Intravenous Human Anti-Murine Immunoglobulin Antibody and who had no preexisting endogenous human anti-murine immunoglobulin antibody.

Patients receiving i.v. human anti-murine immunoglobulin antibody demonstrated a rapid clearance of $^{131}$I-labeled monoclonal antibody from their circulation. The (mean) maximum $^{131}$I blood content was 11.4% of the injected activity in patients receiving human anti-murine immunoglobulin antibody compared to 23.3% in patients not given human anti-murine immunoglobulin antibody. Intravenous human anti-murine immunoglobulin antibody decreased the radiation dose to bone marrow (from $^{131}$I-labeled monoclonal antibody in the vascular compartment) 4-fold.

Following the injection of human anti-murine immunoglobulin antibody, $^{131}$I-monoclonal/human anti-murine immunoglobulin antibody immune complexes were rapidly transported to the liver. Antibody dehalogeneration in the liver was rapid, with 87% of the injected $^{131}$I excreted in 5 days. Despite the efficient hepatic uptake of immune complexes, dehalogeneration of monoclonal antibody was so rapid that the radiation dose to liver parenchyma from circulating $^{131}$I was decreased 4-fold rather than increased. All patients developed endogenous human anti-murine immunoglobulin antibody 2 to 3 weeks after treatment.

The pharmacokinetics of $^{131}$I-labeled MAb administered i.v. were determined by absorption at 280 nm were separated and immediately dialysed against PBS. Finally the anti-HMF G, antibodies were filtered (Millipore, France), and kept at 4°C until used. All samples were tested for sterility and pyrogenicity (Blood Products Laboratory, Elstree, England).

Ethics Committee. All five patients had previously undergone cytoreductive surgery and postoperative chemotherapy for ovarian carcinoma. The abbreviations used are: MAb. monoclonal antibody; HAMA. human anti-murine immunoglobulin antibody; PBS, phosphate buffered saline; ELISA, enzyme-linked immunosorbent assay.

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To whom requests for reprints should be addressed, at Department of Clinical Oncology, Hammersmith Hospital, Du Cane Road, London W12 ONN. UK. Trichloroacetic acid precipitation was used to confirm that over 90% of the antibody was IgG. This was also biologically confirmed, when the immunoreactivity of the antibodies was tested by an ELISA, described later.

Radiolabelling. Iodine-131-labeled HMF G, was supplied by Unipath Ltd., UK. Trichloroacetic acid precipitation was used to confirm that more than 85% $^{131}$I was protein bound. Approximately 2 mg of the administered HMF G, was labeled with 0.5 mCi of $^{131}$I using the N-bromosuccinimide method (14), which enabled the kinetics of the exogenously administered HAMA to be assessed.

Patients. A pilot study to examine the potential usefulness of i.v. HAMA in five patients was approved by the Hammersmith Hospital Ethics Committee. All five patients had previously undergone cytoreductive surgery and postoperative chemotherapy for ovarian carcinoma. The kinetics in these patients were compared with those in a group of 28 patients who received i.p. monoclonal antibody labeled with $^{90}$Y or $^{131}$I. We have found that there is no significant difference between the serum pharmacokinetics of intraperitoneally administered $^{131}$I or $^{90}$Y-labeled antibodies for therapy (15). Informed consent was obtained from all patients. A pretreatment laparoscopy was performed to assess residual disease, and insert a peritoneal catheter for i.p. administration of $^{131}$I-labeled MAb.
Treatment Procedure. The treatment protocol in patients receiving i.p. radiolabeled MAb has been described previously (7, 15). 131Iodine radiolabeled antibody was infused with 1.5 liters of 0.9% saline into the peritoneal cavity. Patients remained in a radiation controlled room (reverse barrier isolation) for 5 days after treatment. The five patients received HAMA at various times after the i.p. infusion of MAb (see Table 1). HAMA was infused i.v. over 20 min in 50 ml of 0.9% saline. Blood samples were taken every 12 h to measure total protein-bound 131I activity, and more frequently following HAMA infusions as shown in Fig. 2. Urine was collected continuously for 5 days following MAb infusion to measure 131I activity excreted. Patients were reviewed weekly after treatment to assess clinical toxicity and to measure hematological and biochemical parameters.

Pharmacokinetics and Dosimetry. The pharmacokinetics of i.p. MAb in patients receiving i.v. HAMA were compared to those in the previous 28 patients who had received i.p. radiolabeled MAb as part of phase I studies of i.p. radioimmunotherapy and did not have a preexisting antihuman immunoglobulin response. Nine of these patients received 111In-labeled MAb, and 19 received 99mTc-labeled MAb. The kinetics were also compared to those of five patients who had been treated with 131I-labeled HMFG1, i.p. for a second time, and had developed endogenous human anti-mouse immunoglobulin antibodies (15).

The 131I activity in blood samples of known weight was determined by counting the activity in a well scintillation counter against standard samples of known 131I activity. The whole blood 131I activity was extrapolated from these measurements by estimating the patients blood volume, according to their body surface area (16).

Urine 131I activity was measured by extrapolating the counts from 2-m1 samples of activity and expressed corrected for radioactive decay, rather than as actual isotope activity. In this way we could directly compare the pharmacokinetics of 131I- and 99mTc-labeled antibody, although their physical half-lives are 193 and 64 h, respectively. Actual blood isotope activity was used to calculate radiation dosimetry. The radiation dose to the hemopoietic bone marrow and liver in the first 5 days after treatment were estimated from the MIRD formulation (17). The radiation dose to bone marrow (D marrow) was calculated as follows:

\[ D_{\text{marrow}} = 0.2 \cdot A \cdot S_{\text{marrow} \to \text{marrow}} + A \cdot S_{\text{whole body} \to \text{marrow}} \]

Hemopoietic bone marrow may contain one-fifth the 131I-labeled MAb activity of blood, as hemopoietic marrow accounts for 2.2% of body weight, and is functionally part of the circulation (18). S is the S factor from the MIRD formulation and A is the integrated blood isotope activity in microcurie-hours for the 100 h after treatment. The radiation dose received by the liver in the first 100 h D liver may be calculated as follows:

\[ D_{\text{liver}} = 0.06 \cdot A \cdot S_{\text{liver} \to \text{liver}} + A \cdot S_{\text{whole body} \to \text{liver}} \]

The liver contains 6% of the blood volume at any one time (18), and its extracellular space is not functionally part of the circulation. Both bone marrow and liver are primarily irradiated by radiolabeled antibody within the organ’s circulation [mostly if particles (90%)], but also receive a contribution from the rest of the body [γ-rays (10%)]. The radiation dose to the marrow and liver can only be calculated in this way if the blood pool is the important radiation source. This is the case for iodine isotopes, as free iodine released from the MAb during catabolism is rapidly excreted in the urine. The radiation dose to organs cannot be calculated accurately from the 99mTc blood activity, as free yttrium released from the MAb accumulates in the liver and bone (19–21).

In three patients the administered HAMA was labeled with 131I. The 125I and 131I activities in blood were measured by counting blood samples in a gamma counter using dual 125I and 131I channels. Blood isotope activity was expressed as total activity. The fraction of 125I or 131I bound to protein was measured by gel filtration, passing 50-μ1 serum samples through a 20-ml Sephadex G-50 column.

In three patients the radiation activity over the liver, heart, and left subclavian vein was monitored by a specially shielded scintillation counter. Measurements were taken from these areas every 20 min after injection of HAMA, until the measured counts had fallen to pre-HAMA levels. Measurements were then taken every 6 h.

Measurement of the Human Anti-mouse Immunoglobulin Antibodies. A blood sample was taken from each patient before, and 14 or 21 days after treatment with radiolabeled MAb. After clotting, the serum was separated and screened for anti-mouse immunoglobulin antibodies using a previously described ELISA method (13). Briefly, microtiter plates (Sterilin, UK) were coated with 5 μg/ml HMFG1 or its F(ab’)2 fragment in bicarbonate buffer pH 9.6 for 3 h at 37°C. The plates were then washed three times with washing buffer (PBS, pH 7.4, 0.02% Tween 20-Sigma, UK), and serum applied at 1:10 serial dilutions. After 2 h incubation at 37°C, the plates were again washed, and a species specific anti-human immunoglobulin reagent, peroxidase linked (Amersham, International, UK) was applied. The conjugate was incubated for 1 h at 37°C, washed and the substrate 2,2'-azino-di(3-ethylbenzthiazoline sulfonate) (Behring, West Germany) added. When the color had developed, the absorption was measured at 405 nm using a Titertek multiscan (Flow Laboratories, UK).

In every microtiter plate, and in parallel with the unknown patient’s sample, purified HAMA of known concentration collected from various patients was added to different concentrations, and a standard curve was plotted. Using this curve as a reference, it was possible to determine the total amount of anti-HMFG1 antibodies in each patient.

Purified HAMA was further tested in order to determine whether it cross-reacted with the Fc part of human IgG. Polyclonal human immunoglobulin G (Sigma, UK) was therefore coated in the microtiter plate, and an ELISA was performed in the same manner as described above, the only difference being that the secondary antibody used, was a peroxidase-conjugated anti-human immunoglobulin M (DAKO, Denmark).

Statistics. The Mann-Whitney test for nonparametric distributions (22) was used to test the significance between radiation doses to organs in patients treated with or without HAMA.

RESULTS

Pharmacokinetics. The pharmacokinetics of radiolabeled MAb in blood following i.p. administration is illustrated in Fig. 1. In the 28 patients receiving MAb for the first time (HAMA

![Fig. 1](image-url)
CLEARANCE OF MURINE MAb FROM BLOOD

Fig. 2. Blood 131I activity following the i.p. injection of 131I-labeled MAb expressed as the percentage of the injected activity. The percentage of I-131 not bound to MAb is also illustrated.

Fig. 3. HAMA concentration in serum expressed as a percentage of each injection. HAMA was given at 17 and 39 h in patient 3 (D), 0 and 39 h in patient 4 (■), and 18 and 41 h in patient 5 (Δ).

Fig. 4. Radiation activity over liver following infusion of 131I labeled MAB. Arrows, time of i.v. injections of HAMA.

Fig. 5. Renal 131I excretion, expressed as the percentage of the injected 131I activity, in HAMA negative patients (D) N = 8, patients with endogenous HAMA ( ■) N = 5, and patients given i.v. HAMA (Δ) N = 5. Bars, ±2 standard errors of the mean.

negative), 23% of the injected isotope activity was in the circulation at 40 h. More than 80% of 131I and 95% of 90Y activity was protein bound, as determined by Sephadex G-50 gel filtration. In our hands using the cyclic anhydride chelate of DPTA for 90Y-labeled MAb, there was no significant difference in the blood pharmacokinetics of patients receiving 131I- and 90Y-labeled antibodies. The percentage of injected isotope activity in the blood was in fact slightly higher for 131I than 90Y (data not shown). The blood pharmacokinetics were altered in the five patients who received i.v. HAMA (Fig. 1).

In patients 1, 3, and 5 who received i.p. labeled HMFG1 prior to HAMA, the initial pharmacokinetics were as expected. The percentage of injected 131I in the circulation of these three patients ranged from 7 to 11% prior to i.v. HAMA. However, 15 min after the injection of HAMA there was a rapid decrease in circulating 131I with only 2.7, 3, and 2.5% of the injected 131I activity in the circulation. One h after the injection of HAMA less than 36% of circulating 131I was protein bound. After clearance of 131I-labeled HMFG1 from the circulation, the blood 131I activity gradually increased and the fraction of protein bound 131I activity increased (Fig. 2).

Patients 2 and 4 received i.v. HAMA immediately before their i.p. treatment, and had altered pharmacokinetics from the beginning of their treatment. A second i.v. injection of HAMA in four patients produced a further clearance of HMFG1, (see Table 1) from the circulation, with predominantly free 131I remaining in the blood (Fig. 2).

When less than 20% of the injected HAMA remains in the circulation (about 7 mg), 131I-labeled HMFG1 rapidly accumulates in the blood. In three patients (patients 3, 4, and 5), the HAMA was labeled with 125I so that the clearance of HAMA from the circulation could be studied. Human anti-murine immunoglobulin is rapidly cleared from the blood (Fig. 3). Hepatic 131I activity was monitored in three patients (Fig. 4).

There was a rapid increase in counts over the liver within 15 min after the injection of HAMA. However this increased 131I activity in the liver lasted only 10 h, following which counts in the liver decreased with a half-life of 18–22 h. Free iodine released from catabolized antibody was excreted in the urine. Fig. 5 compares the 131I excretion in the five patients who received a second treatment and had endogenous HAMA with those given exogenous HAMA in this study, and those treated for the first time. After the administration of exogenous HAMA there is a rapid excretion of 131I so that by 5 days (120 h) almost as much 131I has been excreted as in patients with endogenous HAMA.

Radiation Dose to Bone Marrow and Liver. The radiation dose to bone marrow in patients receiving HAMA was calculated by applying the MIRD formulation to the integrated blood milli-cure hours (see “Materials and Methods”). The mean radiation dose to marrow per millicurie of administered 131I-labeled MAb was 0.21 cGy (range, 0.14–0.25 cGy) in the five patients receiving exogenous HAMA, compared with 0.97 cGy (range, 0.63–
1.09 cGy) in patients receiving a first treatment. The dose to liver from each mCi of 131I-labeled MAb in the circulation was 0.11 cGy (range, 0.07–0.15 cGy). This was significantly lower \( (P = 0.001) \) than the mean radiation dose to the liver in nine patients receiving 131I-labeled MAb with no HAMA, 0.44 cGy (range, 0.30–0.57 cGy).

**DISCUSSION**

The injection of i.v. anti-mouse immunoglobulin antibody increased the clearance of circulating murine monoclonal antibody. This has been reported by other workers (23–25) using rabbit or goat anti-murine antibody. The advantage of using human anti-mouse immunoglobulin antibody is that this could be less immunogenic. By conjugating the HMFGi to Sepharose 4B beads, we were able to isolate human anti-HMFGi antibody and found it to be 3125, 1200, 133, 125, and 66 mg, respectively, for each patient (based on a serum volume of 2.5 liters), which is well above not only the estimated amount of administered HAMA still circulating, but also the total amount of HAMA administered.

**Toxicity.** There was no serious toxicity related to the administration of nine injections of HAMA. Patients 1 and 4 complained of hot flushes immediately after the injection. This was associated with a sinus tachycardia of 110–120. Patients 4 and 5 developed a single episode of rigor 20 min after one of the HAMA injections. These side-effects subsided spontaneously within a few minutes without medical intervention. In addition, patients 1 and 2 complained of lethargy for 10 days after the injection of HAMA. All but one patient demonstrated a degree of myelosuppression in their leukocyte or platelet count following treatment. Myelosuppression was mild with no patient developing more than a 60% fall in either platelets or neutrophils (Fig. 6). The blood count nadir in most cases was observed at 5–6 weeks after treatment.
cavity. Animal studies using a similar model have shown that the i.v. administration of anti-antibodies does not remove i.p. injected antibody from i.p. tumor deposits (28). If further HAMA was required to clear MAb from the circulation (determined by a rise in the protein bound:free formed ratio) then a constant, rather than a 20-min infusion of HAMA should be given.

A reduction in the radiation dose to normal tissue should allow greater isotope activities to be given with increased therapeutic effect. Reduced radiation doses to tissue only occur if the radiolabel is excreted. Several groups (19, 24, 29) including ourselves, are currently working with metal radiolabel chelates such as $^{90}$Y rather than $^{131}$I. Using the present generation of chelating agents only 10–20% of the radiometal is excreted when the MAb is catabolized (19, 24, 29). The administration of HAMA to patients receiving these isotopes could increase radiation toxicity, especially if bone-seeking isotopes such as $^{90}$Y (19, 23, 24, 27) are used. HAMA could only be used with metal chelates if the metal radiolabel remain linked to the chelate after MAb catabolism, so that the complexed metal would be excreted in the urine.

Although the administration of i.v. HAMA was successful in decreasing the potential radiation toxicity in patients, there was no suppression of the patient's own immune response to mouse antibody. All patients developed high titers of endogenous HAMA 2 to 3 weeks after treatment. It is possible that if more HAMA was given prior to the administration of MAB then passive immunization would have been more effective. However, if i.v. HAMA is going to decrease toxicity the mouse antibody must be injected into the peritoneal cavity, and only form complexes with HAMA in the circulation. Injecting MAB into the peritoneal cavity, and presenting it directly to the lymphatics, may make passive immunization extremely difficult to achieve.

In conclusion this study demonstrated that administration of exogenous HAMA is a procedure which could potentially decrease the systemic toxicity seen following the administration of i.p. radiolabeled antibodies. Should this be the case, up to 4-fold higher isotope activities should be given in the future in order to increase the dose of radiation to the i.p. tumor.

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REFERENCES

Clearance of $^{131}$I-labeled Murine Monoclonal Antibody from Patients' Blood by Intravenous Human Anti-Murine Immunoglobulin Antibody


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