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TUMOUR UPTAKE OF MONOCLONAL ANTIBODY AFTER REGIONAL INTRAARTERIAL INJECTION

Biokinetics in the nude rat heterotransplanted with malignant melanoma

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Abstract

Nude rats were heterotransplanted with human melanoma metastases on both thighs. Ten days later a bolus of ^{125}I-labelled monoclonal antibody (MAb) 96.5 was injected through a catheter in the common femoral artery. The femoral vein was clamped for 15 min to obstruct the venous outflow from the injected leg. The specific tissue uptake (%/g) in the tumour on the injected side compared to the non-injected side showed initially higher uptake (ratio 7.2 at 3 h). After 24 h there were no side differences. The tumour to muscle ratio was 2.8 at 3 h when injected and control sides were compared. Intravenous or subcutaneous injection gave similar specific tissue uptake as regional arterial injection after 24 h. Tissue to plasma ratios were similar after intravenous and subcutaneous injection of monoclonal antibodies. Intraarterial injection of a bolus of labelled monoclonal antibodies and obstructing the venous outflow thus increased the tumour uptake during a short period of time during which the contrast enhancement was three-fold.

Key words: Monoclonal antibody, melanoma, intraarterial injection, nude rats.

Most in vivo studies using monoclonal antibodies have used intravenous administration under the assumption that target sites could best be reached via the blood (1–3). Others have tried subcutaneous route with limited success (4, 5). Still others advocate that the intraperitoneal route should be the best in order to reach malignant tissue of i.e. the ovaries and colon (6). Some authors have suggested that intraarterially injected antibodies could improve the tumour uptake (7, 8).

No detailed study of the biokinetics of monoclonal antibodies after intraarterial injection has yet been published and compared to intravenous and subcutaneous injections. We here report a biokinetic study of intraarterial injection of MAb 96.5 in the nude rate heterotransplanted with human melanoma, a model which we have described previously (9, 10).

Material and Methods

Nude rats (Rowett RNu/RNu strain) with a median weight of 234 ± 62 g were used. They were bred and kept in cages with filter tops and provided with autoclaved feed pellets, sterilized drinking water ad libitum, and sterile wood granulate bedding in a temperature-controlled unit receiving particle filtered air. Four to five times during the study the animals were anaesthetized with ether and 0.5–1 ml of blood was withdrawn from the periorbital venous plexa. A human melanoma tumour was established by serial passages in the nude rats. All transplants derived from the same passage and all animals were inoculated with a tumour intramuscularly on both thighs. Experiments started ten days after inoculation.

The monoclonal antibody used was the 96.5 (IgG 2a) specific for p97, a cell surface glycoprotein with a molecular weight of 97 000, present in 60–80% of human melanoma but only in trace amounts in normal tissues (11). Antibodies were purified from the ascites fluid of mice, bearing hybridoma ascites tumours, by affinity chromatography on a Protein A Sepharose CL-4B column (Pharmacia, Uppsala, Sweden) with a stepwise pH gradient elution (11, 12). The purity of the monoclonal antibodies was estimated by agarose electrophoresis. The antibodies were stored at −70°C.

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The monoclonal antibody 96.5 (600 and 630 μg) was labelled with 40–50 MBq ^{125}I using the Chloramine-T method (13). By elution on a Sephadex G 25 column (Pharmacia PD 10) the peak fractions of labelled protein were used for the experiments. The labelling efficiency of the antibody in the two preparations was 50–80% in this study. The antibody was labelled with less than one iodine atom per molecule, thus not interfering with the immunoreactivity (14).

The labelled antibodies were tested for binding to a suspension of cultured melanoma cells H-1477 (Mel-28) in the direct binding assay described by Brown et al. (11). The antibody activity of the labelled protein was expressed as the ratio of bound and total added activity and was in these experiments 65%. In preceding titrations it was shown that the number of cells used represented a large antigen excess.

Experimental design. In total 20 nude rats were used. Under chloral hydrate anesthesia a catheter, Luer 2 FG (Portex Lmt, Hythe, Kent, UK) was introduced through the left renal artery and passed via the aorta to the right femoral artery (Fig. 1a). The external diameter of the catheter was smaller than the inner diameter of the femoral artery thus not obstructing the blood flow to the extremity. When the labelled antibodies were injected, a clamp was placed on the femoral vein, obstructing the venous outflow from the extremity. The injected volume was 0.15 ml, and the catheter was flushed with 0.10 ml saline to ensure that the whole amount of antibodies was injected into the artery. The venous obstruction was released 15 min later, the catheter withdrawn, the renal artery ligated and the abdomen sutured.

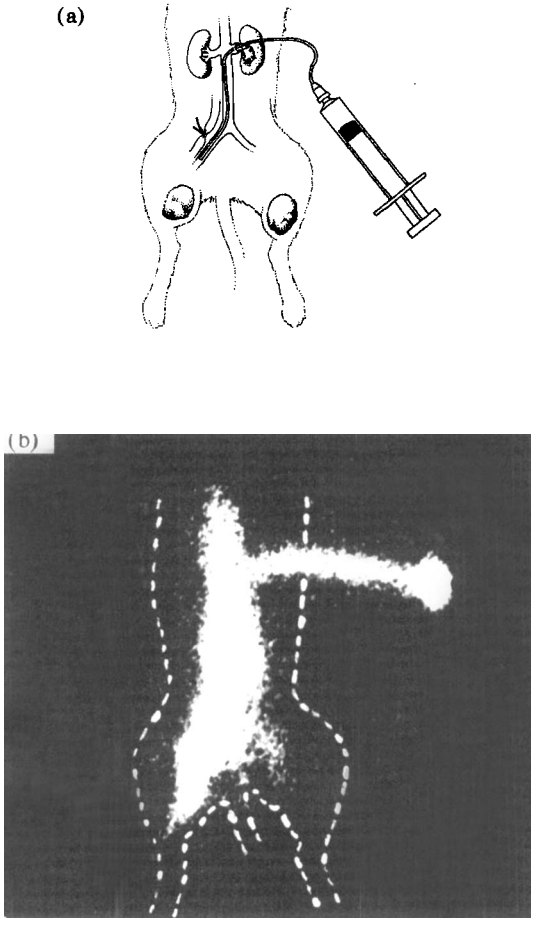

Fig. 1 a) Schematic drawing showing the experimental set up with intraarterial injection of ^{125}I-96.5 monoclonal antibody in the right common fermoral artery. b) Scintillation camera image shows the distribution of activity during the first 5 min after the injection. Note the difference between the two legs of the rat and only minor activity in the upper part of the nude rat.

The mean amount of antibody injected was 38.2 ± 12.5 μg (MAb 96.5), corresponding to 2.6 ± 0.6 MBq. Blood samples from the periorbital venous plexa were centrifuged to separate plasma. The rats were sacrificed at different times (0.5, 1, 3, 6, 24, 48, 72 h) following the injection. Tumours, liver, spleen, lungs, kidney, muscles and bone marrow were removed separately. In each rat ten different lymph nodes were dissected (bilaterally one popliteal, one inguinal and two axillary nodes and from the abdomen one lymph node from the true pelvis and the last one from the hilus of the liver). The rats were not bled and the different organs and tissues were not washed. Each tissue sample was weighed, m_j (g) and measured in an automatic NaI(Tl) sample changer for radioactivity content, A_j (MBq). The specific tissue uptake (STU), %/g, was calculated

$$STU = \frac{1}{A(0)} \times \frac{A_j\, e^{\lambda t}}{m_j} \qquad (1)$$

λ = decay constant for ^{125}I equal to 0.012 d^{-1}
A(0) = injected activity
t = time after injection

A scintillation camera (General Electric Maxi Camera I) was used to follow the initial distribution in the injected leg and subsequent uptake in the rest of the experimental animal. A 25% energy window was centered over the 28 keV photo peak for ^{125}I. The camera was connected to a computer for storage of images (Gamma 11, Digital Equipment Corp., USA).

Results

Following the transplantation the intramuscularly implanted tumours gained a median weight of 0.24 ± 0.18 g (injection side n = 20) and 0.20 ± 0.18 g (control side n = 19). This was in accordance with our previous results (9).

Scintillation camera imaging. Fig. 1b shows the cumulated image of the first 5 min after the intraarterial injection of ^{125}I-96.5 through the catheter in the right femoral artery. The main outflow from the leg was blocked by a

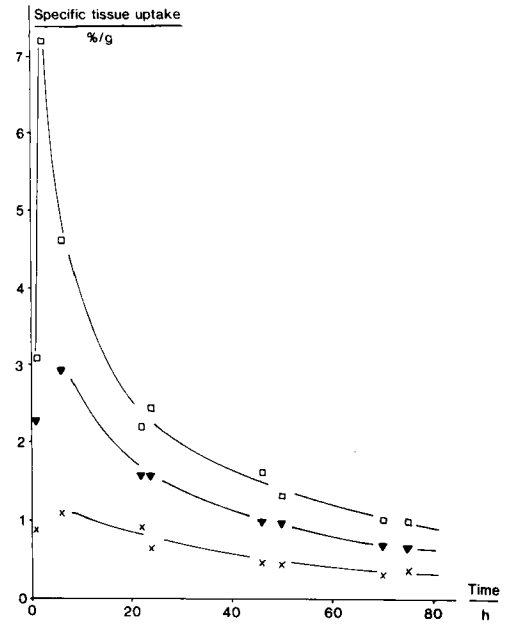

Fig. 2. Specific activity of ^{125}I-96.5 in plasma, whole blood and blood cells during 72 h after intraarterial injection and venous outflow obstruction for 15 min in the nude rat. Note the peak activity at 3 h. □ plasma; ▼ whole blood; × blood cells.

clamp of the femoral vein. Only minor amounts of activity passed through collaterals to the central part of the rat at this early time. In the control leg no major activity could be observed in the image.

Specific tissue uptake (STU). The STU values for plasma, whole blood and blood cells from the centrifuged blood samples are shown in Fig. 2. The peak systemic plasma concentration of the ^{125}I-96.5 was found at 3 h (7.3 ± 0.9%/g of injected activity).

The STU of antibody 96.5 for the different dissected tissues are given in Fig. 3 for liver, lungs, bone marrow, muscle and kidney (except the ligated left side) up to 50 h following injection. Note the initial difference between the tumours on the injected and control side. In lymph nodes there was a slow increase in the uptake during 50 h. The activity at other tissues as kidney, liver, lungs and bone marrow seemed to follow the activity in the blood.

The uptake ratio of intramuscular tumours on the injection side and the control side as function of time are given in Fig. 4. The highest ratio of seven was reached at 3 h. After 24 h the STU values of the two tumours were almost the same giving a ratio close to unity.

In order to evaluate the difference in image contrast between the injected and control leg the tumour to background ratio (here, tumour to muscle) on the injection side and the control side was calculated. The maximum contrast enhancement between the two sides was found at 3 h after injection with a value of three.

Tissue to plasma ratio. The tissue to plasma ratio was calculated according to our previous work (9, 10) and in

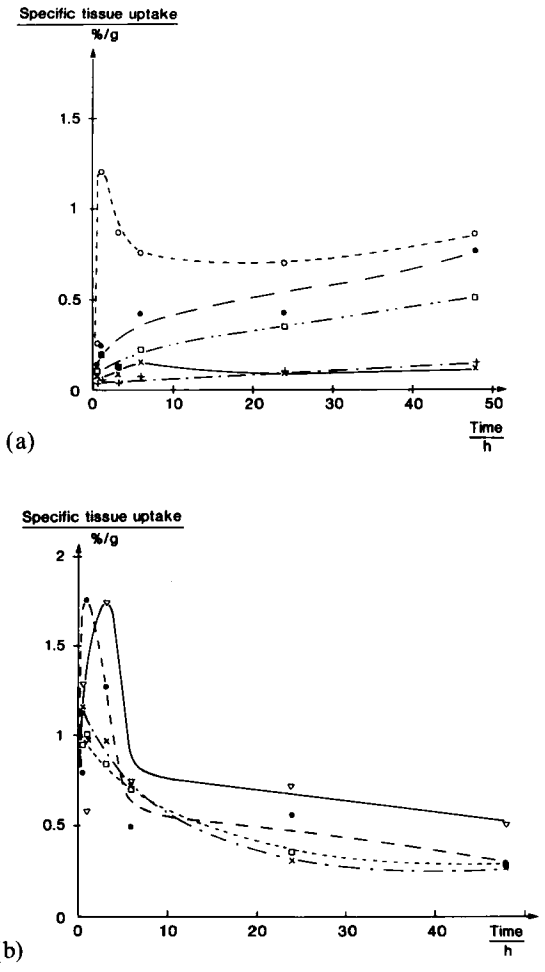

Fig. 3. Time activity curves for specific tissue uptake (STU) of ^{125}I-96.5 in tumours and different tissues after intraarterial injection of ^{125}I-96.5 MAb in the right leg. a) — · · — □ lymph nodes; —— × muscle injection; · — · — + muscle control; - - - - ○ tumour injection; — — ● tumour control. b) - - - - □ kidney dx; — · — × liver; —— ▽ lung; — — — ● bone marrow.

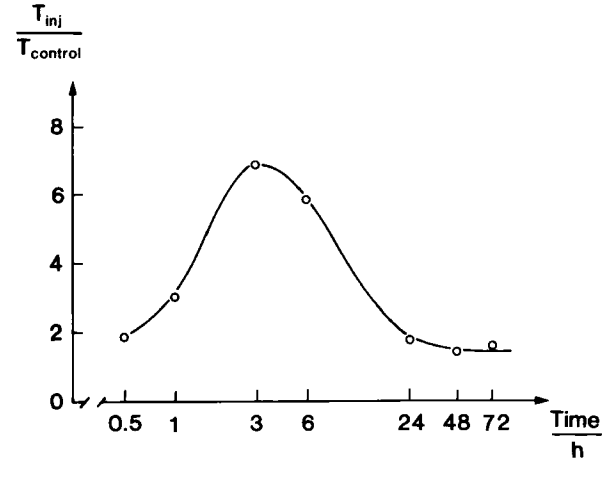

Fig. 4. Ratio between STU-values of ^{125}I-96.5 for tumour on injected and tumour on control side. Note the peak value at 3 h, whereafter the ratio falls and after 24 h equals unity.

Table

The time dependence of the tissue to plasma activity ratio for different modes of injection of ^{125}I labelled monoclonal antibody 96.5 in the nude rat

Tissue	Plasma(t)		
	i.v. injection*	s.c. injection*	i.a. injection**
Lung	0.30	0.30	0.28
Bone marrow	0.15	0.14	0.18
Liver	0.20	0.13	0.15
Lymph nodes	0.001t + 0.19	0.002t + 0.1	0.005t + 0.03
Muscle	0.001t + 0.02	0.001t + 0.01	0.002t
Tumour i.m.	0.45	0.45	—
Tumour injection	—	—	0.007t + 0.18
Tumour noninjection	0.02t + 0.05***	0.013t + 0.02***	0.006t + 0.06

*data see refs. 9 and 10.
**i.a. values 3–72 h only, compared to 24–200 h for i.v. and s.c. injection.
***6–24 h.

the table the equations for the fit of the data are given for different injection techniques and tissues. As can be seen, the tissue to plasma ratios of the specific tissue uptake is constant for lungs, liver and bone marrow and in the same order for the different modes of injection. The tumour to plasma ratio for the tumour on the injected side, as well as the control side, slowly increased over 72 h, indicating that the penetration and binding of the antibodies to the tumour cells is a slow process taking hours to days. A similar slow increase in the ratio is also seen for lymph nodes and muscle.

Discussion

Regional intraarterial injection of cytostatic drugs have been tried for a long time and are advocated by many authors (15). This, in combination with ischemia (degradable mircrospheres or venous tourniquet to obstruct the outflow), has been used by us and others (16, 17).

Weinstein et al. (5) showed that immunoscintigraphy with MAbs could visualize small tumours (2–6 mg) in lymph nodes in guinea pigs. In 1983, Strand et al. (18) showed in the nude rat that subcutaneous injection of monoclonal antibodies could image both tumours and single lymph nodes with a high uptake in an intramuscular tumour with a ratio to the muscle on the contralateral side of 40 : 1.

Since it is known that only a fraction of the injected activity is localized to the tumour after intravenous injection (19), a logical step would be to try to increase the concentration of the antibody by selective injection in the vessels supplying blood to the tumour. Possible advantages of regional injection in a therapy situation are that lower administered activity could be needed and that less competition by circulating antigen might occur. In order to further increase the passage of monoclonal antibodies over the capillary membrane the venous outflow was obstructed. Previous pharmacokinetic studies by us and others have shown that this diminishes the outflow, giving significantly lower systemic concentration of the cytostatic agent (16, 17).

The specific tissue uptake (STU) of the whole antibody 96.5-^{125}I for the tumour on the injected side are in accordance with the previous studies with i.v. and s.c. injections of the same MAb (9, 10). The relatively high initial STU in the bone marrow should be noted. The plasma and blood curves of the specific tissue uptake of ^{125}I-96.5 after this mode of injection was the same as after intravenous and subcutaneous administration, which indicates similar degradation and excretion of the radiolabelled monoclonal antibody after these types of administrations.

As in intravenously and subcutaneously injected rats (9, 10) we observed that the lymph nodes and muscle had a different MAb biokinetics. STU reached peak values much later than in other tissues where the uptake followed the plasma curve (Table).

The tumour uptake on the injected side was at the most seven times higher than on the control side (Fig. 4). When the background (muscle) was accounted for, the contrast enhancement ratio reached a peak value of 2.9 at three hours. This observation underlines the fact that there are additional factors beside immunologic ones which are of great importance for the activity uptake both in tumour and other tissues, such as blood flow and vascular permeability (8–10). The tumour to plasma ratio never exceeded unity after intraarterial injection, not even on the injected side, but a slow increase was seen over time ($T/P(t) = 0.007t + 0.18$, $r = 0.93$, $t < 72$ h, Table). Probably the penetration of the monoclonal antibodies from the capillaries to the tumour cells is a slow process, due to the large size of the macromolecules. IgG has been found to move slower than water by a factor of 10^5 over the capillary

membrane (20). The size of the openings in the endothelial walls varies giving different capillary filtration coefficients. The protein content in the interstitial fluid is directly proportional to the capillary filtration coefficient (20).

Moreover, this study has shown that a higher concentration of labelled monoclonal antibodies increases the passage to the tumour giving a better tumour to background ratio. The advantage, however, is not permanent. Fragments of antibodies would be expected to give even higher uptake in the tumours due to better penetration.

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