup.

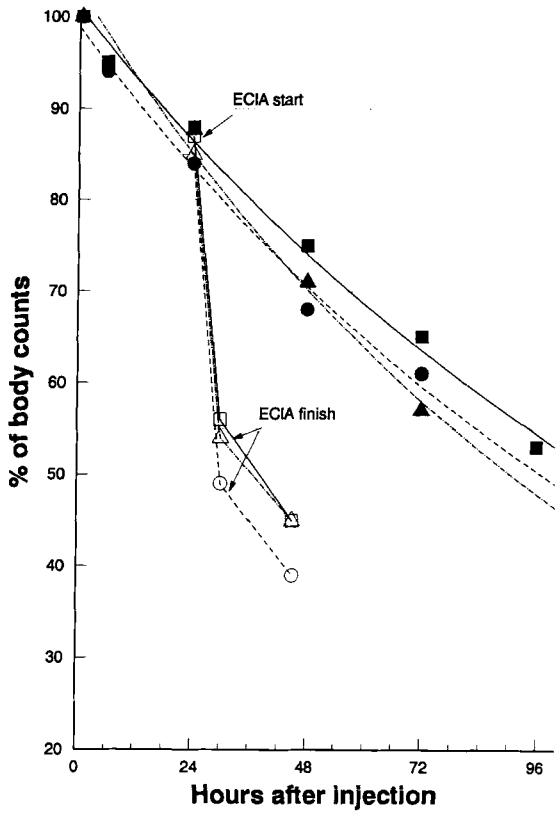

Fig. 3 Whole body activity retention for amounts of 10 μg, 50 μg and 250 μg of ^{125}I-labeled biotinylated L6 MAb with versus without ECIA. ○, 10 μg ECIA; ●, 10 μg contr; △, 50 μg ECIA; ▲, 50 μg contr; □, 250 μg ECIA; ■, 250 μg contr.

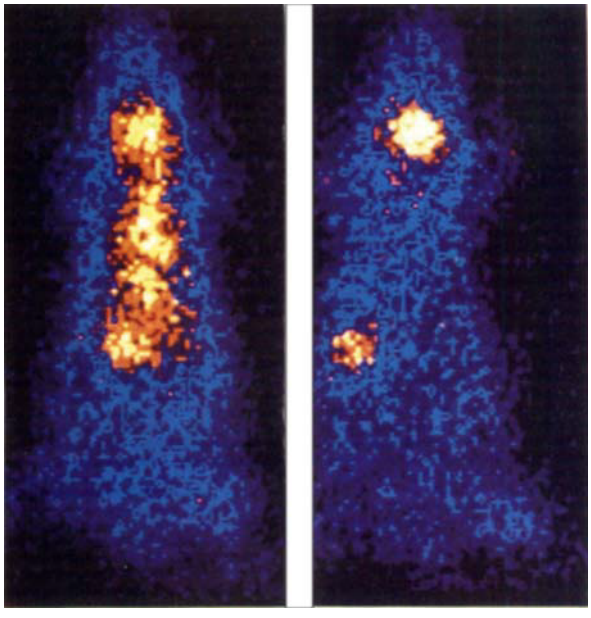

Fig. 4. Immunoscintigrams of a rat injected with 10 μg of ^{125}I-labeled biotinylated L6 MAb before (a) and after (b) ECIA. Scintigraphic visualization of tumors implanted in the well-vascularized region of the kidney (SR tumors) first became possible after the ECIA procedure. Thyroid is unblocked.

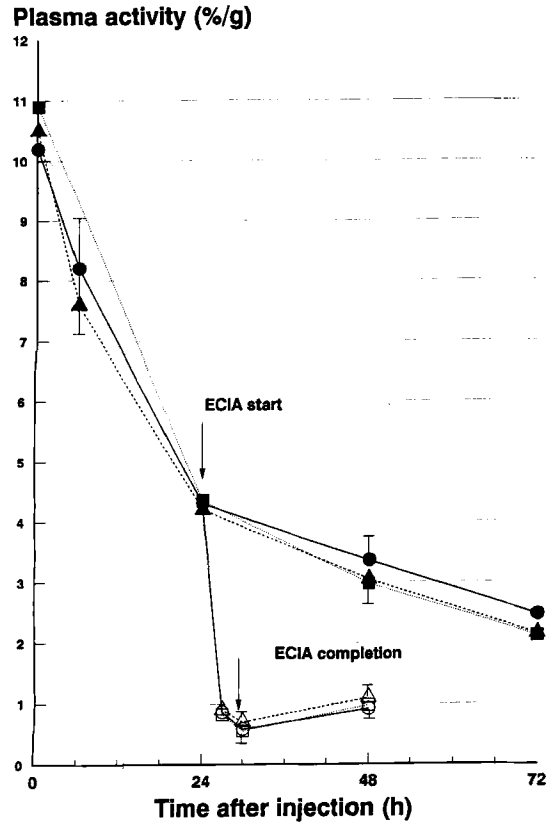

Fig. 5. Plasma biokinetics for different amounts of ^{125}I-labeled biotinylated L6. A small increase in plasma activity was seen 24 h after the start of ECIA. ○, 10 μg ECIA; ●, 10 μg contr; △, 50 μg ECIA; ▲, 50 μg contr; □, 250 μg ECIA; ■, 250 μg contr.

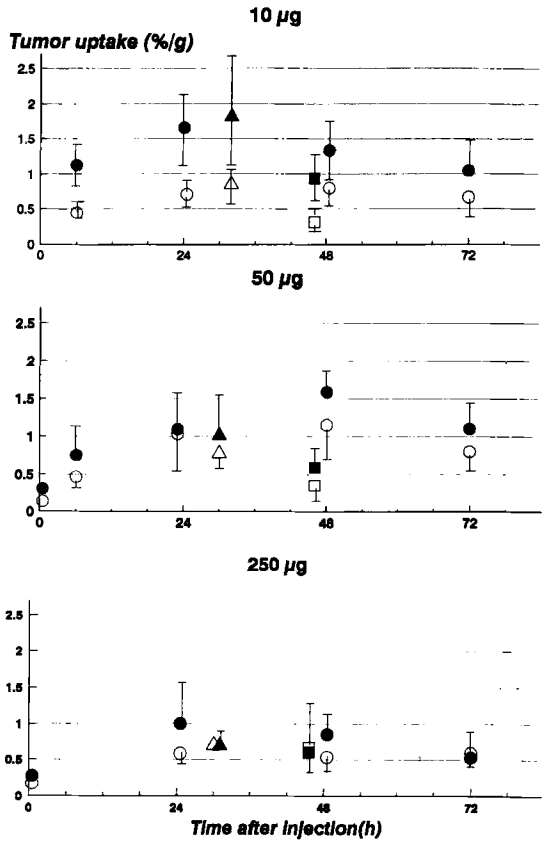

Fig. 6. Biodistribution of the intramuscular (IM) and subrenal capsule (SR) tumors after the injection of three different amounts of L6. ●, SR tumor; ○, IM tumor; ▲, SR tum after ECIA; △, IM tum after ECIA; ■, SR tum 24 h after ECIA; □, IM tum 24 h after ECIA

Neither in controls nor in animals that underwent ECIA, was there any significant difference in the biodistribution of activity in the organs investigated when the 50 and 250 μg subgroups were compared (Fig. 7). The exception was the 10 μg subgroup with ECIA, in which uptake in the organs analyzed was considerably less 24 h after ECIA than in the 250 μg subgroup ($p < 0.05$), and did not show any tendency to increase after ECIA, as was seen in the other two subgroups. The reduction in activity after ECIA in liver, lung, bone marrow and kidney was more pronounced than that in tumors, as reflected by the different T/N ratios (Fig. 8a and b).

The highest T/N ratios were obtained in the 10 μg subgroup, in both with SR and IM tumors. For the SR tumors all T/N ratios improved significantly ($p < 0.05$) after ECIA by a factor of 2.5 to 8.4 (median 3.15) for lungs and by a factor of 6.3 to 35.7 (median 12.6) for bone marrow. The T/N ratios were still elevated 24 h after ECIA. On comparing these T/N ratios with those in control animals at corresponding times (48 h after injection), the improvement was 3.1-fold in the liver ($p < 0.01$), 3-fold in bone marrow ($p < 0.01$), 2.7-fold in kidney ($p < 0.05$), and 2.3-fold in lung ($p < 0.05$). The ECIA procedure improved T/N ratios 2- to 16-fold ($p < 0.05$) in the 250 μg subgroup.

In the 50 μg and 250 μg subgroups, T/N ratios were significantly higher immediately after the procedure than 24 h after the start (Fig. 8a, b), when (with the exception of T/N ratios for the liver in the 250 μg subgroup) they were approximately the same as or even lower than those of the corresponding controls at 48 h.

Discussion

The ECIA technique has the advantage of allowing the control over the period during which unbound circulating MAbs are selectively cleared from plasma. Thus, it is possible to choose both the best moment to start antibody removal, and its duration. In contrast to the use of a column to which an anti-antibody has been conjugated (15), we have developed a novel procedure based on biotinylated radiolabeled antibodies and an avidin-agarose column for adsorption (8, 9). The advantage of this procedure is that it can be used for any antibody system or any combination of antibodies, and there is consequently no need to develop new adsorption columns for each antibody system used. The biotinylation procedure is innocuous and has no significant effect on the binding properties of the antibody (14).

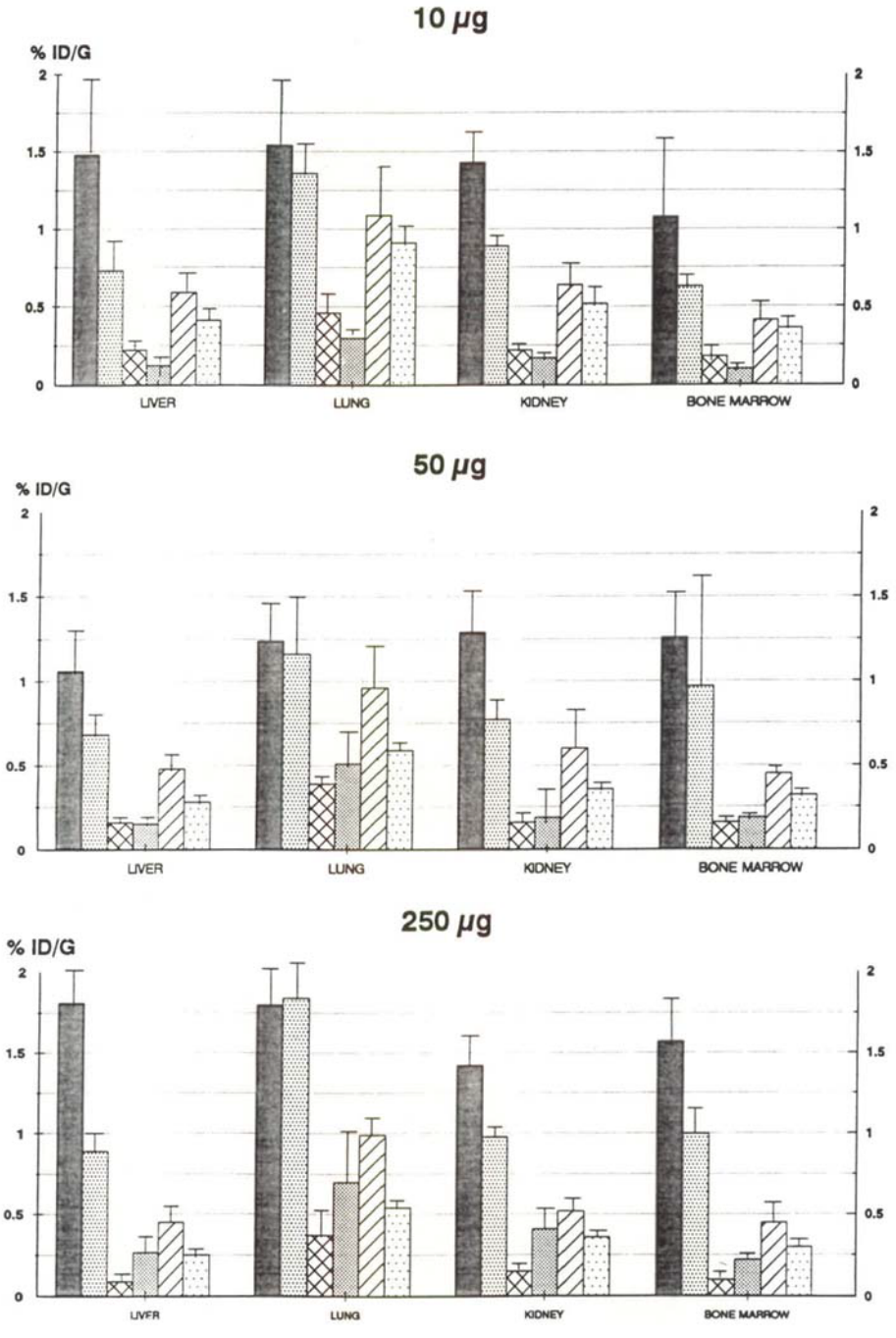

Fig. 7. Normal organ tissue uptake of 10 μg, 50 μg and 250 μg of ^{125}I-labeled biotinylated L6. ■, 6 h (0.5 h for 250 μg); ⊡, 24 h; ⊠, ECIA; ▩, ECIA + 24 h; ▨, 48 h; ⊡, 72 h.

The present findings show our ECIA procedure to be an effective means of removing circulating antibodies (80–95%). The efficacy of the procedure in removing plasma activity was inedpendent of the amount of antibody administered, despite a 25-fold difference in the injected amounts (Fig. 5). In a previous methodological study, we have shown that this system is capable of removing more than 25 mg of antibodies in rats (6). We have thus not found the amount of MAb per se to be an essential limiting factor for the ECIA procedure based on the avidin-biotin reaction.

The main conclusion to be drawn from our findings is that, for both tumor sites, the most favorable tumor-to normal tissue (T/N) ratios, persisting 24 h after start of ECIA were obtained with the lowest amount (10 μg) of L6 (Fig. 8a and b). In the 10 μg subgroup the T/N ratios 24 h after ECIA were still significantly higher than those in controls at 48 h (i.e. animals not undergoing ECIA). This

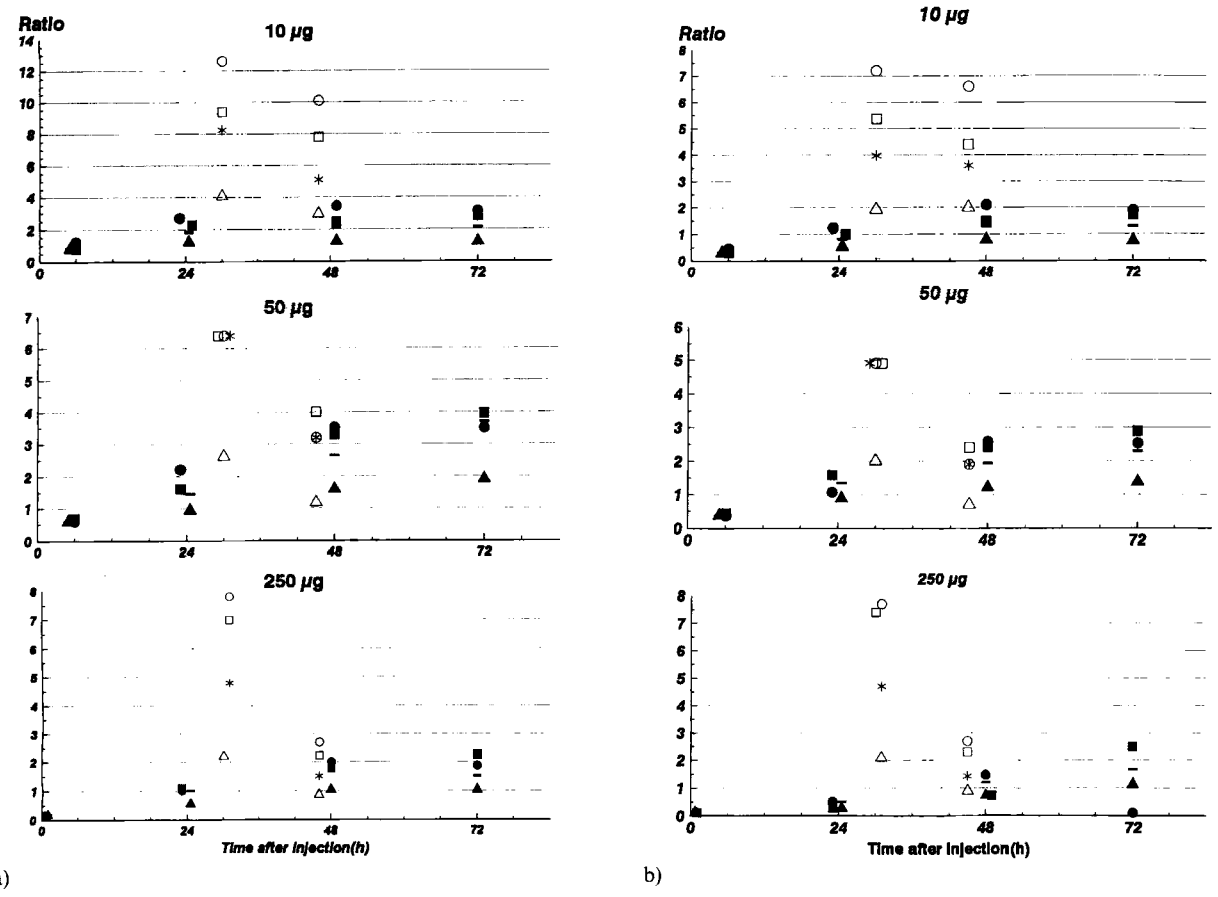

Fig. 8. a) subrenal capsule (SR); b) intramuscular (IM) tumor-to-normal tissue (T/N) ratios for 10 µg, 50 µg and 250 µg of L6. Note the different scale in Fig. 8a. ●, B. marrow; ○, B. marrow after ECIA; ▲, lung; △, lung after ECIA; ■, liver; □, liver after ECIA; —, kidney; ⁎, kidney after ECIA.

result might imply a significant improvement in clinical radioimmunotherapy.

When larger amounts of L6 were injected, the T/N ratios decreased more rapidly during the 24 h following ECIA, mainly due to equilibration between plasma and extravascular (interstitial) space in normal tissue. Thus, by injecting a larger amount of MAb and thereby possibly obtaining a higher degree of tumor saturation (17, 18), this will not necessarily improve the T/N ratio after ECIA. The present results are, to a degree, in agreement with the finding of Jakowatz et al. (19), who did not succeed in improving the tumor-to-blood ratio by increasing the amount of anti-CEA MAb. Because T/N ratios are dependent on uptake and retention of L6 in tumor and normal tissues, these parameters merit further consideration.

A limited supplementary study evaluating radioactivity of the blood content of the tumor and normal organ tissue, i.e. corrected tissue uptake, was carried out in four animals to assess the results of ECIA. This method was previously described in details by our group (20). Based on corrected tissue uptake, we found that 10 to 15% of the activity in the tumors originating from circulating activity in blood, and concluded that the corresponding immediate decrease in tumor activity after ECIA might have been mainly due to the decrease in activity in blood within the tumors.

In blood-rich organs such as the liver, kidney, bone marrow and lung, 60 to 85% of the activity was due to the blood. Immediately after ECIA, a similar reduction in activity was achieved in the organs, critical for radioimmunotherapy (Fig. 7). The increased uptake 24 h after ECIA in liver, kidney and lung when greater amounts of L6 (250 µg) were injected may reflect not only increased radioantibody concentration in blood, but also increased uptake in the reticuloendothelial system (RES) after the redistribution between different compartments, together with binding to readily accessible non-specific sites (21). In absolute terms, the amount of MAb released to plasma from various organs by redistribution is correlated to the amount of MAb administered, as the same fraction of MAb is released to plasma irrespective of the amount injected (Fig. 5). This amount is probably correlated to the uptake in RES until saturation is accomplished. The rates of L6 retention in the lungs were more stable than in the liver, kidney and bone marrow, probably due to the presence of L6 antigen in the vascular or lung endothelium (22, 12). There are several conceivable ex-

planations for the decreased tumor uptake (%i.d./g) after ECIA, apart from decreased activity in the blood pool within the tumor. Activity released due to deiodination (23), or releases of intact antibody from Fc-mediated receptors or from the interstitial fluid within the tumor may be involved. Retention within tumor cells might be prolonged by labeling the antibody with ^{111}In instead of ^{125}I, as suggested by the findings of in vitro experiments (24). Such an ^{111}In-MAb complex will be gradually internalized and degraded in the lysosomes, resulting in catabolic products containing ^{111}In which are retained within the tumor cells.

To sum up, before using a MAb in combination with an ECIA procedure, the biokinetics of different amounts of the injected specific antibody should be evaluated to establish the optimal amounts of MAb to be used, as well as best moment to start ECIA. It can not be taken for granted that the largest amount of MAb will yield the highest tumor tissue uptake (%/g), or the highest tumor-to-normal tissue ratios after ECIA.

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