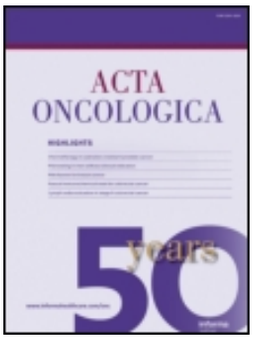

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Biokinetics of the Monoclonal Antibodies MOv 18, OV 185 and OV 197 Labelled with ^{125}I According to the m-MeATE Method or the Iodogen Method in Nude Mice with Ovarian Cancer Xenografts

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The biodistribution of the radiolabelled monoclonal antibodies MOv18, OV185 and OV197 in nude mice with subcutaneous tumours of the human ovarian cancer cell line OVCAR3 was investigated. The early uptake of MOv18 (1–24 h) and the uptake in relation to tumour size were also studied. The antibodies were labelled with ^{125}I according to the Iodogen method or the m-MeATE method, the latter also being suitable for labelling with ^{211}Astatine. The tumour/blood ratio and the localization index for Mov 18 72 h after antibody injection were 2.21 ± 0.25 and 4.62 ± 1.27, respectively. This is significantly higher than for the other two specific antibodies. The early tumour uptake of MOv18 was low with a tumour/blood ratio of 0.23 ± 0.04 after 6 h, and the uptake was higher in small tumours. The two labelling methods were found to be equivalent. We conclude that MOv18 labelled according the m-MeATE method should be suitable for further therapeutic studies with ^{211}Astatine in nude mice.

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Epithelial ovarian cancer is the leading cause of death in women with gynaecologic malignancy. Most of the patients (60–70%) have advanced disease at the time of diagnosis. The standard treatment for advanced ovarian cancer is maximally cytoreductive surgery followed by at least six months of cytotoxic intravenous chemotherapy. The response rate is high, but it is very difficult to completely eradicate the tumour and the 5-year survival is only about 25%. Hence there is a great need for alternative treatment methods. In most patients the tumour dissemination is restricted to the peritoneum and ovarian cancer is well suited for local treatment.

Treatment with monoclonal antibodies has been reported in several malignancies. Complete remission has been seen in lymphoid and haematologic malignancies but very seldom in solid tumours. Probably one can only expect to see a therapeutic effect of antibodies in patients with minimal residual tumour. Thus this therapy is well suited for treatment of cancer with micrometastases only. The antibody by itself can be cytotoxic but as a rule it is necessary to link it to toxic substances: radionuclides, antineoplastic drugs or biological toxins. Among the radionuclides, the beta-emitter ^{131}I has been best studied. However, this radionuclide has disadvantages and other radionuclides have been tried with promising results (1, 2). An interesting radionuclide is ^{211}Astatine (^{211}At), an alpha-emitter with a half-life time of 7.2 h (3). Compared to beta-emitters, ^{211}At has a considerably higher linear energy transfer and a short range of less than 100 microns. ^{211}At is probably suitable only for treatment of micrometastases.

There are several reports on radioimmunotherapy of ovarian cancer both experimentally in nude mice with xenografts of ovarian cancer cell lines and clinically in patients (4–6). An antibody with high specificity is probably a prerequisite for a good therapeutic effect and several monoclonal antibodies have been raised against different antigens on ovarian cancer cells. Among them OC 125 which binds to the surface antigen CA 125 is best studied. There are several other antibodies against the same antigen, which has two major antigenic domains, A and B. The antibodies are classified as OC 125-like (group A) or M11-like (group B). The antibody OV 197 is unique and

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binds to a third domain designated C. (7, 8) MOv18 is a monoclonal antibody reacting with a surface antigen which is expressed on about 90% of all human ovarian carcinomas (9). It has been used for immunoscintigraphy (10) and radioimmunotherapy (6) in patients with ovarian cancer, with promising results.

Conventionally, the antibodies have been labelled with Iodine according to the Iodogen method. A problem is the loss of the nuclide from the antibody leading to a high uptake in the thyroid and the stomach. Zalutsky et al. have developed several other binding methods, which give a more stable bound. These labelling methods have improved the affinity of the antibodies, which has resulted in increased uptake in tumour tissues. The ATE-method using N-succinyl-3-(tri-n-butylstannyl) benzoate is suitable for labelling antibodies with ^{211}At (11).

Tumour size seems to influence the uptake of radiolabelled antibody. In a study of the human colon, melanoma and lymphoid tumours growing in nude mice, Hagan et al. (12) have shown that the uptake of specific antibodies was inversely proportional to tumour size. This was less clear with an unspecific antibody. They state that small tumours of about similar size should be used in animal experiments. In another study Chiou has found that the calculated absorbed dose was higher and the therapeutic effect better when ^{131}I-labelled antibodies were given to nude mice with small xenografts of human renal cell carcinoma compared to heavier ones (13).

The aim of the present study was to find a suitable model for planned studies of the therapeutic efficacy of ^{211}At bound to monoclonal antibodies in nude mice with subcutaneous and intraperitoneal ovarian cancer xenografts. For this purpose we have investigated the binding of three different antibodies (MOv 18, OV 185 and OV 197) to OVCAR 3 cells in nude mice with subcutaneous tumours. The biodistribution of MOv 18 during the first 24 h after intravenous injection was studied and the Iodogen- and m-MeATE-binding methods were compared. Furthermore, we have studied the uptake in tumour xenografts in relation to tumour size.

MATERIAL AND METHODS

Monoclonal antibodies

MOv18 is a murine monoclonal antibody first characterized by Miotti et al. It recognizes a membrane folate-binding glycoprotein of 38 kDa and reacts with about 90% of human ovarian carcinomas (9, 14, 15). The antibody was kindly provided by Professor S. O. Warnar, Centocor B/V, Leyden.

The murine antibody OV 185 binds to the B and OV 197 to the C antigenic domains of the ovarian cancer surface antigen CA 125 (7). C242 is an antibody that recognizes a membrane glycoprotein on cells of human colon, pancreatic and cervical cancers. It does not bind to ovarian cancer cells. The antibodies were kindly provided by CanAg Diagnostics, Gothenburg (OV 185 and OV 197) and by Pharmacia AB, Lund (C242).

Labelling of antibodies

The antibodies were labelled using the intermediate reagent N-succinimidyl-3-(trimethyl stannyl)benzoate, m-MeATE, earlier described (16). The ester, 0,5 nmole in chloroform, was added to a glass vial (Chromacol, UK) and the solvent was evaporated under a gentle stream of nitrogen. Then 10 μl (35 nmole) of chloramine-T in ethyl acetate (EtOAc), dimetylformamide (DMF) and acetic acid (HAc) with a volume ratio of 94:5:1 was added to the vial. Carrier-free Na^{125}I (IMS 300, Amersham, UK), was added (1–2 μl = 5–15 MBq) and the reaction was allowed to proceed for 15 min under agitation. The reaction was stopped by adding 1 μl (35 nmole) of $Na_2S_2O_5$ and the organic fraction was evaporated. Antibody (50–100 μg) was then added to the crude labelling mixture. The conjugation mixture was incubated for 30 min under gentle agitation at room temperature.

For a head to head comparison between the conventional and conjugate methods, antibodies were also subjected to conventional iodination using Iodogen as oxidizing agent. Iodogen, 10 μg, was immobilized in Eppendorf tubes. Antibody (50–100 μg) and 5–15 MBq Na^{125}I(IMS 30, Amersham, UK) or 10–20 MBq Na^{131}I(IBS30, Amersham UK) was added to the precoated tubes. The reaction was allowed to proceed for 2 min at room temperature.

The labelled antibodies were purified by passage over a Sephadex G-25 PD-10 Column (Pharmacia, Sweden) and analysed by TCA precipitation. The immunoreactivity of all labelled antibodies was determined by a cell-binding assay using OVCAR-3 cells. In some cases the immunoreactivity fraction was established according to Lindmo et al. (17).

Tumours and animals

Xenografts were established from the human ovarian cancer cell line NIH:OVCAR 3 injected subcutaneously (s.c.) in both flanks of 7–8 week-old Balb/c nu/nu mice. The tumours were serially transferred by implanting fragments of tumour tissue with a diameter of 2–3 mm s.c. into one or both flanks of subsequent mice under anaesthesia. At the end of the experiments the mice were killed by cervical dislocation. Blood was collected and tumours and organs were dissected and weighed.

Measurement of radioactivity

All measurements of radioactivity were performed using a gamma counter (Wizard 1480, Wallace) and a dose calibrator (Capitec CRC-15R, ScanflexMedical).

Corrections for background and radioactive decay were made for all measurements.

Uptake of the radionuclide was expressed as the fraction of the injected activity per unit mass of the blood and tissue (%ID/g). The tissue to blood ratio (%ID/g_{tissue}/%ID/g_{blood}) and localization index were calculated. The latter was defined as the tumour to blood ratio for specific antibody divided by the tumour to blood ratio for unspecific antibody.

Biodistribution studies

Comparison of four antibodies (experiment 1). About 4 weeks after transplantation, 13 animals were injected via the tail vein with 0.05 ml containing 2 μg ^{125}I-labelled specific antibody MOv 18, OV 197 (4 animals with each antibody) or OV 185 (5 animals). All the animals were also injected with the unspecific antibody C242 labelled with ^{131}I. All antibodies were labelled according to the Iodogen method. The injected activity varied between 107 and 387 kBq for ^{125}I and 209 and 292 kBq for ^{131}I. After 72 h the mice were killed. All the animals except one had two tumours. The tumour weight at the time of removal was 0.138 g $\pm$ 0.092 g (mean and SD) and varied between 0.090 and 0.380 g.

Comparison of two labelling methods: m-MeATE and Iodogen (experiment 2). Six tumour-bearing mice were injected with ^{125}I-MOv18 labelled according to the m-MeATE or the Iodogen-method, 3 in each group. The injected activities were 155–166 kBq and 410–432 kBq, respectively. Seventy-two hours later the animals were killed The animals had bilateral tumours and the tumour weight was 0.558 $\pm$ 0.422 g (range 0.060–1.446 g).

Early uptake of MOv 18 (experiment 3). Twelve mice with bilateral tumours were injected with ^{125}I-MOv 18 labelled according to the m-MeATE method. The injected activity was 159–185 kBq. After 1, 3, 6 and 24 hours the mice were killed, 3 at each time point. The tumour weight was 0.527 $\pm$ 0.284 g (range 0.118–1.212).

Uptake in relation to tumour size (experiment 4). Forty-five mice were transplanted bilaterally with fragments of tumour tissue. Two to three weeks later they were injected with ^{125}I antibody, labelled according to the m-MeATE method. Six, 24 and 72 h after injection, 25, 10 and 10 mice were killed and handled as above. There were 35, 17 and 18 tumours weighing 0.053 $\pm$ 0.034, 0.084 $\pm$ 0.057 and 0.130 $\pm$ 0.071 in the three groups, respectively. Some of the animals had no or only one viable tumour. The injected activity was 30–58 kBq in the first and 133–180 kBq in the other two groups.

RESULTS

Radioiodination

Radioiodination of the antibodies gave a labelling yield of 40–70% for the conjugate method and 50–80% for the Iodogen method. The radiochemical purity was always over 95% as determined by TCA precipitation.

Table 1

Tissue distribution of intravenously administered ^{125}I-labelled(Ov187, OV195, MOv18) or ^{131}I-labelled(C242) antibody (% ID/g, mean and SD, n = 13 mice) after 72 h

Organ	Antibody			
	OV 185	OV 197	MOv 18	C 242
Blood	2.84 $\pm$ 0.58	1.98 $\pm$ 0.12	2.36 $\pm$ 0.17	5.11 $\pm$ 1.03
Lungs	1.06 $\pm$ 0.41	0.67 $\pm$ 0.18	0.77 $\pm$ 0.22	1.67 $\pm$ 0.70
Liver	0.66 $\pm$ 0.13	0.52 $\pm$ 0.06	0.48 $\pm$ 0.07	1.19 $\pm$ 0.23
Spleen	0.55 $\pm$ 0.13	0.44 $\pm$ 0.08	0.47 $\pm$ 0.05	0.98 $\pm$ 0.24
Kidney	0.49 $\pm$ 0.08	0.49 $\pm$ 0.12	0.53 $\pm$ 0.10	1.07 $\pm$ 0.22
Muscle	0.17 $\pm$ 0.04	0.24 $\pm$ 0.05	0.24 $\pm$ 0.08	0.43 $\pm$ 0.13
Tumour	2.12 $\pm$ 0.42	1.93 $\pm$ 0.32	5.21 $\pm$ 0.74	2.23 $\pm$ 0.65

All antibodies showed specific binding to the OVCAR-3 cells, with cell-bound fractions from 8 to 89%. Immunoreactive fractions ranged from 0.33 to 0.89 for OV185, 0.08 to 0.59 for MOv18 and 0.21–0.41 for OV197.

Biodistribution

Comparison of four antibodies. The uptake of ^{125}I- or ^{131}I-labelled antibody 72 h after injection is shown in Table 1. The highest uptake in tumour tissue was for MOv 18 with 5.21 $\pm$ 0.74% ID/g compared with 2.12 $\pm$ 0.42, 1.93 $\pm$ 0.32 and 2.23 $\pm$ 0.65 for OV 185, OV 197 and C242, respectively. Table 2 shows the mean tumour/blood ratio. The ratio was 2.21 $\pm$ 0.25 for MOv 18, 0.76 $\pm$ 0.17 for OV185, 0.97 $\pm$ 0.17 for OV 197 and 0.45 $\pm$ 0.15 for C242. The differences between MOv 18 and the other antibodies were statistically significant ($p < 0.0001$, unpaired t-test). The localization index was 4.62 $\pm$ 1.27 for MOv 18 compared with 2.41 $\pm$ 1.02 for OV185 ($p = 0.0008$) and 2.09 $\pm$ 0.64 for OV197($p = 0.0004$).

Comparison of the m-MeATE and Iodogen labelling methods. Seventy-two hours after injection the biodistribution of m-MeATE and Iodogen-labelled MAbs was compared. Three mice with bilateral tumours were used in each group. The tumour uptake and tumour/blood ratio are

Table 2

Tissue to blood ratio of intravenously administered ^{125}I-labelled(OV187, OV195, MOv18) or ^{131}I-labelled (C242) antibody (mean and SD, n = 13 mice) after 72 h

Organ	Antibody			
	OV 185	OV 197	MOv 18	C242
Blood	1	1	1	1
Lungs	0.36 $\pm$ 0.06	0.33 $\pm$ 0.07	0.32 $\pm$ 0.08	0.32 $\pm$ 0.07
Liver	0.23 $\pm$ 0.01	0.26 $\pm$ 0.02	0.20 $\pm$ 0.03	0.23 $\pm$ 0.02
Spleen	0.19 $\pm$ 0.01	0.22 $\pm$ 0.03	0.20 $\pm$ 0.03	0.19 $\pm$ 0.02
Kidney	0.17 $\pm$ 0.02	0.25 $\pm$ 0.06	0.23 $\pm$ 0.05	0.21 $\pm$ 0.05
Muscle	0.06 $\pm$ 0.01	0.12 $\pm$ 0.03	0.18 $\pm$ 0.04	0.09 $\pm$ 0.03
Tumour	0.76 $\pm$ 0.17	0.97 $\pm$ 0.17	2.21 $\pm$ 0.25	0.45 $\pm$ 0.15

Table 3

Comparison of the Iodogen and ATE labelling methods. Tissue distribution (%ID/g) and tissue to blood ratio of intravenously administered ^{125}I-labelled MOv18 (mean and SD, n = 3 mice for each group) after 72 h

Organ	Tissue distribution		Tissue to blood ratio	
	Iodogen	ATE	Iodogen	ATE
Blood	7.21 ± 2.71	6.56 ± 1.89	1	1
Lungs	3.22 ± 1.59	2.64 ± 0.34	0.45 ± 0.20	0.42 ± 0.11
Liver	1.64 ± 0.60	1.21 ± 0.18	0.23 ± 0.03	0.19 ± 0.06
Spleen	1.36 ± 0.30	1.28 ± 0.37	0.20 ± 0.04	0.22 ± 0.13
Kidney	1.57 ± 0.42	1.27 ± 0.22	0.22 ± 0.04	0.20 ± 0.06
Muscle	1.01 ± 0.32	0.86 ± 0.24	0.15 ± 0.06	0.13 ± 0.02
Tumour	10.47 ± 3.18	9.90 ± 4.24	1.59 ± 0.23	1.47 ± 0.33

shown in Table 3. The uptake in tumour tissue was 9.90 ± 4.24 and 10.47 ± 3.18%ID/g, respectively. The uptake in other tissues and the tissue/blood ratios were also very similar for the two methods.

Early uptake of MOv18. The biodistribution of ^{125}I-labelled MOv 18 during the first 24 h after intravenous injection is shown in Table 4 and Fig. 1. The uptake in tumour tissue was low after one hour but increased to 7.69 ± 2.46% ID/g after 24 h. The tumour/blood ratio was 0.74 ± 0.20 at the same time point (Table 5 and Fig. 1).

Uptake in relation to tumour size. The uptake of radiolabelled antibody after 6 h was 6.00 ± 2.15% ID/g tissue (range 2.37%–11.60%) and the tumour/blood ratio 0.39 ± 0.14. The correlation between uptake and tumour size was weak with a correlation coefficient of −0.321 (Fig. 2). The uptake and tumour/blood ratio were significantly higher than in the larger tumours in experiment 3 ($p = 0.015$ and 0.011, respectively) at the same time point. After 24 and 72 h the correlation was slightly better with correlation coefficients of −0.439 and −0.558 (Fig. 3, 72 h). For the correlation between the tumour/blood ratio and tumour weight, the correlation coefficients were −0.277, −0.422 and −0.606 after 6, 24 and 72 h, respectively.

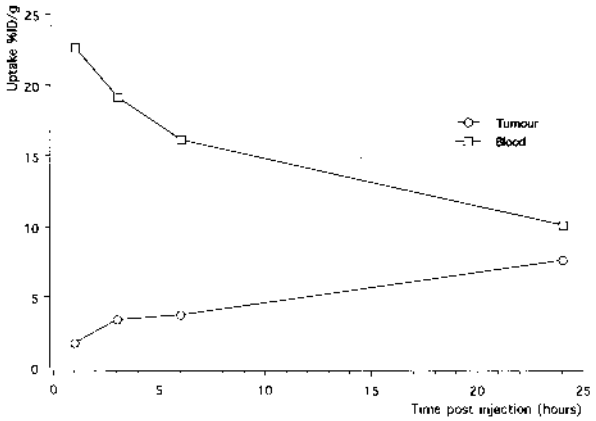

Fig. 1. Uptake of labelled MOv 18 given as mean %ID/g vs. time post-injection.

DISCUSSION

There are several specific monoclonal antibodies raised against human ovarian cancer cells. MOv18, which reacts with a folate-binding protein on the surface of about 90% of all ovarian cancers, was characterized by Miotti et al. (14). Mov18 is well studied and has been used for immunoscintigraphy (10) and clinical radioimmunotherapy studies (6).

The two antibodies OV185 and OV197 have been developed by CanAg Diagnostics and are much less studied. However, they are directed against the same antigen as OC125, which has been used in several experimental and clinical studies (18–21).

We tried to estimate the immunoreactivity for MOv18 on OVCAR3 cells according to Lindmo (17). However, the fraction varied between 0.08 and 0.59 in successive experiments. Other authors have also reported low and variable figures. Mantovani et al. (22) have found an immunoreactivity of 12 to 21% on OvCa432 and between 22 and 56% on IGROV1-cells. The same authors state that the OVCAR3 cells have a high spontaneous release of the antigen recognized by MOv18 during the incubation period (23).

Table 4

Tissue distribution of intravenously administered ^{125}I-labelled MOv18 (%ID/g, mean and SD, n = 12 mice)

Organ	Hours post injection			
	1	3	6	24
Blood	22.58 ± 6.84	19.19 ± 1.05	16.21 ± 0.92	10.12 ± 0.97
Lungs	10.52 ± 3.76	12.28 ± 1.58	8.15 ± 0.32	4.65 ± 1.05
Liver	5.26 ± 1.33	5.32 ± 0.81	4.26 ± 0.36	2.20 ± 0.47
Spleen	3.66 ± 1.08	3.77 ± 0.56	4.19 ± 0.07	2.23 ± 0.38
Kidney	5.18 ± 1.57	4.39 ± 0.42	3.69 ± 0.33	2.01 ± 0.21
Muscle	0.78 ± 0.09	1.03 ± 0.25	1.59 ± 0.44	1.19 ± 0.17
Tumour	1.79 ± 0.81	3.42 ± 0.48	3.76 ± 0.60	7.69 ± 2.46

Table 5

Tissue to blood ratio of intravenously administered ^{125}I-labelled MOv18 (mean and SD, n = 12 mice)

Organ	Hours post injection			
	1	3	6	24
Blood	1	1	1	1
Lungs	0.46 ± 0.07	0.64 ± 0.02	0.50 ± 0.05	0.46 ± 0.09
Liver	0.24 ± 0.02	0.28 ± 0.03	0.26 ± 0.03	0.22 ± 0.03
Spleen	0.16 ± 0.00	0.20 ± 0.03	0.26 ± 0.02	0.22 ± 0.02
Kidney	0.23 ± 0.01	0.23 ± 0.01	0.23 ± 0.02	0.20 ± 0.01
Muscle	0.04 ± 0.01	0.05 ± 0.01	0.10 ± 0.02	0.12 ± 0.01
Tumour	0.08 ± 0.02	0.18 ± 0.02	0.23 ± 0.04	0.74 ± 0.20

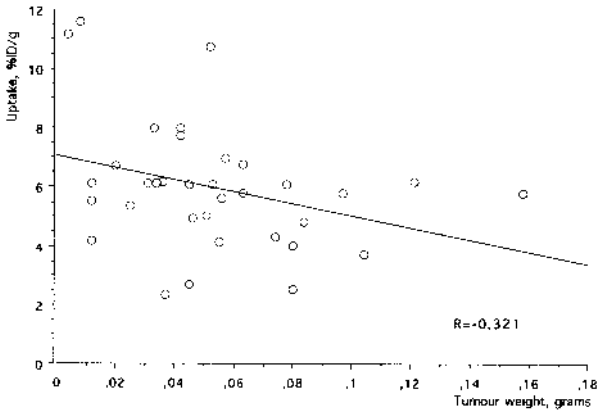

Fig. 2. Correlation between tumour weight and uptake of radiolabelled Mov18 6 h after injection.

This could probably explain the low and variable immunoreactivity seen in other studies as well as our own. The tumour/blood quotient for Mov 18 72 h after injection was 2.21 ± 0.25. This is higher than the quotient for the other two specific antibodies OV185 and OV197 and for the unspecific antibody C242. The localization index was 4.64 ± 1.27 for MOv18, which is about twice as high as that for the other two specific antibodies. This is comparable to other studies using MOv18 or other specific antibodies (24–26). However, for therapy studies with a short-lived nuclide such as ^{211}At, with a half-life of 7.2 h, the biodistribution during the first 24 h after injection is crucial. The early uptake in tumour tissue was low, as suspected, with a tumour/blood ratio of only 0.23 ± 0.04 (0.39 ± 0.14 for small tumours) after 6 h and 0.74 ± 0.20 after 24 h. This makes it difficult to carry out treatment studies on subcutaneous tumours using ^{211}At bound to intact antibody. However, this does not rule out that ^{211}At is a good candidate for intraperitoneal treatment of micrometastases.

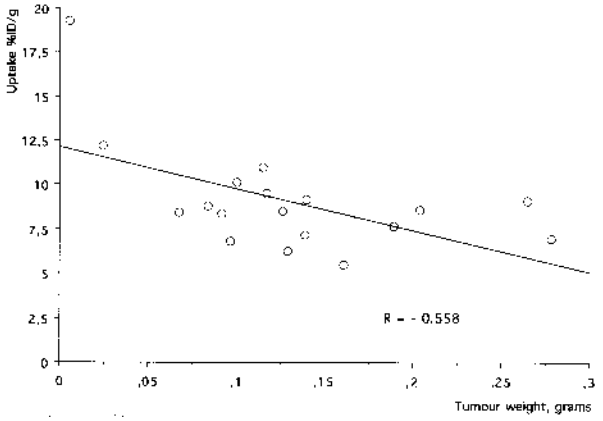

Fig. 3. Correlation between tumour weight and uptake of radiolabelled Mov 18 72 h after injection.

The uptake of radiolabelled antibody in small tumours 6 h after injection varied considerably and there was no correlation between tumour size and uptake. However, when comparing two experiments with different mean tumour sizes, the uptake and tumour/blood quotient were significantly higher in the small tumours at the same time point. Seventy-two hours after injection a weak correlation was seen with a coefficient of -0.558. This is in accordance with other studies, in which an inverse relationship between tumour size and antibody uptake has been found, probably due to better blood perfusion in small tumours (12, 27).

The antibodies were labelled according to the Iodogen method and the m-MeATE method, a modification of the ATE method, described by Zalutsky et al. (11, 28). The uptake of antibody after 72 h in subcutaneous tumours and normal organs was similar with the two methods. This is contrary to the results of Zalutsky et al. In one study nude mice with xenografts of the ovarian cancer cell line OVCAR 3 were injected with $F(ab')_2$ fragments of the monoclonal antibody OC 125 labelled according to the two methods. With the ATE method there was a decrease in thyroid uptake and an increase in tumour uptake, and a superior tumour to tissue ratio was seen. Zalutsky et al. concluded that this was probably due to a combination of improvements in inertness to deiodination in vivo, rate of clearance of labelled catabolites and antibody affinity (29). Similar results were seen in another study of the antibody 81C6 in nude mice with xenografts of a human glioma cell line (30).

The interpretation of our results is that MOv 18 is a suitable antibody for further studies. The Iodogen and m-MeATE labelling methods are equivalent with respect to antibody kinetics and capacity to bind to tumour tissue. An advantage of the m-MeATE method is that the iodide is not released (not shown in this study). We conclude that it should be possible to label the antibodies with ^{211}At according to the m-MeATE method with preserved high binding capacity.

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