Kinetics of Radioiodinated Monoclonal antibodies in the Rat: Influence of Tumour Growth and Reticuloendothelial System Host Modulation

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KINETICS OF RADIOIODINATED MONOCLONAL ANTIBODIES IN THE RAT

Influence of tumour growth and reticuloendothelial system host modulation

S. B. HOLMBERG, L. HAFSTRÖM, E. FORSSSELL ARONSSON, J. GRETARSDOTTIR, L. JACOBSSON, S. MATTSSON and L. LINDHOLM

Abstract

This experimental study in rats examines the influence of tumour growth and RES function modulation on the kinetics of iodinated MAb IgG1 C241. The study was designed to investigate unspecific accumulation in liver and blood. C241 is raised against human colon adenocarcinoma COLO 205 and reacts with SiLewis tumor-associated antigen, also known as tumour-associated antigen 19-9. In 26 rats, 2 μg 125I MAb C241 (iodobead labelling method) was given i.v. Blood, organ and tumour content was measured at 0.5, 24, 72 and 144 h. In 61 rats, 10 μg 131I MAb C241 (iodogen labelling method) was given i.v. The rats were divided into a non-tumour and a tumour-bearing group and subjected to RES function modulation with Zymosan stimulation or methyl palmitate depression. A syngeneic nitrosoguanidine-induced colorectal carcinoma—mean 11 g—was growing in back subcutaneous tissue and hind leg musculature. Serum content of tumour-associated antigen was not found on IRMA testing and tumour content of SiLewis ganglioside antigen was found only on lipid binding phase assay. The half-time in blood of iodinated MAb C241 was three days. In-vivo release of iodine was tested by plasma separation on a gel column. More than 90% of the iodine was in the IgG fraction. The activity distribution was almost in equilibrium after 24 h. A tumour/blood activity concentration ratio of 0.5 and liver/blood ratio of 0.3 remained at 72 h and 144 h. Radionuclide accumulation was equally low in the macrophage-rich liver and the kidneys. Tumour-bearing animals had significantly lower blood content (0.37 versus 0.99% g⁻¹) and liver content (0.09 versus 0.31% g⁻¹) at 144 h than non-tumour-bearing rats. The whole body content at 144 h was also lower (24% versus 35% of administered activity) (p=0.10). Modulation of RES function had no significant influence on the whole body, blood or liver content of 131I MAb C241 activity in non-tumour-bearing animals. In tumour-bearing animals, RES stimulation with Zymosan increased the whole body, liver and blood content of 131I activity. The two tested methods of iodination gave similar results.

Key words: Monoclonal antibodies, radioiodinated, kinetics, rat, tumour growth, RES.

Several tumour-associated antigens (TAA) have been identified and monoclonal antibodies (MAb) raised against them. Such tumour antigens are now used for serological diagnosis of primary and recurrent cancer disease. Antibodies labelled with radionuclides can be used for imaging tumours in vivo. Trials with MAb conjugated to radionuclides with a therapeutic radiation effect have also been reported.

Imaging with MAb for diagnosis of a solid tumour requires a TAA that is basically fixed to the cell surface of a tumour cell and is in contact with blood or interstitial fluid. The MAb-TAA binding has to be stable and specific to allow for sufficient tumour/normal tissue or tumour/blood ratios to be established. However, only a small portion of administered MAb will be transported to the tumour and most will be distributed to blood and interstitial fluid. Unspecific uptake in normal tissues and remaining activity in blood must be limited (1, 2).

MAb kinetics are influenced by many factors, viz. the type of antibody, fragmentation, labelling and antigen-antibody specificity. Host factors, such as the function of the RES macrophage–monocyte system may be an important denominator of MAb kinetics, since foreign substances, including MAb and MAb antigen complexes, are phagocytosed by the RES (2, 3). Activation of RES phagocytic function could improve clearance of MAb-antigen complexes and allow for better tumour/blood ratios. Depression of the RES, on the other hand, could mean longer exposure of MAb in the bloodstream and thus better possibilities of finding cellularly fixed tumour antigens. Iodination of the monoclonal antibody with a radionuclide can lead to alterations of the specificity and kinetics of the antibody (4). These changes in antibody specificity might also lead to changes in liver uptake and blood radionuclide content.

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Stimulation of RES phagocytic function can be achieved with unspecific immunostimulants such as BCG, cytokines and yeast extracts such as Zymosan. The active component of Zymosan is glucan (5). Depression of RES function can also be achieved with several substances, among them methyl palmitate (6). Tumour growth and therapy directed at tumour growth, i.e. surgery, irradiation and cytotoxic drugs, influence RES function (7, 8). In early phases there is a stimulation and in later phases depression is seen in disseminated cancer (9).

The aim of this experimental study was to examine host factors in the kinetics of iodine-labelled tumour unspecific MAb, especially liver uptake and blood content. The study was designed to investigate the influence of tumour growth and RES macrophage function.

An experimental tumour with no tumour-associated antigen identified in blood or tumour was used. MAb were raised against human colon adenocarcinoma. Two different methods of iodination with 125I and 131I were used.

Material and Methods

Animals. Inbred female and male Wistar/FU rats weighing 200–250 g were used. The animals were maintained on a normal day and night cycle and were fed with water and pellets ad libitum.

Experimental tumour. A syngeneic experimental nitrosguanidine-induced colonic adenocarcinoma was used (10). The tumour was maintained viable by deep-freezing and then, after thawing, by passage transplantation every 14th day. This study used tumour generations 28 to 34. Tumours were transplanted to back subcutaneous tissue and left hind leg musculature.

Monoclonal antibody (MAb). MAb C241 IgGl mouse antibody raised against human colon adenocarcinoma COLO 205 was used. C241 reacts with SiLea, which is also known as 19-9 antigen (11, 12).

Radiolabelling, series A. MAb C241 IgGl was labelled with 125I using the lodobead method (4, 13). N-chlorobenzensulfonamide in 6 beads is then mixed with 100 µg antibody and iodide for 15 min. Each animal was injected with 2 µg of MAb corresponding to 0.2 MBq of 125I.

Radiolabelling, series B. MAb C241 IgGl was labelled with 131I using the lodogen method (4, 14). 50 µg lodogen (1,3,4,6-tetrachloro-3,6-diphenylglycoluril) is dried and coated and iodide and 100 µg antibody added for 5 min. Each animal was injected with 10 µg of MAb corresponding to an 131I-activity of 0.5–3.0 MBq.

Release of iodine in vitro was tested by thin layer chromatography and in vivo by separation of plasma on a gel column (Sephacryl S-300).

RES modulation. Zymosan, a yeast cell derivative with the active component glucan, was given in a dose of 3 mg per 100 g rat weight i.v. 3 days before MAb C241 injection.

Table 1

| Study design. Number of rats and mean tumour weight in each group (series B) |
|---------------------------------|----------------|----------------|
|                                | Untreated     | Zymosan        | Methyl palmitate |
| Non-tumour                      | 7             | 10             | 9               |
| Tumour                          | 10            | 14             | 11              |
| Mean tumour weight g±SD         | 11±2          | 10±2           | 12±2            |

Table 2

<table>
<thead>
<tr>
<th>Time after injection (h)</th>
<th>Percentage activity bound to immunoglobulin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>125I</td>
</tr>
<tr>
<td>0.5</td>
<td>98</td>
</tr>
<tr>
<td>24</td>
<td>96</td>
</tr>
<tr>
<td>72</td>
<td>98</td>
</tr>
<tr>
<td>144</td>
<td>97</td>
</tr>
</tbody>
</table>

Methyl palmitate was given in a dose of 100 mg per 100 g rat weight i.v. one day before MAb C241 injection.

SiLea antigen determination. Serum analysis of SiLea antigen was made with an immunoradiometric (IRMA) assay (11). The tumour antigen content was examined with an immunohistochemical technique (15). Determination of SiLea ganglioside antigen was performed by ganglioside isolation (16) and solid-phase binding assay (17).

Radionuclide activity determination. Blood and organ samples were measured in a gamma counter equipped with a 3 by 3 inch sodium iodide well crystal and a single channel pulse height spectrometer. Whole body activity was registered with a gamma camera with a medium energy parallel hole collimator.

Experimental series A. 26 tumour-bearing rats were given 125I MAb C241 IgGl in the tail vein. The animals were killed after 0.5, 24, 72 or 144 h. Tumour, liver, spleen, kidney and blood were collected and the radionuclide concentrations measured.

Experimental series B. Sixty-one Wistar/FU non-tumour or tumour-bearing rats were randomly allocated to no treatment or RES modulation treatment. All groups were given 131I MAb C241 IgGl in the tail vein. The animals were killed after 6 days. Liver, tumour and blood were collected and the radionuclide concentrations registered. Blood was also collected at 0.5, 24, 72 and 144 h for determination of half-time. The study design of series B is shown in Table 1.

Statistics. Groups were compared using Student's t-test. Factorial analysis with ANOVA was made in study B.
Table 3
Distribution of administered ¹²³I activity in percentage of administered activity per gram of tissue at 0.5, 24, 72 and 144 h after i.v. injection of labelled MAb. Mean %±SD

<table>
<thead>
<tr>
<th></th>
<th>0.5 h</th>
<th>24 h</th>
<th>72 h</th>
<th>144 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>5.06±0.79</td>
<td>1.38±0.35</td>
<td>1.18±0.03</td>
<td>0.61±0.29</td>
</tr>
<tr>
<td>Tumour</td>
<td>0.29±0.04</td>
<td>0.73±0.09</td>
<td>0.40±0.18</td>
<td>0.23±0.04</td>
</tr>
<tr>
<td>Liver</td>
<td>2.01±0.41</td>
<td>0.41±0.05</td>
<td>0.23±0.09</td>
<td>0.13±0.03</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.46±0.04</td>
<td>0.34±0.03</td>
<td>0.20±0.07</td>
<td>0.07±0.02</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.91±0.24</td>
<td>0.38±0.06</td>
<td>0.26±0.07</td>
<td>0.14±0.02</td>
</tr>
</tbody>
</table>

Table 4
Whole body content percentage of administered ¹²³I activity after 144 h. Values given as mean %±SD

<table>
<thead>
<tr>
<th></th>
<th>Untreated</th>
<th>Zymosan</th>
<th>Methyl palmitate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-tumour</td>
<td>35±13</td>
<td>29±12</td>
<td>42±3</td>
</tr>
<tr>
<td>Tumour</td>
<td>24±13</td>
<td>38±7</td>
<td>33±12</td>
</tr>
</tbody>
</table>

* = statistically significant; p<0.05.

Table 5
Radionuclide concentration in blood in percentage of administered ¹³¹I activity per g after 144 h. Mean %±SD

<table>
<thead>
<tr>
<th></th>
<th>Untreated</th>
<th>Zymosan</th>
<th>Methyl palmitate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-tumour</td>
<td>0.99±0.44</td>
<td>0.74±0.31</td>
<td>0.87±0.34</td>
</tr>
<tr>
<td>Tumour</td>
<td>0.37±0.35</td>
<td>0.66±0.29</td>
<td>0.24±0.15</td>
</tr>
</tbody>
</table>

* = statistically significant; p<0.05.

Table 6
Radionuclide concentration in liver in percentage of administered ¹³¹I activity per g after 144 h. Mean %±SD

<table>
<thead>
<tr>
<th></th>
<th>Untreated</th>
<th>Zymosan</th>
<th>Methyl palmitate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-tumour</td>
<td>0.31±0.09</td>
<td>0.19±0.03</td>
<td>0.28±0.06</td>
</tr>
<tr>
<td>Tumour</td>
<td>0.09±0.02</td>
<td>0.17±0.02</td>
<td>0.08±0.01</td>
</tr>
</tbody>
</table>

* = statistically significant; p<0.05.

Table 7
Tumour/blood ¹³¹I activity concentration ratio at 144 h

<table>
<thead>
<tr>
<th></th>
<th>Untreated</th>
<th>Zymosan</th>
<th>Methyl palmitate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumour</td>
<td>0.54±0.12</td>
<td>0.53±0.17</td>
<td>0.71±0.34</td>
</tr>
</tbody>
</table>

Table 8
Liver/blood ¹³¹I activity concentration ratio at 144 h

<table>
<thead>
<tr>
<th></th>
<th>Untreated</th>
<th>Zymosan</th>
<th>Methyl palmitate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-tumour</td>
<td>0.31±0.11</td>
<td>0.27±0.05</td>
<td>0.31±0.09</td>
</tr>
<tr>
<td>Tumour</td>
<td>0.29±0.15</td>
<td>0.29±0.17</td>
<td>0.45±0.24</td>
</tr>
</tbody>
</table>

Results

Serum analyses from non-tumour and tumour-bearing animals with IRMA showed no SiLe⁴ antigen. Immunohistochemical study on tumour was negative for tumour-associated antigen. Solid phase binding assay was weakly positive for SiLe⁴ ganglioside antigen.

Gel chromatography of plasma showed 2 distinct peaks, one at the position of IgG and one at the position of iodide. More than 90% of radioactivity was found at the position of IgG in all plasma measurements (Table 2).

In series A, the concentration of ¹²³I at 0.5 h given relative to the administered activity was 5.1±0.8% g⁻¹ in the blood and 2.0±0.4% g⁻¹ in the liver. At 144 h, the relative concentration was 0.61±0.29% g⁻¹ in the blood and 0.13±0.03% g⁻¹ in the liver (Table 3). The half-time of ¹²³I MAb C241 IgG1 in blood was 88 h measured between 72 and 144 h after injection.

In series B, the remaining fraction of ¹³¹I in the whole body at 144 h was 35±13% of administered activity in non-tumour-bearing untreated animals. Tumour-bearing untreated rats had no significantly faster elimination, 24±13% remaining (p=0.10). In the tumour-bearing, Zymosan-treated, group the remaining activity was 38±7% (p<0.05 compared to tumour-bearing untreated rats) (Table 4).

The ¹³¹I concentration relative to administered activity in blood at 144 h was 0.99±0.44% g⁻¹ in untreated non-tumour-bearing untreated animals. Tumour-bearing untreated rats had no significantly faster elimination, 24±13% remaining (p=0.10). In the tumour-bearing, Zymosan-treated, group the remaining activity was 38±7% (p<0.05 compared to tumour-bearing untreated rats) (Table 4).

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The ¹³¹I concentration relative to administered activity in blood at 144 h was 0.99±0.44% g⁻¹ in untreated non-tumour-bearing rats and 0.37±0.35% g⁻¹ (p<0.05) in tumour-bearing rats. Zymosan-treated tumour-bearing rats had a higher radionuclide concentration, 0.66±0.29% g⁻¹ (p<0.05), compared to untreated tumour-bearing rats (Table 5). The half-time of ¹³¹I MAb C241 IgG1 in blood in untreated tumour-bearing rats was 62 h between 72 and 144 h after injection.

In series B, the remaining fraction of ¹³¹I in the whole body at 144 h was 35±13% of administered activity in non-tumour-bearing untreated animals. Tumour-bearing untreated rats had no significantly faster elimination, 24±13% remaining (p=0.10). In the tumour-bearing, Zymosan-treated, group the remaining activity was 38±7% (p<0.05 compared to tumour-bearing untreated rats) (Table 4).

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The ¹³¹I concentration relative to administered activity in the liver at 144 h was 0.31±0.09% g⁻¹ in untreated non-tumour-bearing animals and 0.09±0.02% g⁻¹ (p<0.05) in tumour-bearing animals. Zymosan-treated tumour-bearing rats had a higher liver radionuclide concentration, 0.17±0.02% g⁻¹ (p<0.05), compared to tumour-bearing untreated rats (Table 6).

The tumour/blood concentration ratio was approximately 0.5 and the liver/blood ratio approximately 0.3.
There were no statistically significant differences between untreated and RES modulated rats or between non-tumour and tumour-bearing rats (Tables 7, 8).

Discussion

Host factors such as the RES macrophage monocyte system and tumour growth may be of importance in the kinetics of iodinated MAb. In this study, established agents used to achieve RES macrophage activation and depression have been utilised to study the influence on iodinated MAb kinetics.

The tumour model with a syngeneic colonic adenocarcinoma in rats has no SiLe4 tumour-associated antigen in serum. This makes it suitable for kinetic studies since circulating MAb C241-antigen complexes will not be formed. This experimental tumour did not express the tumour-associated SiLe4 ganglioside antigen on immunohistochemical examination. The accumulation of MAb in tumour is thus ‘unspecific’, i.e. not dependent on antigen-antibody reaction. This experimental model can be used and may even be advantageous in studies of the host factor aspects of MAb kinetics. A high specific tumour uptake could endanger the interpretation of these host factor aspects of MAb kinetics.

A half-time in blood between one day and several weeks for IgGl antibodies has been shown. The faster elimination was seen with iodinated mouse antibodies given to humans and the slower elimination with idiotypic antibodies (18). In this study, both the 125I- and 131I-labelled C241 antibody half-life was about 3 days. It is important to consider that elimination of radionuclide may be due to either dehalogenation of intact MAb or release of iodine on degradation of MAb. Free iodine is accumulated in the thyroid and otherwise rapidly excreted (19). No more than 10% free iodide was found in plasma at all times and the other 90% of the iodide was bound to IgG. The radionuclide concentration in different organs, i.e. liver, spleen and kidney, thus mainly reflects the distribution of iodinated MAb.

The tumour/blood and organ/blood ratios were stable from 24 h to 144 h and this is interpreted as an equilibrium of the iodinated MAb concentration between tumour, organs and blood, indicating no specific uptake. The fact that the organ concentration was lower than the blood concentration further supports an unspecific uptake. The distribution was equally low to macrophage-rich organs like the liver and the spleen and macrophage-poor organs like the kidneys. The somewhat higher radioactivity content in tumour tissue than in liver and kidney is probably explained by more interstitial fluid per gram in the experimental tumour.

Since no organ accumulation was registered in series A, whole body activity was used in series B to evaluate MAb kinetics. 131I was then used because of its better radiation properties when using the gamma camera to register whole body activity. This implies higher activity and then a higher MAb amount to maintain the iodination level of about 0.3 iodine atoms per antibody. A second method of iodination was used for reasons of comparison.

The comparison of MAb kinetics between non-tumour and tumour-bearing rats showed that the elimination of radionuclide was faster in tumour-bearing animals. One explanation might be the increased RES macrophage activity usually seen in tumour-bearing animals (9). In a previous study, the growth of this tumour increased the RES phagocytic rate by about 30%, measured with dynamic RES scintigraphy (20). On the other hand in this study, RES modulation with Zymosan and methyl palmitate had no significant influence on the blood or organ content of radionuclide in non-tumour-bearing rats. This indicates that conventional ideas about the importance of RES in the elimination of MAb might not be the only explanation.

Among tumour-bearing rats, Zymosan-treated rats had a slower whole body elimination, higher liver uptake and higher blood content, which is also contradictory to the hypothesis that RES stimulation leads to faster MAb elimination. In a previous study, Zymosan had an unspecific suppressive effect on tumour growth, especially tumour take (21). The influence of Zymosan on MAb kinetics in tumour-bearing rats might then be explained by suppression effects of tumour growth and hence normalised kinetics of MAb.

RES modulation had no significant influence on tumour/blood ratios in tumour-bearing animals. Nor were there any significant differences in liver/blood ratios between non-tumour, tumour-bearing and RES-modulated rats. This signifies the equilibrium between tumour, blood and organ content of iodinated MAb.

This study, using iodinated monoclonal antibody in non-tumour and tumour-bearing rats, has shown low uptake in RES macrophage-rich organs like the liver and the spleen, and similar uptake in non-RES organs like the kidneys. RES stimulation or depression caused no significant changes, which led to the conclusion that the RES macrophage system has little influence on iodinated MAb kinetics in this experimental model. Tumour growth, on the other hand, led to faster elimination of iodinated MAb. Zymosan treatment of tumour-bearing rats reduced this faster elimination.

No major difference in kinetics between the two types of iodination of MAb could be identified in this study.

ACKNOWLEDGEMENTS

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REFERENCES