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Preparation and quality control of ^{211}At-labelled and ^{125}I-labelled monoclonal antibodies. Biodistribution in mice carrying human osteosarcoma xenografts.

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Summary.

Two anti-osteosarcoma monoclonal antibodies (TP-3 IgG and TP-1 F(ab')$_2$) were labelled with the α-particle emitting radionuclide ^{211}At and, for comparison of stability, with ^{125}I using the N-succinimidyl-3-(trimethylstannyl)benzoate intermediate. The quality of the final preparations was measured with immunoreactivity analyses using intact osteosarcoma cells. Immunoreactivity was well retained with values in the range of 65% to 85% for ^{211}At-labelled and ^{125}I-labelled TP-3 IgG and approximately 60% for both ^{211}At-labelled and ^{125}I-labelled TP-1 F(ab)$_2$. Tumour uptake and retention as well as normal tissue distribution in mice with osteosarcoma xenografts were measured. The uptake of the two radionuclides in tumour was similar, while there was a slight general increase in normal tissue activity at later points for the ^{211}At-labelled MoAbs compared to the ^{125}I-labelled MoAbs, probably caused by a minor release of free ^{211}At from the

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MoAb preparations. The stable retention in tumor tissue demonstrated in this study indicates that ^{211}At-labelled MoAbs may have potential in the treatment of tumours that allow a rapid uptake.

Key words: Astatine-211; α-particle emitting radioimmunoconjugate; osteosarcoma.

Introduction.

Since the advent of the hybridoma technology (1) monoclonal antibodies (MoAbs) have been extensively studied as carriers of radionuclides for diagnostic and therapeutic applications (2,3). Clinical radioimmunotherapy (RIT) with MoAbs has been partly successful (4-6), but many problems concerning their practical use are still unsolved (7). One problem is the *in vivo* detachment of the radionuclide from the conjugate observed for the most frequently used labelling methods (9). Another problem is the low dose rate delivered to single tumour cells and micrometastases from the β-emitting radionuclides used up to now (8).

The latter problem with RIT may be overcome by introducing α-particle emitters instead of the β-emitters used today, since the radiotoxicity of α-particles is virtually independent of dose rate (10). Moreover, the high energy radiation is absorbed within a radius of < 100 μm from the site of disintegration indicating that a very limited number of disintegrations has to occur nearby a cell to cause cell-death (11).

In recent years a new method for labelling MoAbs with the α-emitting radionuclide ^{211}At based on the bifunctional compound N-succinimidyl-3-(trimethylstannyl)benzoate has been developed (12,13). This method (termed the ATE-method) is a two-step procedure. Firstly, the trimethylstannyl group is substituted with ^{211}At and the product, N-succinimidyl-3-[^{211}At]astatobenzoate is purified. Secondly, the N-succinimidyl ester function is used to conjugate the astatobenzoic unit to lysine residues on MoAbs under mildly basic conditions. This method has been reported to give ^{211}At-MoAb conjugates with well preserved immunoreactivity and *in vivo* localizing capacity (14).

In an earlier report it was demonstrated that the anti-sarcoma MoAbs TP-3 and TP-1 labelled with ^{125}I and ^{131}I using the Iodo-Gen method localize selectively in osteosarcoma xenografts at day 1 to 4 after intravenous administration (15). In the present study we wanted to investigate the biodistribution of TP-3 IgG and TP-1 $F(ab')_2$ within the timeframe relevant to the 7.21 h halflife of ^{211}At, in order to evaluate these MoAbs as carriers for this radionuclide. To gain information about the stability of the conjugate, the biodistribution of the ^{211}At-MoAbs was at selected time points compared to that of ^{125}I-MoAbs also labelled using the NSTB intermediate.

Materials and methods.

Monoclonal antibodies

TP-1 (IgG 2a) and TP-3 (IgG 2b) are two antibodies which bind to two different epitopes on an antigen expressed on the surface of osteosarcoma cells (16). The production and purification of the antibodies has been described elsewere (17). The $F(ab')_2$ fragments were obtained by digestion with pepsin as previously described (15).

Radiolabelling of monoclonal antibodies

^{211}At was produced at the cyclotron at the Department of Physics at Oslo University using the $^{209}Bi(\alpha,2n)^{211}At$ reaction at 28 MeV α-energy. The radionuclide was separated from the bismuth target using a dry distillation procedure previously described in detail (18). The labelling of MoAbs was performed according to described procedures (14) Briefly, ^{211}At in 0.3 ml chloroform was added to 0.5 μmol N-succinimidyl-3-(trimethylstannyl)benzoate (NSTB) and 30 μmol *tert*-butylhydroperoxide and thoroughly mixed for 20 min. Then the chloroform was removed by evaporation and the product, N-succinimidyl-[^{211}At]astatobenzoate, was purified on a sep-pak silicagel cartridge (Waters) by elution with 30 ml hexane, 30 ml 8% ethylacetate in hexane and finally 12 ml of 30% ethylacetate in hexane collected in 1 ml samples. Samples 3-5 contained the main activity and were pooled into a reaction vial and evaporated to dryness.

Approximately 0.4 mg of TP-3 IgG or TP-1 F(ab')$_2$ antibody in 100 μl of borate buffer (pH 8.5-9.0) was added to the reaction vial which was then incubated on a shaker for 20 min before the reaction was terminated with 0.3 ml 0.2 M glycine in borate buffer (pH 8.5-9.0). The labelled MoAb was then purified on a Sephadex G-25 PD 10 column eluted with 0.1 M phosphate buffered saline (PBS, pH 7.4). The labelling with ^{125}I (Amersham) was done in a similar manner except that an aqueous ^{125}I solution was added to the chloroform containing the reactants. The pH was adjusted to 5-6 by means of acetic acid at the start of the reaction with NSTB.

Immunoreactivity measurement

The quality of the preparations was controlled with immunoreactivity measurements using OHS osteosarcoma cells according to published procedures (19). Briefly, 0.25 ml in 2 ml test tubes in triplicates of seven different concentrations varying between $9 \cdot 10^4$ and $6 \cdot 10^6$ cells per ml were incubated with a fixed amount of radiolabelled MoAb. After 2 h the incubation was stopped and the the total radioactivity per test tube was measured. Cells were washed three times with PBS, and the cell-bound radioactivity was then measured. The immunoreactivity was determined at infinite antigen excess by linear extrapolation to the ordinate of the regression line derived from the experimental points (Figure 1). Binding experiments were also carried out with antigen negative cells to estimate cross-reactivity. The K13 murine hybridoma cell line (20) was used as negative control.

Biodistribution measurements

Male BALB/c athymic (*nu/nu*) mice with a bodyweight of 25-30 g carrying one or two OHS human osteosarcoma xenografts were used in the biodistribution studies. The biodistribution experiments were performed two to three weeks after subcutaneous implantation of tumour tissue, when the tumour sizes varied from 5-20 mm in diameter. Animals were selected to give both small and large tumours within the same group. The

radiolabelled antibody preparations were injected into the tail vein. Animals were sacrificed by cervical dislocation and dissected at different times within the first 42 h after administration of the antibodies.

Statistical evaluation

The students t-test with the level $P < 0.05$ was used to determine any significant difference between the biodistribution values.

Results.

The production yield of ^{211}At measured on target after the end of the cyclotron irradiations was 11±2 MBq/(μA•h) (mean ± SD) as measured from two experiments. After distillation for 1 h, 40-60% of the target activity was recovered in the chloroform from which 40-60% was finally measured in the purified ^{211}At-MoAb conjugate fraction after the labelling procedure. This gives an overall yield from target to final MoAb-preparation of 16-36% (12-27% decay-corrected).

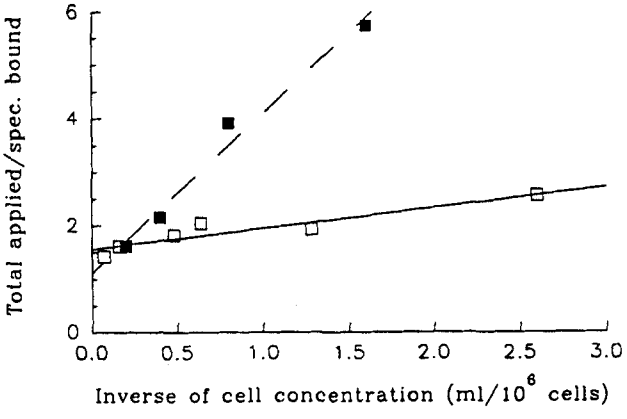

Figure 1 Immunoreactivity for incubation of 10 ng/ml ^{211}At-labelled TP-3 IgG (□, continous line) and 110 ng/ml ^{125}I-labelled TP-3 IgG (■, dashed line) with various concentrations of OHS osteosarcoma cells. Each point represents the mean of a triplicate.

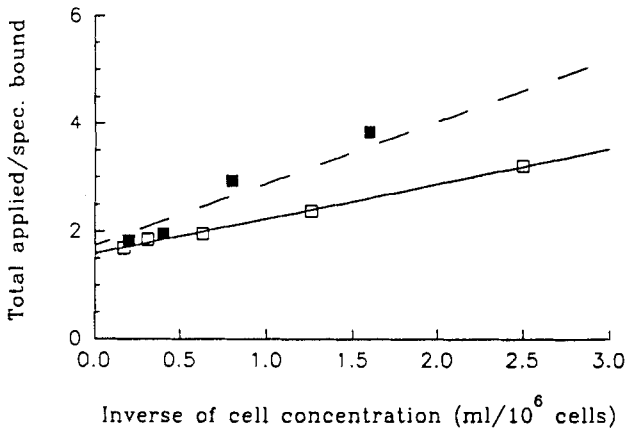

Figure 2 Immunoreactivity for incubation of 21 ng/ml of ^{211}At-labelled TP-1 F(ab')$_2$ (□, continous line) and 40 ng ^{125}I-labelled TP-1 (Fab')$_2$ (■, dashed line) with various concentrations of OHS osteosarcoma cells. Each point represents the mean of a triplicate.

Immunoreactivity plots for TP-3 IgG and TP-1 F(ab')$_2$ labelled with ^{211}At and ^{125}I are presented in Figure 1 and Figure 2. The immunoreactivity for the batch with ^{211}At-TP-3 IgG was approximately 60% and for the batch with ^{125}I-TP-3 IgG approximately 80%. The immunoreactivities for ^{211}At-TP-1 F(ab')$_2$ and ^{125}I-TP-1 F(ab')$_2$ were approximately 60% for both batches. Cross-reactivity with antigen negative cells was less than 5%.

The biodistribution in terms of percent of injected dose per g tissue (% ID/g) for ^{211}At-TP-3 IgG and ^{211}At-TP-1 F(ab')$_2$ is presented in Table 1 and Table 2. The uptake of ^{211}At-labelled MoAbs in different tissues was measured up to 40 h in order to more clearly detect any dehalogenation which would give an increased uptake in tissues accumulating elementary astatine (e. g. stomach and neck including thyroid).

The tumour uptake of ^{211}At-TP-3 IgG increased up to 16 h, whereafter the level was essentially stable up to 40 h post injection. The ^{211}At-TP-3 level in blood was high up to 24 h but decreased significantly from 24 h to 40 h. The other tissues had a clearance similar to the blood. Exceptions were stomach and neck (i.e. thyroid) where the level of radioactivity was maintained up to 40 hours.

Table 1 Distribution of ^{211}At-labelled TP-3 IgG in athymic mice with OHS osteosarcoma xenografts[a]

tissue	3.5 h	16 h	24 h	40 h
blood	20.2 ± 5.4	17.8 ± 3.9	15.1 ± 4.3	7.3 ± 4.3
heart	7.3 ± 2.0	4.8 ± 1.3	3.7 ± 0.3	1.9 ± 0.5
kidney	9.6 ± 3.7	4.4 ± 0.4	5.0 ± 0.6	2.7 ± 1.0
liver	6.3 ± 1.3	3.4 ± 0.3	3.5 ± 0.1	1.3 ± 0.5
lungs	11.4 ± 2.0	7.5 ± 1.0	9.1 ± 2.0	4.9 ± 1.2
neck	11.6 ± 8.5	5.4 ± 4.8	10.0 ± 4.6	5.8 ± 3.7
spleen	7.5 ± 3.4	5.0 ± 0.6	6.8 ± 4.2	3.1 ± 0.4
stomach	8.2 ± 6.0	4.9 ± 1.7	6.5 ± 1.3	5.8 ± 0.9
tumour	4.6 ± 2.6	10.3 ± 2.2	13.9 ± 4.3	11.0 ± 5.5

[a]Results were obtained for n = 3 mice per point and are given as mean ± SD of the percent injected dose/g (% ID/g), corrected for decay of ^{211}At.

The retention of activity in tumour was high without any significant difference at the points measured after injection of ^{211}At-TP-1 F(ab')$_2$, although the blood clearance was quite rapid with a decrease from 12.1% ID/g at 3.5 h to 2.5% ID/g at 16 h. The different normal tissues showed some retention of activity compared to blood while neck and stomach had some accumulation of activity.

Table 2 Distribution of ^{211}At-labelled TP-1 F(ab')$_2$ in athymic mice with OHS osteosarcoma xenografts[a]

tissue	3.5 h	16 h	24 h	40 h
blood	12.1 ± 2.5	2.5 ± 0.7	1.6 ± 0.7	0.7 ± 0.1
heart	4.7 ± 0.8	1.5 ± 0.6	1.5 ± 0.8	0.6 ± 0.3
kidney	7.8 ± 2.1	2.8 ± 1.2	2.0 ± 0.4	1.4 ± 0.1
liver	7.2 ± 2.0	1.8 ± 0.3	1.2 ± 0.3	0.7 ± 0.1
lungs	8.4 ± 2.5	3.6 ± 2.2	4.5 ± 1.9	3.9 ± 1.0
neck	3.5 ± 2.2	4.3 ± 3.5	26.8 ± 16.3	8.2 ± 7.7
spleen	7.5 ± 1.6	3.9 ± 1.0	3.3 ± 1.2	2.0 ± 0.5
stomach	3.3 ± 2.0	5.3 ± 1.2	4.3 ± 2.3	6.7 ± 2.2
tumour	6.1 ± 2.2	7.2 ± 2.2	5.5 ± 1.6	5.2 ± 1.1

[a]Results were obtained for n = 3-5 mice per point and are given as mean ± SD of the percent of injected dose/g (% ID/g), corrected for decay of ^{211}At.

The biodistribution of the two different ^{211}At-labelled antibodies was similar at 3.5 h except for blood where ^{211}At-TP-3 IgG had significantly higher values. At later time-points ^{211}At-TP-3 IgG gave in general higher tissue activity than ^{211}At-TP-1 F(ab')$_2$. This was probably caused by a more rapid renal clearance of the smaller F(ab')$_2$ molecule reflected by the difference in radioactivity in the blood at the later time-points.

Table 3 Distribution of ^{125}I-labelled TP-3 IgG and ^{125}I-labelled TP-1 F(ab')$_2$ in athymic mice with osteosarcoma xenografts[a]

	3.5 h		24 h	
tissue	TP-3 IgG	TP-1 F(ab')$_2$	TP-3 IgG	TP-1 F(ab')$_2$
blood	18.1 ± 2.0	11.0 ± 2.2	12.9 ± 3.5	1.7 ± 0.9
heart	3.9 ± 1.1	3.7 ± 0.5	3.3 ± 0.7	0.4 ± 0.2
kidney	4.5 ± 0.6	6.5 ± 1.0	4.8 ± 1.5	1.0 ± 0.2
liver	4.2 ± 0.9	4.9 ± 0.7	3.3 ± 1.1	0.5 ± 0.1
lungs	5.0 ± 1.4	8.5 ± 1.5	4.6 ± 1.3	1.6 ± 1.2
neck	3.1 ± 0.9	3.3 ± 0.7	5.2 ± 1.4	0.8 ± 0.1
spleen	3.7 ± 0.5	4.9 ± 1.2	3.4 ± 1.0	0.8 ± 0.3
stomach	0.9 ± 0.4	0.8 ± 0.1	0.9 ± 0.4	0.2 ± 0.1
tumour	6.0 ± 4.0	4.1 ± 1.9	18.7 ± 5.8	4.1 ± 1.4

[a]Results were obtained for n = 3-6 mice per point and are given as mean ± SD of the percent injected dose/g (% ID/g), corrected for decay of ^{211}At.

The biodistribution of ^{125}I labelled TP-3 IgG and TP-1 F(ab')$_2$ is presented in Table 3. Generally, the distribution was similar to that of the ^{211}At-MoAbs. There was a significantly higher uptake of radioactivity in tissues like stomach, neck and lung at late points associated with the ^{211}At-MoAb conjugates compared to the ^{125}I-MoAb conjugates.

Discussion.

The immunoreactivity plots of ^{211}At-labelled and ^{125}I-labelled TP-3 vary due to differences in concentration of MoAb, but this difference does not influence the interception value with the ordinate (19). The difference in immunoreactivity of the ^{211}At-TP-3 and the ^{125}I-TP-3 is most likely caused by a difference in the amount of free radionuclide in the two batches. Because of differences between I^- and At^- in affinity to sulfhydryl groups on proteins the gel filtration purification may be less effective as a means of separating free ^{211}At from MoAb (21). The use of HPLC separation to purify the NS[^{211}At]AB before MoAb conjugation (14) and another HPLC separation to purify the final radioimmunoconjugate (22) may give higher quality preparations.

The biodistributions of ^{211}At-TP-3 IgG and ^{211}At-TP-1 F(ab')$_2$ presented here indicate that favourable tumour to tissue ratios can be achieved, although the penetration barriers associated with solid tumours of the size used here did not allow an early uptake suitable for RIT with ^{211}At.

The degree of tumour uptake demonstrated in this study was comparable with similar studies (14,23). The uptake and retention in tumour tissue demonstrated a high tumour-affinity of the ^{211}At-MoAb conjugate. While the radioactivity was cleared from other tissues it was strongly retained in tumour tissue up to 40 hours after injection, indicating a high stability of the radioimmunoconjugates.

The biodistribution of the ^{211}At-labelled and ^{125}I-labelled MoAbs was similar for most tissues. The stomach and neck (containing the thyroid) were the only tissues showing differences, with slightly higher retention of ^{211}At at 24 h and 40 h. Earlier studies showed that free ^{211}At to a large extent accumulated in these tissues (13,23). No thyroid or stomach blocking agents were given to the animals, and minor amounts of free ^{211}At could therefore easily be spotted by an increase of radioactivity in thyroid and stomach. A small fraction of free ^{211}At occuring either as a coinjected radiochemical impurity or because a catabolism product of the ^{211}At-MoAb conjugate may have been the cause of

the thyroid and stomach uptake seen in this study. Enzymatic dehalogenation is a less likely cause since the radioactivity was bound in tumour tissue and because there was no significant de-astatination in serum and wholeblood of ^{211}At-labelled aminated polymer particles labelled via the same procedures as presented here (18).

Because of the short half-life of the nuclide, treatment of solid tumours with ^{211}At-MoAbs through intravenous injection is not a realistic option. The most likely application is in treatment of single cells, completely vascularized cancers and micrometastatic cancers with small penetration barriers allowing a rapid uptake of MoAbs. Another possible approach would be direct intracavitary and intratumour injections (24) in inoperable tumours. The short half-life of the nuclide may then be beneficial because a large fraction of the radioactivity will have decayed before the ^{211}At-Moab conjugate has cleared from the tumour.

In conclusion, the uptake and retention of ^{211}At-MoAb conjugates in tumour tissue and the distribution in normal tissues indicate that ^{211}At may have a potential in radioimmunotherapy of malignancies allowing a rapid uptake of MoAbs. The uptake in stomach and thyroid must be monitored carefully, since a high uptake in these tissues indicates release of free ^{211}At. To minimize the amount of free ^{211}At in the radioimmunoconjugate preparations improved purification procedures by using HPLC purification of the final ^{211}At-MoAb should be developed.

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