III. 2. Tissue Distribution Study of Murine Monoclonal anti-GD_3 Antibody in Nude Mice Bearing Human Melanoma Xenografts

Ojima F., Ido T., Kijima-Suda I.**, and Nakagawa Y.*

Department of Pharmacy, Yamagata University Hospital
*Cyclotron and Radioisotope Center, Tohoku University**
*Tokyo Pharmaceutical Reserch Laboratories, Nissin Food Products Co., LTD.***

Tissue distribution of radiolabeled monoclonal anti-GD_3 antibody (IgM) was studied in normal mice and nude mice bearing the human melanoma xenograft. Tissue-to-blood distribution ratios of the antibody in the liver, spleen and kidney increased with time in both normal and melanoma transplanted mice, however, no significant changes were observed in other tissues. Specific accumulation of the antibody to human melanoma (HMV-II) was observed 4 and 5 days after injection. On the other hand, no specific accumulation of standard murine IgM to HMV- II was observed in mice bearing the HMV-II xenograft for 5 days after injection. Since the tissue-to-blood ratio of the distribution ratio became larger than that of other tissues 4 and 5 days after administration, 4 days after the administration of the antibody were required for immunoscintigraphy. Accumulation of the antibody to other human melanoma cells (HMV-I, HMY-1 and MEL188) inoculated into mice was also observed 4 days after the antibody administration. The monoclonal anti-GD_3 antibody would be useful in immunodetection or immunotherapy.

Introduction

The anti-tumor marker monoclonal antibody (mAb) has been developed for immunodetection in human tumors[1,2]. Monoclonal Ab is useful for in vivo immunodetection and the in vitro immunoassay system[1-4].

Disialoganglioside GD_3 was reported to be associated on the cell surface of lymphoblastic leukemia and other tumor cells[3]. The anti-GD_3 mAb reacts with human melanoma cells and some other human cells in vitro as analyzed by the fluoresein immunodetection system[3]. Therefore, GD_3 is an important tumor marker associated with human melanoma. There are many differences around the GD_3 on the cell surface situations between in vivo and in vitro. It is necessary to investigate tissue distribution of the mAb in nude mice bearing human tumor xenografts for immunoimaging and immunothrapeutics[5]. We examined the tissue accumulation of the anti-GD_3 mAb in human melanoma-bearing nude

mice in vivo and the timing necessary for detecting human melanoma using immunoscintigraphy.

Materials and methods

Anti-GD_3 mAb

Anti-GD_3 murine mAb (IgM) established in our group was purified from the ascitic fluid of male BALB/c mice (Funabashi Farm) previously inoculated with antibody-producing hybridoma. The mAb was partially purified by Sephacryl S-300 (Pharmacia) gel exclusion chromatography. This mAb reacts to ganglioside GD_3 in vitro.

Tumor models

Human melanoma cells were cultured and subcutaneously injected (10^7/100μL/mouse) in the back of male BALB/c nu/nu mice (Funabashi Farm). The mice were given 0.1 % NaI solution 3 days before mAb injection to reduce specific uptake of iodine into the thyroid. Melanoma transplanted nude mice were used in this study at 3-4 weeks after transplantation. Three or four mice were used in each study.

Radioiodination of anti-GD_3 mAb

Radioiodination of anti-CD_3 mAb was performed using an ENZYMOBEADS (Radioiodination System, Bio-Rad). One hundred μL of mAb solution and 37 MBq of ^{131}I were added to 100μL of ENZYMOBEADS suspension. The iodination was started by the addition of 1 % β-D-glucose (25μL) and then the reaction mixture was allowed to stand for 30 min at room temperature. After centrifugation, the supernatant was applied to a Sephadex G-25M column (PD-10, Pharmacia) to purify the labeled mAb with phosphate buffered saline as the elution buffer. Immunoreactivity of the labeled mAb was determined by comparison with the residual radioactivity in the wells coated with GD_3 or no GD_3 after incubation at 37°C for 120 min followed by three washings. The labeled mAb solution was filtered to sterilize it before administration to the mice through the tail vein (7.5-11.5μg/100μL/mouse).

Investigation of the tissue distribution of ant-GD_3 mAb in the mice bearing human melanoma.

HMV-II bearing nude mice were sacrificed on 3, 4 and 5 days after administration of labeled mAb through the tail vein. Blood, liver, heart, pancreas, spleen, small and large intestines, kidney, brain, muscle and bone were taken. The solid tissues were washed with 0.9 % NaCl, wiped and weighed. The samples and labeled mAb solution were counted using an auto-well gamma counter. Tissue distribution was expressed as the differential absorption ratio (DAR, counts of tissue/total injected counts) × (g body wt/g tissue) for normalizing the body weight of each mouse. The tissue-blood ratio was also obtained to determine if accumulation would occur. The tissue distribution of anti-GD_3 mAb was also measured in the HMY-1 and MEL188 human melanoma bearing mice 4 days after the mAb adminstration.

Standard murine IgM was iodinated using the same method as mentioned above and its tissue distribution ratio in HMV-II melanoma bearing mice were examined on 4 days after one hundred μL of standard murine IgM solution injection through the tail vein to certain specificity of anti-GD_3 mAb accmulation to HMV-II.

Autoradiography

In the HMV-I and HMV-II inoculated mice, the mice were sacrificed on 4 days after labeled mAb injection and frozen in hexane-dry ice to make a 3 % sodium carboxy methyl cellulose block. Slice samples were cut from the frozen whole body block with a -20°C cryotome and exposed to X-ray film.

Results

Time course of tissue distribution of labeled anti-GD_3 mAb in the normal mice

Biodistribution of labeled ant-GD_3 mAb in normal mice was investigated on 1, 2, 3 and 4 days after injection. Radioactivities in the tissues all decreased with time (Fig. 1), however, the tissue-to-blood radioactivity ratios were the same level with time except for the liver, spleen and kidney (Fig. 2). The tissue-to-blood radioactivity ratios of the liver, spleen and kidney increased with time (Fig. 2).

Time course of tissue distribution of labeled anti-GD_3 mAb in the nude mice bearing HMV-II xenografts

The results of the tissue distribution study of the labeled mAb are shown in Fig. 3. The distribution ratio of the radioactivity declined with time in all tissues except for HMV-II and liver. The tissue-to-blood ratio is shown in Fig. 4. In the HMV-II, liver, spleen and kidney, the tissue-to-blood ratio increased with time, however, no changes were observed in any of other tissues on 3, 4 and 5 days after mAb injection.

Autoradiography

The accumulation of the anti-GD_3 mAb was observed in nude mice bearing both HMV-I and HMV-II human melanoma 4 days after mAb administration. Fig. 5 shows the whole body autoradiogram. In the melanoma, a nonuniform uptake was observed, however, the accumulation was uniform in the other normal tissues. The radioactivity ratios of the other tissues to liver in HMV-I bearing mice are shown in Table 1. In the HMV-I, the mean melanoma to liver ratio was 1.1 and the maximum was 3.9. The mean melanoma to liver ratio was 7.9, and the maximum distribution ratio to liver was more than 10 in the HMV-II (Table 1).

Tissue distribution of labeled murin standard IgM antibody in the nude mice bearing HMV-II xenografts

The tissue-to-blood distribution ratios of labeled murine standard IgM and anti-GD_3 Ab in HMV-II bearing nude mice 4 days after antibody injection are represented in Fig. 6. No specific accumulation in the murine standara IgM was observed in HMV-II compared to the anti-GD_3 mAb distribution ratio.

Distribution ratio of Ab in HMY-1 and MEL188 human melanoma

The tissue-to-blood distribution ratios of anti-GD_3 mAb in HMY-1 and MEL188 bearing nude mice 4 days after mAb injection are represented in Fig. 7. The mean distribution ratio of mAb in HMY-1 was same as in the blood, however, specific accumulation of mAb was also seen in the MEL188 tissue.

Discussion

The tissue distribution of mAb is of significant importance in order to develop the mAb for delivering radionuclei or anti tumaor drugs to objective tumors. The immunoscintigraphy and/or immunotherapy of tumors would depend on the ratio of specific radioactivity delivered to tumors versus normal tissues. The murine monoclonal anti-GD_3 mAb was investigated for its biodistribution in the normal mice and nude mice bearing some human melanoma xenografts in this study.

In the HMV-II study, the accumulation of labeled mAb seemed to occur 3 days after injection. Since no specific accumulation was seen in standard murine IgM in HMV-II bearing nude mice, the accumulation of antibody in HMV-II was specific for anti-GD_3 mAb. Because the concentration of the radioactivity in the blood was higher than that of HMV-II, it was not suitable for immunoscintigraphy till 3 days after administration. Compared to all other tissues, the highest accumulation of mAb in HMV-II was observed on 4 and 5 days after injection. The tissue accumulation dose was higher on 4 days after mAb injection compared to the tissue accumulation ratio on 5 days after mAb injection. Therefore, it was suitable for the immunodetection of melanoma in this IgM subclass murine anti-GD_3 mAb on 4 days after injection. An autoradiography and tissue distribution study was performed 4 days after mAb administration. At the point of the mean distribution ratio, the HMV-I and HMY-1 human melanoma seems not to be detectable with this mAb, but as shown in the autoradiograms and Table 1, nonuniform accumulation in the melanoma tissues were seen, however, the accumulation was uniform in other normal tissues. This suggested that the highest accumulating regions in the melanoma tissue were detectable with immunoscintigraphy. These results indicated that the melanoma region was detectable despite their shape and volume were not exactly detectable. The nonuniform accumulation in the melanoma was based on the capillaries in the tumor tissue and affinity of antibody and cell

surface antigen. For example, a high affinity antibody is difficult to separate from the antigen near the capillary and diffuse into inner region of the tumor tissues.

The accumulation ratio of this mAb was approximately 1 % of the injected dose per gram wet tissue 4 days after injection. A lager accumulation ratio was reported when using the IgG subclass Ab[6,7]. A higher accumulation was necessary for immunotherapeutics. The mAb used in this study was the IgM subclass, because of its high molecular weight, it is difficult for it to pass through the capillaries in the melanoma tissues. It is necessary to study the biodistribution of Fab or F(ab') obtained from enzyme digestion in the melanoma-bearing nude mice and other radiolabeling methods[1,8-11].

References

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Table 1. Ratio of tissue radioactivity intensity compared to liver in HMV-I and HMV-II bearing mice in autoradiograms (Fig. 5).

Melanoma	Radioactivity intensity ratio to liver
HMV-I	Mean (whole): 1.1, Most accumulated region: 3.9
	Blood: 0.93, Lung: 0.64, Spleen: 0.59, Kidney: 0.59
HMV-II	Mean (whole): 7.9, Most accumulated region: > 10

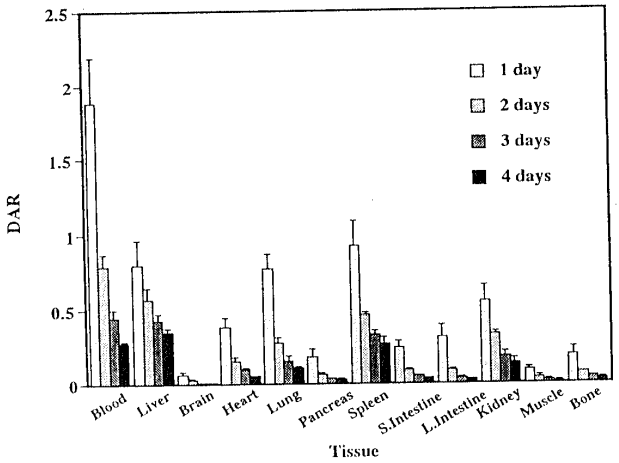


Fig. 1. Time course of tissue distribution ratio of anti-GD_3 ^{131}I mAb in normal mice.

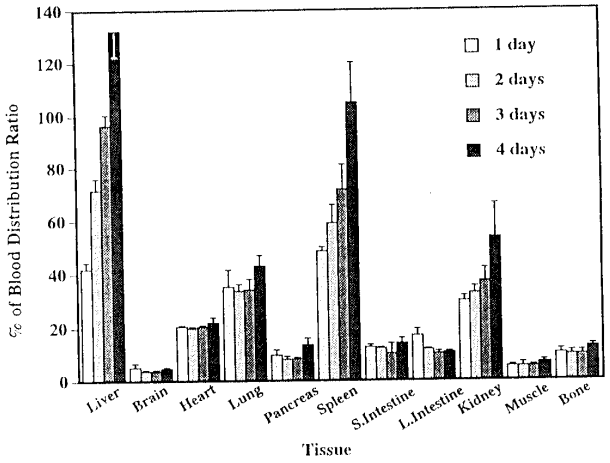


Fig. 2. Time course of tissue distribution ratio of ^{131}I anti-GD_3 mAb compared to blood radioactivity in normal mice.

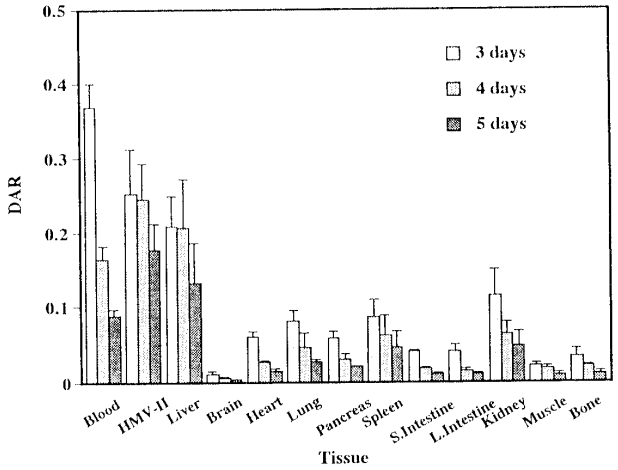


Fig. 3. Time course of tissue distribution of anti-GD_3 mAb labeled with ^{131}I in HMV-II bearing mice.

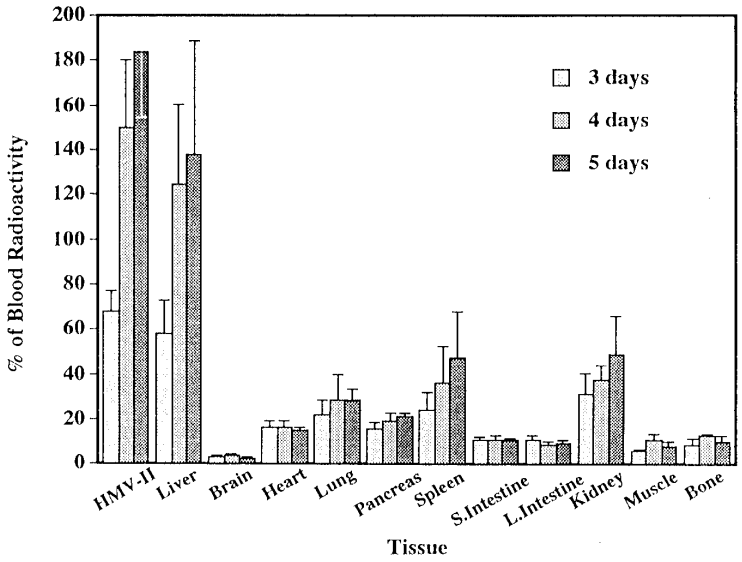
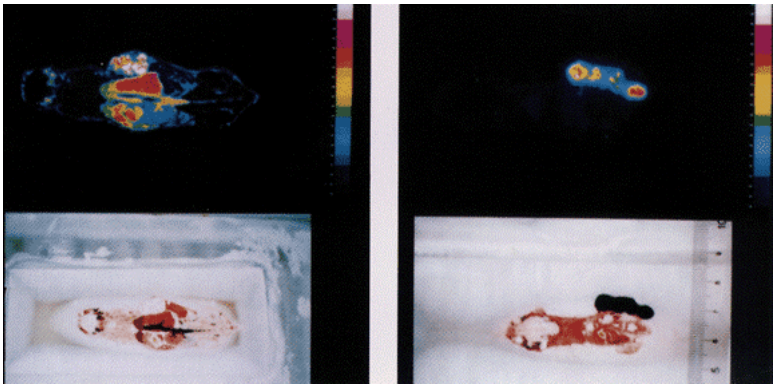


Fig. 4. Time course of tissue distribution ratio of anti-GD_3 mAb labeled with ^{131}I compared to blood in HMV-II bearing mice.



(A) (B)

Fig. 5. Autoradiograms of whole body 4 days after ^{131}I anti-GD_3 mAb injection in HMV-I (A) and HMV-II (B) bearing mouse. Arrows indicate the melanoma.

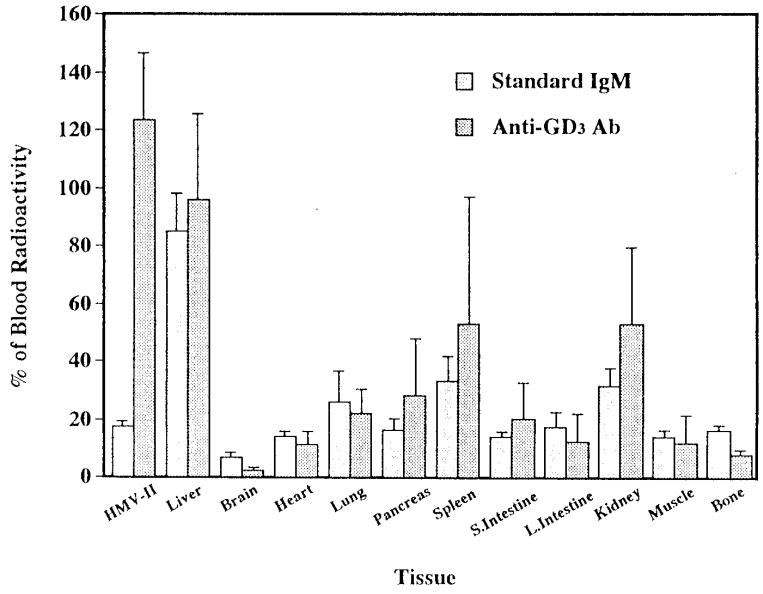


Fig. 6. Tissue distribution ratio of standard murine IgM and anti-GD_3 mAb compared to blood 4 days after injection in HMV-II bearing mice.

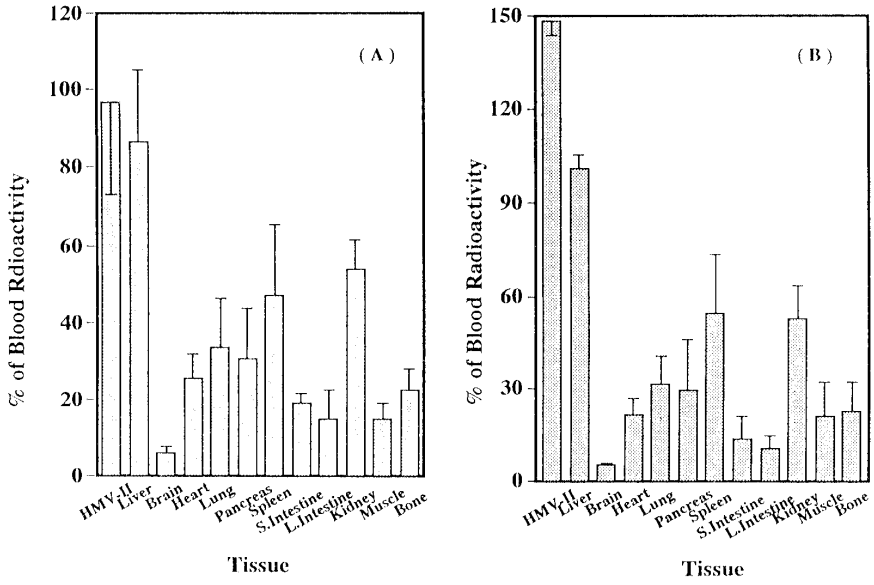


Fig. 7. Tissue distribution ratio of ^{131}I anti-GD_3 mAb compared to blood radioactivity in HMY-1 (A) and MEL188 (B) bearing mice 4 days after injection.