A General, Extracorporeal Immunoadsorption Method to Increase the Tumor-to-Normal Tissue Ratio in Radioimmunoimaging and Radioimmunotherapy

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The aim of this study was to investigate a new extracorporeal immunoadsorption method to improve tumor-to-normal tissue ratios in radioimmunoimaging (RII) and radioimmunotherapy (RIT). We have developed and investigated a general method using biotinylated antibodies and an agarose-avidin column for extracorporeal immunoadsorption. The studies were made in an animal model and extracorporeal immunoadsorption (ECIA) was performed 24 or 48 hr after the injection of ^{125}I-labeled biotinylated antibodies. In athymic rats, heterotransplanted with human malignant melanoma, 90%–95% of the circulating activity was removed with ECIA. The tumor-to-normal tissue ratios at 24 hr was increased 4 times (from 1.2 to 5.1) in the liver, 2.5 times (0.7 to 1.8) in the lung, 4 times (1 to 4) in the kidneys and 4 times (1.4 to 5) in the bone marrow. Whole body activity was reduced by 40%–50%. Tumor-to-organ ratios at 48 hr were increased 3.5 times (from 1.5 to 5.2) in the liver, 2 times (0.9 to 1.7) in the lung, 3 times (1.3 to 3.8) in the kidneys and 4 times (1.4 to 5.5) in the bone marrow. Whole body activity was reduced by 35% when ECIA was performed 48 hr after injection. This study proves that an important reduction in background activity, and thereby an improvement in the tumor-to-background ratio, can be achieved by using this generally applicable, biotin-avidin ECIA method. For RII, the improved ratio increases the possibilities of detecting tumors and metastases in blood-rich organs. For RIT, the procedure may lead to a decreased absorbed dose to bone marrow and other critical organs.

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Radiolabeled monoclonal antibodies (Mabs) are widely used in the diagnosis and therapy of malignant tumors (*1,2*). Significant therapeutic results have been achieved by several groups (*3–8*), but in many cases specific targeting to tumor is low and the possibility of reaching sufficiently high absorbed doses to tumors without causing harmful effects on critical organs such as the bone marrow is limited. Extensive work has been done and is currently being performed in various animal models to develop methods to improve diagnostic and therapeutic results with radiolabeled Mabs. Different factors to increase tumor uptake of Mabs and to get a more homogeneous distribution of Mab in tumors have been considered: the route of injection, the amount of Mab, the use of fragments and chimeric antibodies, the use of a second antibody and labeling methods (*9*). To increase the ratio, tumor uptake has to be increased, background activity reduced, or preferably both.

The idea of reducing blood background has been previously investigated. Different methods have been presented to achieve this: use of a second antibody (*10,11*), in which radiolabeled antibodies were injected and allowed to accumulate in tumors and then the second antibody was injected, resulting in the formation of complexes with the circulating, labeled antibodies and a rapid clearance of the complexes from the circulation. Another method is the administration of biotinylated antibodies followed by an injection of avidin after antibody localization. The circulating biotinylated Mabs and avidin form complexes that are removed from the circulation (*11*). The drawback of these methods is that the complexes formed are accumulated in the liver and spleen, thus increasing background activity in those organs.

The use of plasmapheresis or extracorporeal immunoadsorption (ECIA) to reduce blood background has been presented in both experimental studies (*12*) and in clinical trials (*13,14*). This method causes no complex formation with increased background and would be more favorable compared to the other methods.

We previously presented a theoretical basis for the use of ECIA to reduce uptake in normal tissue (*15,16*). By using a compartment model, we showed that tumor-to-organ ratios could be increased, especially for blood-rich organs. The calculated reduction of normal tissue activity

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was then verified in an experimental model (*17*). Ovalbumin was linked to a Sepharose column and 90% of circulating anti-ovalbumin was adsorbed. In that experimental model, we showed that it was possible to reduce normal tissue uptake four to eight times.

In this study, we have further developed the ECIA procedure into a more general method. Here we used the method of biotinylated, radiolabeled antibodies and an avidin agarrose column for adsorption in a model with tumor-bearing athymic rats.

TABLE 1
Experimental Setup

Time after injection	Injected antibody	ECIA treatment	Number of rats
24 hr	I-96.5	no	4
24 hr	I-96.5-biotin	no	5
24 hr	I-96.5-biotin	yes	6
48 hr	I-96.5	no	4
48 hr	I-96.5-biotin	no	4
48 hr	I-96.5-biotin	yes	4

MATERIALS AND METHOD

Animals

Male and female athymic nude rats (RNu/RNu, n = 27) with a mean weight of 220 ± 40 g were used. The rats were provided with standard feed pellets and water ad libitum. During the experiments, the rats were kept in cages with air filters.

Tumors

The rats were heterotransplanted with human melanoma tumors (UMT10). Tumor cells were passaged in nude rats after thawing and a coarse suspension of the tumor cells, in a volume of 0.4 ml, was inocculated subcutaneously into the right thigh and intramuscularly into the left thigh. The antibody was injected intravenously 3 wk later. The tumors had then reached a mean weight of 0.84 ± 0.79 g (range 0.025–3.383, n = 27) for subcutaneous tumors and 1.19 ± 0.94 g (range 0.015–3.081, n = 24) for intramuscular tumors.

Mab and Radiolabeling

The Mab used in this study was 96.5 (mouse IgG2a) (*18*), which is specific for a cell-surface glycoprotein, with a molecular weight of 97,000 present in 60%–80% of human melanoma. The Mab was labeled with ^{125}I according to the Cloramine-T method (*19,20*). Three hundred and fifty micrograms of the Mab was labeled with 37 MBq ^{125}I (Amersham, UK). The antibody was then separated from free iodine on a Sephadex G25 column (PD10, Pharmacia, Sweden).

Biotinylation

After iodination, the Mab was conjugated with biotin (*21*), in a 0.1 *M* $NaHCO_3$, 0.15 *M* NaCl buffer, pH = 8.3. Biotin reagent (N-hydroxysuccinimido-biotin, Sigma, St. Louis, MO) in DMSO, 0.125 mg/mg protein, was added to the antibody and DMSO was added to give a final concentration of 10%. The solution was incubated for 30–60 min at room temperature and then in a refrigerator (+4°) overnight. The antibody was separated from unbound biotin reagent on a Sephadex G25 gel column (PD10, Pharmacia, Sweden). After conjugation, the binding of the biotinylated antibodies to agarose-avidin (Agarose-avidin D, Vector, Burlingame, CA) was tested by mixing a small sample of the radiolabeled and biotinylated antibody with 50–100 μl of agarose-avidin. After 10 min of incubation, the agarose avidin was washed three times and the supernatant removed. The avidin-agarose gel was then measured for activity in an automatic NaI(Tl) well, sample changer along with a standard sample and the binding efficiency was calculated.

Cell Binding Assay

The binding capacity of the antibodies was tested on tissue-cultured melanoma cells. The labeled and biotinylated antibodies (approx 20 ng) were incubated on ice in a suspension of $2 \cdot 10^6$ melanoma cells (UMT10) for 60 min. The cell suspension was then washed three times and the activity bound to the cells was measured in a NaI(Tl) well sample changer.

Control Study

The biodistribution of ^{125}I-labeled antibodies and ^{125}I-labeled biotinylated antibodies was investigated in control groups of animals at 24 and 48 hr after injection (Table 1). The Mab was injected intravenously into the femoral vein of tumor-bearing animals. Blood samples were drawn 2–5 min after injection and then at daily intervals. At 24 or 48 hr after injection, the animals were killed and dissected. Eight lymph nodes, four different muscle samples, thyroid, liver, kidneys, spleen, lung, heart, tumors and bone marrow were removed and measured for activity in an automatic (NaI(Tl) well sample changer. Activity uptake was calculated as:

$$\text{Uptake} = \frac{A}{A_0}\frac{1}{m} e^{-\ln 2 \cdot t/T} \text{ [\%/g]},$$

where A = activity in sample (kBq), A_0 = injected activity (kBq), m = mass of sample (g) and $e^{-\ln 2 \cdot t/T}$ = decay correction, where t = elapsed time between measurement of sample and time of injection and T = physical half-life.

Catheterization

For the extracorporeal treatment, the rats were catheterized under anesthesia with Mebumal (30–40 mg/kg) and Hypnorm (0.15–0.30 ml/kg) with an arterial (arteria carotis) and a vein (vena jugularis) catheter to gain blood access (*22*). The catheters were flushed every second day with heparinized normal saline (20 IE/ml) to avoid coagulation in the catheters. The catheterization was performed 3–4 days before the ECIA procedure to allow the rats to recover completely from the operation before ECIA was performed.

Extracorporeal Immunoadsorption

One or two days after catheterization, the rats were injected with 3–5 MBq (50 μg) of the ^{125}I-labeled and biotinylated Mab. Twenty-four or 48 hr after injection, ECIA was performed to remove circulating antibodies. A column with 1–1.5 ml of Agarose-avidin-D (Vector) was used as an adsorbent. The experimental setup is shown schematically in Figure 1. The catheters were connected to the extracorporeal circuit using a swivel-tethering system while the rats were anesthetized with ether. The animals were unanesthetized during the ECIA procedure. The animals were imaged on a scintillation camera (General

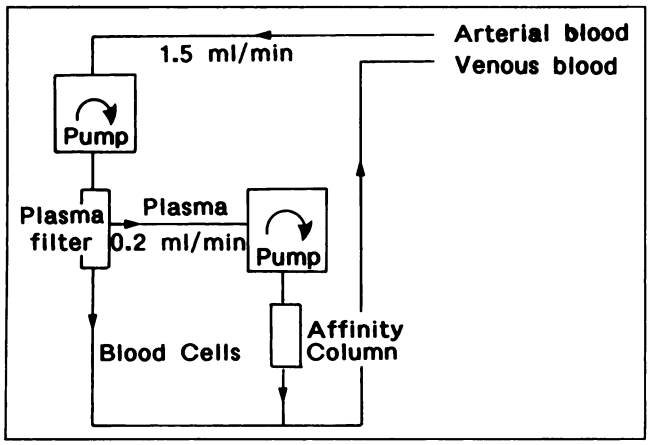

FIGURE 1. Experimental setup. Arterial blood is pumped at a flow rate of 1.5 ml/min through a plasma filter and plasma is pumped through the adsorbent column at a flow rate of 0.2 ml/min. After adsorption, blood cells and plasma are mixed and returned via an air trap to the vein catheter and back into the animal.

Electric 400T, GE, Milwaukee, WI) just after injection, after 24 hr, just before and immediately after ECIA.

ECIA was performed as described by Nilsson et al. (*22*) and Norrgren et al. (*17*). Briefly, the blood is pumped from the arterial catheter through a hollow-fiber plasma filter at a flow rate of 1.5 ml/min. The plasma is separated and passed through the adsorbent column at a flow rate of 0.2 ml/min. The plasma is then mixed with the blood and returned via the venous catheter. Blood samples were drawn immediately after injection, at 24 hr after injection, just before ECIA, after half of the ECIA procedure and immediately after ECIA was completed. After ECIA, the rats were killed, dissected and the tissues were removed and measured for activity in accordance with the procedure for the control animals.

RESULTS

Radiolabeling efficiency was 70%, and on average 0.2 iodine atoms were bound per antibody molecule. After radiolabeling and biotinylation of Mab, more than 90% of the activity was bound to the agarose-avidin. In the cell binding test, 30% of the antibodies were bound to the melanoma cell suspension. The cell binding results are in accordance with results reported by Larson et al. (*18*), and by Ingvar et al. (*23*).

Scintillation camera images from one of the rats illustrate activity distribution at 24 hr after injection of radiolabeled biotinylated antibodies, before ECIA (Fig. 2A) and after ECIA (Fig. 2B). The images clearly show the reduction in the blood background, which is achieved, especially in the abdominal region, with ECIA. Whole body activity was reduced by 48% in this case, and the plasma activity concentration was reduced by 91% (from 2.58%/g to 0.23%/g). The intramuscular tumor (indicated by an arrow) is more clearly visualized after immunoadsorption. The thyroid was not blocked during any of the experiments.

The whole-body retention for iodinated antibodies in nude rats was similar to our previous results reported by Ingvar et al. (*23*), with a whole-body, effective half-life of 110 hr and a plasma concentration of 3.5%/g at 24 hr after injection and 2.5%/g at 48 hr. For biotinylated antibodies, the whole-body, effective half-life was approximately 54 hr. The plasma concentration was 2.7%/g at 24 hr and 2%/g at 48 hr. In Figure 3, the whole-body retention is given for iodinated antibodies, biotinylated iodinated antibodies and whole-body content after ECIA performed 24 hr after the injection of antibodies (Fig. 3A) and 48 hr after injection (Fig. 3B). The reduction in whole-body content due to the immunoadsorption was 40%–50% at 24 hr and 35% at 48 hr.

In Figure 4, plasma retention, i.e., the activity concentration in the plasma versus time, is given for the control animals and for the ECIA animals. In Figure 4A, ECIA was started 24 hr after injection of Mab and in Figure 4B, 48 hr after injection. The major portion of the plasma activity content (approximately 85%) was removed during the first half of ECIA. During the second half of the

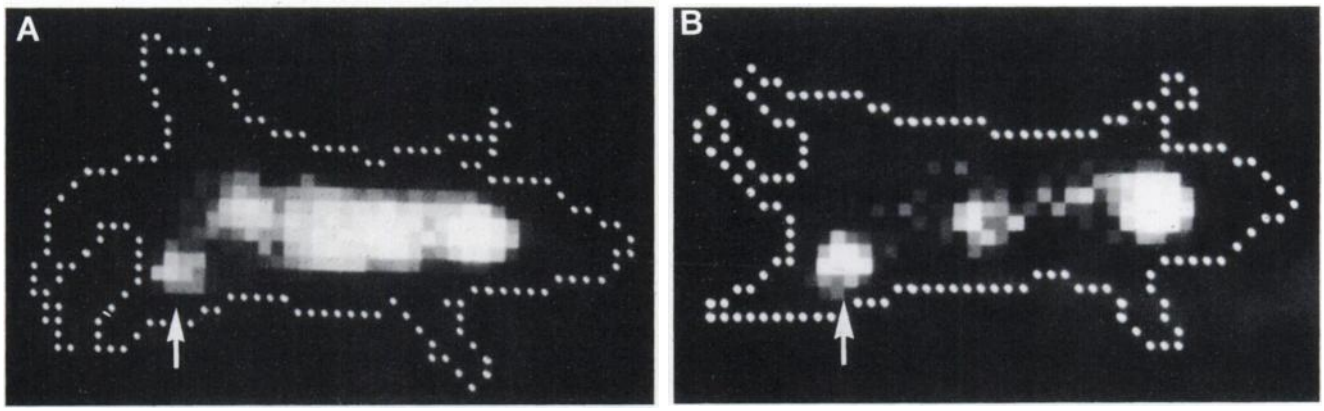

FIGURE 2. Scintillation camera images before (A) and after ECIA (B). The contours of the rats are indicated. The intramuscular tumor in the thigh (arrow) is clearly visualized after ECIA. The whole-body activity is reduced by 48% of the activity just before the start of ECIA. In particular, clearance from the abdominal region is important.

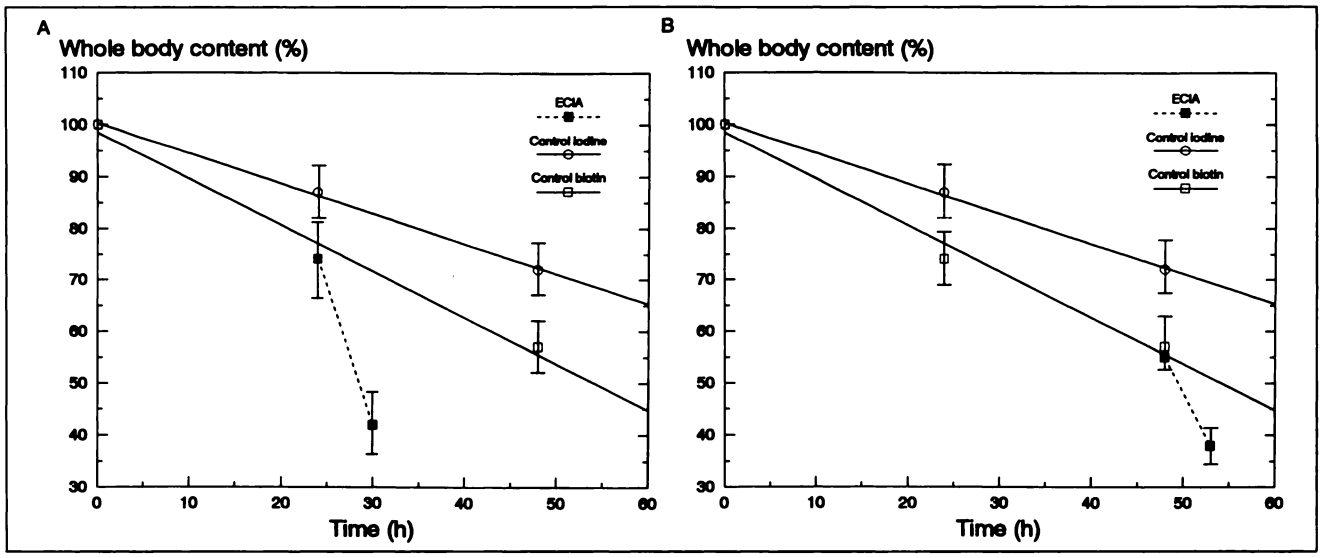

FIGURE 3. Whole-body retention for control groups and after ECIA (A) 24 hr and (B) 48 hr after injection.

procedure, another 5%–15% was removed, resulting in a total of 90%–95% depletion in plasma activity.

The biodistribution for the various dissected tissues is given in Table 2 for 24 and 48 hr. Activity uptake in muscle, kidneys, liver, lung, subcutaneous tumor, intramuscular tumor and bone marrow (in percent of injected activity per gram tissue (%/g) corrected for decay of ^{125}I) is given. The error range is ±1 s.d. The ^{125}I-96.5 antibodies (iodine control group) have longer whole-body and plasma retention times when compared to the ^{125}I-96.5 biotinylated antibodies (biotin control group), and a lower tissue uptake is seen for the biotin control group. Iodine-125-96.5 biotinylated antibody uptake was reduced during the ECIA procedure by 75%–80% of the initial value for kidneys, liver, lung and bone marrow in comparison to the biotin control group at 24 hr postinjection. For tu-

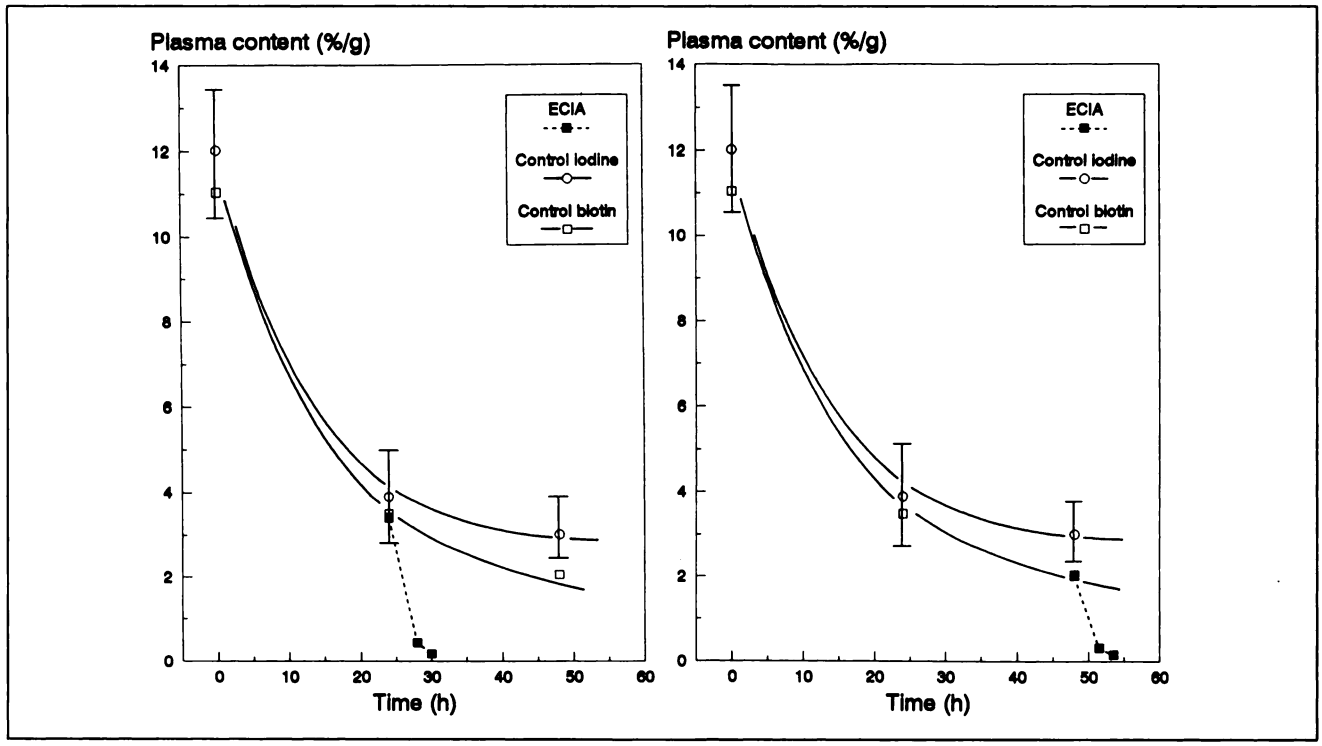

FIGURE 4. Plasma activity concentration (%/g) for control rats with ^{125}I-96.5 antibodies and control rats with ^{125}I-96.5-biotin antibodies. Plasma concentrations after ECIA at 24 hr (A) and ECIA after 48 hr (B) are shown.

TABLE 2
Uptake (%/g) in Different Tissues 24 and 48 Hours After Injection

Tissue	24 hr		
	ECIA	Control biotin	Control iodine
Muscle	0.06 ± 0.03	0.06 ± 0.03	0.07 ± 0.03
Kidney	0.11 ± 0.01	0.55 ± 0.13	0.61 ± 0.02
Liver	0.09 ± 0.02	0.47 ± 0.09	0.59 ± 0.02
Lung	0.22 ± 0.04	0.83 ± 0.30	1.05 ± 0.14
Subcutaneous tumor	0.41 ± 0.17	0.55 ± 0.11	0.88 ± 0.24
Intramuscular tumor	0.49 ± 0.14	0.70 ± 0.32	0.78 ± 0.11
Bone marrow	0.11 ± 0.04	0.40 ± 0.10	0.49 ± 0.07
	48 hr		
	ECIA	Control biotin	Control iodine
Muscle	0.05 ± 0.03	0.08 ± 0.02	0.07 ± 0.02
Kidney	0.07 ± 0.02	0.38 ± 0.04	0.54 ± 0.13
Liver	0.06 ± 0.02	0.34 ± 0.05	0.52 ± 0.13
Lung	0.16 ± 0.07	0.54 ± 0.06	0.88 ± 0.16
Subcutaneous tumor	0.26 ± 0.11	0.51 ± 0.03	1.33 ± 0.30
Intramuscular tumor	0.27 ± 0.19	0.45 ± 0.08	1.26 ± 0.42
Bone marrow	0.05 ± 0.10	0.36 ± 0.04	0.46 ± 0.10

mors, the reduction was only 20%–25%. Corresponding data for ECIA at 48 hr after injection was 65%–85% and 40%–50%, respectively.

To illustrate the contrast enhancement in a situation where tumors are present in an organ or close to an organ in the abdominal region, we calculated the tumor-to-normal tissue ratio for kidney, liver, lung and bone marrow. The values for subcutaneous tumors were used for the calculations. In Figure 5, the ratios achieved with ECIA at 24 and 48 hr are presented along with the ratios at the same times in control animals. Here we can see that the ratio for ^{125}I-95.5 biotinylated Mab was increased about 2.5 times in the lung and 4 times in the liver, kidney and bone marrow at 24 hr and 3.5 times in the liver, 2 times in the lung, 3 times in the kidneys and 4 times in the bone marrow at 48 hr.

DISCUSSION

A general problem when using Mabs RII and RIT is high background activity due mainly to circulating antibodies and Mab in the extravascular volume and, often to

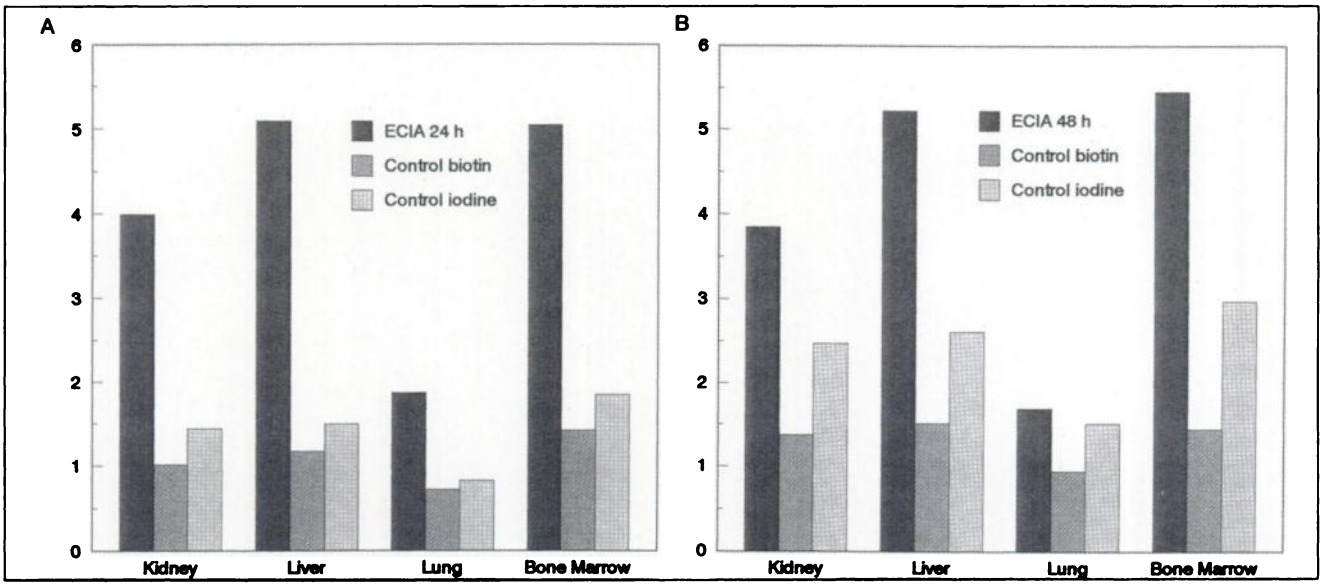

FIGURE 5. Subcutaneous tumor-to-tissue ratio for kidney, liver, lung and bone marrow after ECIA and for control groups at 24 hr (A) and at 48 hr after injection (B).

a much lesser extent, to actual antibody binding to relevant epitopes expressed by normal cells. A reduction of the amount of antibodies in circulation can be achieved by plasma exchange, selective ECIA or by the administration of, for example, antibodies binding selectively to the Mab. Plasmapheresis is a routine procedure and is widely used to treat autoimmune diseases. The drawback of using this method is the need to replace plasma.

ECIA has been studied in rabbits by Charlton et al. (*24*) and in rats by Nilsson et al. (*22*). The advantage of using this method in combination with RII and RIT is the possibility of designing a system so that only the Mab used in that particular RII or RIT protocol is adsorbed from the circulation.

Plasma exchange has been used to improve Mab tumor-to-background ratios in a nude rat model with human ovarian carcinoma by Henry et al. (*12*). They showed a drop in tumor activity of 5%, in blood activity of 79%, in liver of 85%, in kidneys of 77% and in lungs of 72%. The tumor-to-background ratios were improved considerably. The reduction in normal tissue is in accordance with our results. Their lower tumor reduction as compared to our results might be due to differences in tumor and antibody properties and to biokinetics.

We have shown in the present model that the tumor uptake was reduced by 20%–25%, while kidney, liver, lung and bone marrow uptake was reduced by 75%–80% and plasma reduction was 90%–95%, when ECIA was performed 24 hr after the injection of Mab. In our previous biokinetic study (*23*) we used ^{99m}Tc-labeled red blood cells to correct for circulating blood activity in different tissues. The corrected ^{125}I-96.5 uptake in tumors was 10%–20% lower than uncorrected data. Our corrected, specific tissue uptake showed an approximate 80% reduction in kidney, liver and bone marrow. The same animal and tumor model was used in both studies. The results of the RBC method seem to correlate well with the activity reduction after ECIA achieved in this study.

ECIA has been reported in two patient studies. Lear et al. (*14*) studied seven patients with lung or breast carcinoma. They performed ECIA using goat anti-mouse antibody for adsorption 6 to 24 hr after the injection of ^{111}In-labeled Mab and reported a 68% reduction in plasma activity and a 10% reduction in tumor activity following ECIA. In a study by DeNardo et al. (*13*), treatment of B-cell malignancies was performed with high doses of ^{131}I-labeled Lym-1 antibodies. A total activity of 29.6 GBq (800 mCi) was used with and without immunophoresis. The circulating ^{131}I-Mab was effectively removed by immunophoresis.

The general method for ECIA described in this study using biotinylated antibodies and an agarose-avidin column can considerably improve tumor-to-tissue ratios. The biotinylation procedure is mild and has no significant influence on the binding properties of the antibody (*21*). ECIA methods are advantageous over other background reduction methods in that they do not cause increased uptake in tissues (such as the liver and spleen), as do systems in which second antibodies and biotin-avidin are administered (*10,11*). Our ECIA method is general in that the agarose-avidin column can be used for any antibody system, and an anti-antibody coupled to the column is not required. Thus, one does not need to develop new adsorption columns for each antibody system used.

In this study, image improvement, especially activity reduction in the abdominal region, is important. Many tumors metastasize in the liver, where normally a very high background is present for several days postinjection of whole antibodies. ECIA would increase imaging possibilities in this region. Our experiments show only a small reduction in tumor uptake, while blood background is reduced several times. This indicates the ability to better detect smaller metastases and metastases with lower activity concentration because of better image contrast.

The procedure used here is efficient for the depletion of circulating antibodies. However, antibodies present in the extravascular space can not be reached by a single ECIA, as can be seen from the whole-body retention curves which show that 50%–60% of the activity still remains in the body after ECIA, while 95% of the blood activity is removed. If ECIA is repeated 24 hr after the first procedure when equilibrium of Mab between the extravascular space and plasma has been reached (*22,24*), it would be possible to remove an additional 25% of the injected activity. This might be a possible step to further improve detectability in RII.

In summary, we have developed a simple, generalized model for ECIA to improve tumor-to-normal tissue ratios in which different parameters and new therapeutic concepts using ECIA can easily be investigated and evaluated for later implementation in clinical studies.

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