Pharmacokinetics of the Mouse Monoclonal Antibody 17-1A in Cancer Patients Receiving Various Treatment Schedules¹

Jan-Erik Frödin, Ann-Kari Lefvert, and Håkan Mellstedt²

Department of Oncology (Radiumhemmet) [J.-E. F., H. M.] and Immunologic Research Laboratory [J.-E. F., A.-K. L., H. M.], Karolinska Hospital, Stockholm, Sweden

ABSTRACT

Twenty-four patients with metastatic colorectal carcinoma were treated with repeated doses (200–500 mg) of the mouse monoclonal antibody (MAb) 17-1A. Four different treatment schedules were used. The total dose was 1, 3.6, 7.6, and 12 g, respectively. Altogether, 263 infusions were administered. The interindividual variations in the maximum serum concentration at 2 h (max2h) were large. The mean max2h value after an infusion of 200 mg was 55 ± 5 μg/ml and after 500 mg, 132 ± 7 μg/ml. Max2h concentration correlated inversely with the half-life of MAb 17-1A (P < 0.001). The mean for 200 mg was 25.9 ± 1.4 h and after the administration of 500 mg, 19.8 ± 1.0 h. The total area under the concentration versus time curve increased when high doses were administered on a continuous basis, in comparison with spaced infusions, thus increasing the exposure of the tumor tissues to MAb 17-1A. The pharmacokinetics of mouse MAb 17-1A are best described by a one-compartment model. All patients developed anti-mouse IgG antibodies and most also IgM antibodies. In the more intensive treatment schedules, the IgG antibody response was suppressed. Induction of high titers of anti-mouse antibodies did not cause clinical problems. Neither did they affect the pharmacokinetics of MAb 17-1A at these dose levels. Therapy was tolerated well. The side effects were mild and of short duration. The gastrointestinal adverse reactions were dose dependent and correlated to serum max2h concentration. Allergic reactions were rare and easily clinically manageable.

INTRODUCTION

Tumor cells may possibly differ from normal cells in their expression of surface antigenic structures. Antigens of importance for growth and differentiation may possibly be expressed at a high density during fetal stages but may be down-regulated and hardly detectable in mature cells (1). Such antigens might be reexpressed on malignant cells and thus serve as tumor-associated antigens. They can be exploited not only for diagnostic purposes but also as targets for immunotherapy. After the hybridoma technique was introduced (2), many MAb against tumor-associated antigens have been produced and some of them have been used for therapy (3, 4). Hitherto almost all of them are of murine origin.

In principle there are two different ways in which MAb might be applied in tumor therapy. Either the MAb may be used as a carrier of a cytotoxic agent (5, 6) or the MAb might be used naked and, after the binding to tumor cells, may induce immune reactions which destroy the tumor cells (7, 8). Varying doses of single and multiple infusions have been given to patients. The pharmacokinetics and humoral immune responses have been analyzed using both unconjugated and radiolabeled MAb or fragments or MAb for diagnostic purposes. Comparatively small doses of MAb have been given (9–15). The circulating half-lives of MAb varied between 11 and 35 h, although longer half-lives have been reported. Most patients developed antinouse antibodies.

CO17-1A is a M, 37,000 glycoprotein which might play a role in the transport of calcium across the cell membrane. It is expressed with a low frequency in normal gastrointestinal tissues, mainly on the colon mucosa (16). In most tumors of gastrointestinal origin, it is expressed with a high frequency and at a high cell density (17). A mouse MAb (IgG2A) for therapy directed against the antigen has been produced (4).

One of the main effector mechanisms for tumor cell destruction in vivo using unconjugated MAb seems to be antibody-dependent cellular cytotoxicity (18). In vitro, higher MAB saturation on the tumor cell surface induced a better cell killing in antibody-dependent cellular cytotoxicity (19). In vivo only a small amount of the MAB reaches the tumor cells, usually less than 1% (11). In an attempt to establish an optimal dose schedule for MAb 17-1A in the treatment of patients with metastatic colorectal carcinoma, with the aim of increasing the tumor cell saturation of MAb 17-1A, we have gradually increased the total amount of MAB 17-1A given to the patients as well as the exposure time of tumor tissue to MAB 17-1A. In this paper we report on the pharmacokinetics and humoral immune response of these MAb 17-1A schedules, as well as the side effects. The therapeutic results have partly been described elsewhere (20). The complete clinical results will be published in the near future.

MATERIALS AND METHODS

Patients, Material, and Administration of MAB 17-1A. Twenty-four patients with metastatic colorectal carcinomas were entered in the study. All patients had metastatic disease not accessible to surgery. The median age was 64.5 years (range, 32–81 years). There were 11 men and 13 women. The median time from primary surgery was 10 months (range, 1–75 months). Two patients had received chemotherapy and one of the others, radiotherapy for their metastases. All the others were untreated except for surgery. No other treatment was given during MAB therapy. The tumors of all patients expressed CO17-1A.

Four different treatment schedules were used. In treatment schedule I, 10 patients received a first infusion of 400 mg followed by three infusions of 200 mg, for a total dose of 1 g of MAB 17-1A. The infusions were given at intervals of 6 weeks and with autologous mononuclear cells diluted in balanced salt solution with 1% human serum albumin to a final volume of 225 ml, as described previously (21). In treatment schedule II, 5 patients received 400 mg on days 1, 3, and 6. A second course began on day 22 and the third course on day 43. The total dose was 3.6 g. The first infusion in each course of schedule II was given together with autologous mononuclear cells; the other two infusions were in physiological saline diluted to a final volume of 250 ml. In treatment schedule III, 1 patient received 200 mg and 6 patients received 400 mg every other day, for total doses of 4.8 and 7.6 g, respectively. In treatment schedule IV, 2 patients received 500 mg on Mondays, Wednesdays, and Fridays for 8 consecutive weeks, for a total dose of 12 g. In treatment schedules III and IV, the MAB was

Received 12/29/89; revised 3/28/90.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 The study was approved by the Ethical Committee of the Karolinska Institute. The study was supported by grants from the Swedish Cancer Society, the Cancer Society in Stockholm, the King Gustav V Jubilee Fund, the Karolinska Institute Foundations, and the Alex and Eva Wallerströms Foundations.

2 To whom requests for reprints should be addressed; at Department of Oncology (Radiumhemmet), Karolinska Hospital, S-104 01 Stockholm, Sweden.

3 The abbreviations used are: MAb, monoclonal antibody(ies); VBS-T, veronal-buffered saline-Tween; AUC, area under the curve; max2h, concentration, concentration at 2 h; ELISA, enzyme-linked immunosorbent assay.

4 H. Koprowski, personal communication.
given in physiological saline, as described above. The infusion period varied between 10 and 60 min. The planned total doses and number of infusions are shown in Table 2. Due to disease complications and/or disease progression, 7 of 10 patients on treatment schedule I, 3 of 5 patients on treatment schedule II, and 3 of 7 patients on treatment schedule III did not receive the full prospected course. A total of 263 infusions were given. Complete pharmacokinetic data were available after 231 infusions. After an additional 11 (total 242) infusions, only serum max2h concentration was available (see Fig. 9). However, side effects could be recorded after all 263 infusions (see Table 3). In all patients, blood samples were taken before the infusion. After the infusion, blood was drawn after 1 and 2 h (schedules I and II), after 2 h (schedules III and IV), and after 16 h and thereafter once a week. In some patients more frequent samples were taken (every 15 min for the first 4 h, then every hour for the following 4 h, each 4 h for the subsequent 20 h, and thereafter twice a day for 1 week).

Mouse Immunoglobulin and Anti-Mouse Immunoglobulin Analyses. Mouse immunoglobulin (MAb 17-1A) and class-specific human anti-mouse antibodies in serum were assayed in an ELISA, using flat-bottomed microtiter ELISA plates (Dynatech) coated at 4°C overnight with goat anti-mouse IgG (Nordic, Tilburg, The Netherlands) for mouse IgG (MAb 17-1A) determination and with MAB 17-1A for human anti-mouse IgG or IgM analyses. After blocking with 0.5% bovine serum albumin in coating buffer for 1 h at 37°C, diluted samples to be tested were added for 2 h at 37°C. Finally, the plates were reacted for 2 h at 37°C with goat anti-mouse IgG peroxidase conjugate (Nordic), alkaline phosphatase conjugates (Sigma, St. Louis, MO), rabbit anti-human IgG or IgM peroxidase conjugates (Nordic), or alkaline phosphatase conjugates (Sigma). After enzyme reaction for 20 min using 1,2-phenyldiamine dihydrochloride (0.55 mg/ml) in Tris-HCl buffer, pH 7.6, containing 30 μl H2O2/100 ml of volume or p-nitrophenylphosphate (1 mg/ml; Sigma) in diethanolamine buffer, pH 7.4, the absorbance at 492 nm was measured using an automatic ELISA reader (Titertec; Multiscan). Absorbance of a normal control serum pool for anti-mouse IgG antibodies was 0.34 ± 0.18 and for IgM antibodies was 0.09 ± 0.06. The background, determined by incubating the antigen-coated wells with medium alone, was subtracted.

Immune Complex Analysis. A solution of 6.25 mg/liter of Clq in 0.05 M carbonate buffer, pH 9.6, was prepared, and 200 μl were dispensed in flat-bottomed polystyrene wells, with a capacity of 300 μl (no. M179A; Cooke Laboratory Products, Alexandria, VA), contained in 96-well styrofoam holders (Cooke). After incubation for 3 h at 37°C, the wells were washed 3 times with cold VBS-T (0.15 mM Ca, 0.05 mM Mg, pH 7.2; Merieux, Charbonnieres-les-Bains, France; containing 0.05% Tween), filled with 100 μl of 0.1% gelatin solution in VBS-T, and incubated for 2 h at room temperature. Following 3 washes with VBS-T, the wells were immediately inverted and drained. Twenty-five-μl serum samples were incubated with 50 μl of 0.2 M EDTA at pH 8.3 for 30 min at 37°C. Duplicate 200-μl aliquots of a final 1:20 dilution of the sample sera (in VBS-T) were dispensed into the Clq-coated wells and incubated with goat anti-human IgG alkaline phosphatase conjugates (Sigma) for 2 h at room temperature, followed by 3 washes with cold VBS-T. Finally, the plates were reacted with enzyme as described above and the absorbance at 405 nm was measured.

Serum Immune Reactivity against CO17-1A. The human colorectal carcinoma cell line SW948, expressing the antigen CO17-1A, was trypsinized with 0.1% trypsin (Flow Laboratories, Scotland) in PBS for 3-4 min and washed twice in PBS. Fifty μl of the diluted serum sample were added to 5 × 10^6 cells and incubated at 4°C for 30 min and the cells were washed twice in PBS. The cell pellet was further incubated at 4°C for 30 min with 50 μl absorbed fluoresceinated goat anti-mouse IgG (Becton-Dickinson, Mountain View, CA) and then washed twice in PBS. Then, 5 × 10^5 cells were analyzed by flow cytometry (FACScan; Becton-Dickinson) at 480 nm with a flow rate of <300 cells/s. As a measurement of intact MAb 17-1A in serum, the immune reactivity of the patient serum against the cell line SW948 was expressed as the highest dilution staining ≥10% cells. Pretreatment serum and serum from healthy control donors did not stain SW948. Moreover, the patient serum containing MAB 17-1A did not stain unrelated cell lines, i.e., cells not expressing CO17-1A (KS62 and M5).

Statistics and Pharmacokinetic Analyses. Analyses of differences between means were made by the Mann-Whitney U test. The linear regression model was used to estimate correlation between independent observations. The AUC was calculated according to trapezoidal and Simpson's rules (22). To make the AUC values comparable when repeated doses were given at short intervals, the breakpoint of the x-axis was calculated from the slope of the line.

Evaluation of Side Effects. Side effects were evaluated by supervising the patients during the infusion and interviewing the patients. To reveal a possible correlation between the serum max2h concentration of MAB 17-1A and gastrointestinal symptoms (nausea, vomiting, abdominal pain, and diarrhea), the World Health Organization scale was used (23).

RESULTS

The serum pharmacokinetics after a single infusion of 400 mg MAB 17-1A are shown in Fig. 1 (schedule I). During the first 3-4 h after an infusion of MAB 17-1A, the serum concentration of MAB 17-1A remained at a fairly constant level. When this information was obtained and because the patients in these studies were exposed to a large number of blood drawings, it was decided to reduce the blood drawings for mouse immunoglobulin analysis, as indicated in "Material and Methods." Maximum serum values are given as the max2h concentration. The max2h serum concentration after a single infusion of 400 mg was between 8 and 425 μg/ml and after 200 mg was 6-156 μg/ml (schedule I).

The pharmacokinetics of MAB 17-1A for schedule II are shown in Fig. 2. In schedule II the max2h concentration ranged between 43 and 474 μg/ml and the nadir values from 3 to 92 μg/ml. There was a tendency towards a decrease of the max2h concentration with repeated infusions, although the differences
were not statistically significant.

The pharmacokinetics of MAb 17-1A for schedule III are shown in Fig. 3. The max_{2h} values varied between 25 and 185 μg/ml and the nadir values from 5 to 49 μg/ml. A pharmacokinetic curve for treatment schedule IV is shown in Fig. 4. Max_{2h} values varied from 69 to 266 μg/ml and nadir values from 5 to 62 μg/ml. In schedules III and IV, there was a statistically significant (P < 0.001) decline in the serum concentration at 2 h with repeated infusions.

A summary of serum max_{2h} concentrations, circulating MAb 17-1A half-lives (t_{1/2}), and AUC at different dose levels is shown in Table 1. The exposure to MAb 17-1A in the different treatment protocols, expressed as total AUC, is given in Table 2.

Thus, a high dose given on a continuous basis increased the exposure of tumor tissues to MAb 17-1A. No correlation was observed between serum max_{2h} concentration and body surface area or body weight. The serum half-life of MAb 17-1A correlated strongly with the serum max_{2h} concentration when all infusions were taken into consideration (r = -0.447; P < 0.001; n = 231). A similar correlation was also found when only the max_{2h} concentrations after the first infusion (r = -0.599; P < 0.01; n = 22) were analyzed. Thus, a high max_{2h} concentration resulted in a short half-life. In the majority of cases, MAb 17-1A was not detectable in serum after 1 week from the last infusion. However, in 23% of the cases analyzed, MAb 17-1A could be found in serum for 2 weeks.

Serum factors might alter the immune reactivity (binding specificity) of MAb 17-1A. Our ELISA method for the detection of MAb 17-1A did not allow any conclusion as to whether the specific antigen binding capacity was preserved. A semi-quantitative technique for measuring the immune reactivity was set up. As can be seen from Fig. 2, the decline of the immune reactivity (specific binding to SW948) paralleled the decrease in MAb 17-1A. This was the case for all 130 serum samples of the 5 patients tested. Thus, immune-reactive antibodies were present in the serum as long as MAb 17-1A was detected.

Prior to therapy no patients had anti-mouse antibodies. During treatment all patients developed IgG anti-17-1A antibodies and most also developed IgM antibodies. The antibody responses for schedule I have partly been reported previously (20). IgM antibodies appeared on days 5–10 (Figs. 5–7). A late reinduction of an IgM response (specific binding to SW948) could be noted in some patients (Figs. 6 and 7). In one patient (schedule IV) no IgM response, only an IgG response, was recorded. Anti-17-1A IgG antibodies after a first single infusion appeared on about day 10. However, in 2 of 10 patients (schedule I) no IgG antibodies were detected until after the second infusion 6 weeks later. Maximum IgG response after the first infusion was noted just before the second infusion, i.e., day 36 (data not shown). Repeated infusions of

---

**Table 1** Pharmacokinetic data for MAb 17-1A

<table>
<thead>
<tr>
<th>Infused amount of MAb 17-1A (mg)</th>
<th>No. of infusions</th>
<th>Serum max_{2h} concentration (µg/ml)</th>
<th>t_{1/2} (h)</th>
<th>AUC (h x µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>41</td>
<td>55 ± 5* (6-156)</td>
<td>25.9 ± 1.4</td>
<td>2,045 ± 161</td>
</tr>
<tr>
<td>300</td>
<td>12</td>
<td>69 ± 6 (34-100)</td>
<td>28.6 ± 3.8</td>
<td>3,681 ± 568</td>
</tr>
<tr>
<td>400</td>
<td>134</td>
<td>104 ± 6 (8-474)</td>
<td>20.7 ± 0.7</td>
<td>3,771 ± 253</td>
</tr>
<tr>
<td>500</td>
<td>44</td>
<td>132 ± 7 (69-266)</td>
<td>19.8 ± 1.0</td>
<td>4,975 ± 340</td>
</tr>
</tbody>
</table>

* Mean ± SE.

* Range.

* In treatment schedule III, one patient had a dose reduction from 400 to 300 mg/infusion.

---

**Table 2** Comparison of total AUC for the four different treatment schedules

<table>
<thead>
<tr>
<th>Treatment schedule</th>
<th>Total dose planned (mg)</th>
<th>Infusions planned</th>
<th>No. of patients</th>
<th>Treatment period (days)*</th>
<th>Total AUC (h x µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1,000</td>
<td>4</td>
<td>10</td>
<td>126</td>
<td>6,849 ± 1,157* (1,463-10,454)*</td>
</tr>
<tr>
<td>II</td>
<td>3,600</td>
<td>9</td>
<td>5</td>
<td>48</td>
<td>35,054 ± 9,958 (18,075-78,745)</td>
</tr>
<tr>
<td>III</td>
<td>4,800</td>
<td>24</td>
<td>1</td>
<td>47</td>
<td>43,171</td>
</tr>
<tr>
<td>IV</td>
<td>12,000</td>
<td>24</td>
<td>2</td>
<td>54</td>
<td>92,030 ± 12,462 (74,406-109,654)</td>
</tr>
</tbody>
</table>

* No. of days from the first to the last infusion.

* If a patient did not receive a complete course, the data were standardized to make comparisons possible.

* Mean ± SE.

* Range.
related to immune complexes were noted. One patient on
was probably due to binding of the human antibodies to MAb
of MAb 17-1A were given every other day for a total of 19 infusions.

antibody titers for one patient (schedule III). Ü, the infusion period when 400 mg
infusion of 200 mg MAb 17-1A.

reticuloendothelial system. However, no clinical symptoms
ules I and II). Immediately after an infusion, both IgG and IgM
antigen-antibody complex formation is not a likely explanation.
Our data seem to fit best with a one-compartment model (Fig.
three models have been suggested (9, 12, 14, 15). A possible
netics of mouse antibodies are best described by a one-, two-

DISCUSSION
There has been discussion as to whether the pharmacoki-
etics of mouse antibodies are best described by a one-, two-

PHARMACOKINETICS OF MAb 17-1A

Fig. 6. Kinetics of human anti-mouse 17-1A IgM (••••) and IgG ( — )
antibody titers for one patient (schedule III). Ü, the infusion period when 400 mg
of MAb 17-1A were given every other day for a total of 19 infusions. Arrow, infusion of 200 mg MAb 17-1A.

Fig. 7. Kinetics of human anti-mouse 17-1A IgM (••••) and IgG ( — )
antibody titers for one patient (schedule III). Ü, the infusion period when 400 mg
of MAb 17-1A were given every other day for a total of 19 infusions. Arrow, infusion of 200 mg MAb 17-1A.

Fig. 8. Kinetics of human anti-mouse IgG antibody titers (mean) for patients receiving schedule I. Arrows, infusions of MAb 17-1A. Patients were given 2
infusions (— — —) (n = 2), 3 infusions (— — —) (n = 5), or 4 infusions (— — —) (n = 3).

MAb 17-1A at intervals of 6 weeks gradually increased the IgG
response (Fig. 8). The same was found for patients receiving
treatment schedule II (Fig. 5). When infusions were given every
other or every third day, the IgG response was delayed for 30–50 days (Figs. 6 and 7). Moreover, the antibody titers remained
at a lower level in those patients given MAb 17-1A continuously
(schedules III and IV), as compared to spaced infusions (sched-
ules I and II). Immediately after an infusion, both IgG and IgM
antibody titers decreased. This is clearly shown in Fig. 5. This
was probably due to binding of the human antibodies to MAb
17-1A and clearance of the antigen-antibody complex by the
reticuloendothelial system. However, no clinical symptoms
were recorded.

There were only two occasions on which clinical symptoms
related to immune complexes were noted. One patient on
schedule III received two additional infusions, at an interval of
4 weeks, 7 months after the initial infusion series. One h after
the second infusion, he developed severe bilateral pain in his
triceps muscles. Later, his blood pressure fell, peripheral cy-

4869
complexes (C1q-binding technique) could only be demonstrated on two occasions. Thus, repeated infusions of mouse MAb 17-1A can be given safely, with regard to immune-related side effects.

One of the aims of the study was to analyze whether increasing doses given on a continuous basis increased the exposure of the tumor to MAB 17-1A. Based on the results presented in Tables 1 and 2, we could demonstrate that this was the case. However, this does not seem to correlate with the therapeutic outcome. The reason for that is unclear. Increasing doses gave a higher tumor cell saturation in vivo (26), which was one of the aims. However, prolonged and high dose mouse MAB exposure to the immune system might suppress various effector functions and thereby impair killer functions activated by MAb.

All patients developed anti-mouse IgG antibodies and most of them, also IgM. The interindividual variations were large. When giving spaced doses (schedules I and II), the pattern was as expected. After each infusion or infusion period, the amounts of circulating IgG antibodies increased. For treatment schedules III and IV the development of IgG antibodies was delayed. Peak titer was noted 70–110 days after institution of therapy. Moreover, there was a tendency towards lower antibody titers in schedules III and IV in comparison with I and II. A similar relation has also been observed by other workers (10). However, the suppressing effect of continuous high doses of MAb 17-1A can be given safely, with regard to immune-related side effects, allergic/anaphylactoid-like reactions, and immune complex-related symptoms were clinically easily manageable and left no sequela.

Taken together, the pharmacokinetics of mouse MAB 17-1A are best described by a one-compartment model. Extremely high and repeated infusions can be given with safety.

**ACKNOWLEDGMENTS**

We wish to express our sincere gratitude to Prof. H. Koprowski and Prof. Z. Steplewski for providing us with MAB 17-1A. For reviewing the pharmacological data and for valuable discussion, we acknowledge the clinical pharmacologist Dr. Curt Peterson. For expert technical assistance we thank Harriet Grabó and Gun Ersmark and for excellent secretarial help Gunilla Burén and Marie Karlsson.

**REFERENCES**

13. Hnatowich, D. J., Gionet, M., Ruszkowski, M., Siebecker, D. A., Roche, J.,


Pharmacokinetics of the Mouse Monoclonal Antibody 17-1A in Cancer Patients Receiving Various Treatment Schedules

Jan-Erik Frödin, Ann-Kari Lefvert and Håkan Mellstedt