Biodistribution of Radiolabeled Monoclonal Antibody after Intraperitoneal Administration in Nude Mice with Hepatic Metastasis from Human Colon Cancer

KAZUHIKO YOSHIDA, TOURU FUJIKAWA, GEORGE YOSHIZAWA, AKIHIRO TANABEA, and KENJI SAKURAI

First Department of Surgery, The Jikei University School of Medicine, Tokyo, Japan

Abstract: The utility of the intraperitoneal (IP) administration of radiolabeled monoclonal antibody (mAb) for hepatic metastasis from human colon cancer was evaluated in congenital athymic mice. The intrasplenic injection of HT-29 LMM metastatic human colon cancer cell line reproducibly results in hepatic metastasis formation in nude mice. HT-29-15, a murine mAb of IgG1 class reactive with the HT-29 LMM cell line was labeled with iodine 125. One μg/2 μCi of labeled HT-29-15 was injected intraperitoneally into mice with hepatic metastases, and additionally IP administration of the same dose of I-125 labeled HT-29-15 with increased volume and intravenous (IV) administration of dose quantity of HT-29-15 were performed. Blood samples were obtained at 1, 3, 5 hours, and the animals were sacrificed on days 1, 3, and 5. The per cent of injected dose per gram (%ID/g) of blood after IP administration of 1-125 labeled HT-29-15 reached the same level of %ID/g after IV administration by 5 hours. The transfer from the peritoneal cavity to blood was delayed by increasing the volume injected. From day 1 to day 3, there was a progressive increase for hepatic metastasis/blood ratios of I-125 labeled HT-29-15 in each group. There was no difference in the hepatic metastasis/blood ratios among the three groups. IP administration of specific mAb, therefore, provides the same level of tumor uptake in hepatic metastasis from colorectal cancer, and would be advantageous in patients with both hepatic metastasis and peritoneal implants in which radioimmunodetection and radioimmunotherapy are appropriate.

Key Words: Monoclonal antibody, nude mice, hepatic metastasis, colon cancer, HT-29LMM

Introduction

The prevalence of colon cancer continues to increase in Japan. Liver metastasis has been found to represent the primary cause of therapeutic failure; in patients dying of colorectal cancer 48 per cent will have future liver involvement.\(^\text{1}\) Both the detection and therapy of hepatic metastasis of colorectal cancer remain important goals of clinical oncology.

Since the initial success reported by Goldenberg et al. for the clinical localization of primary and secondary colorectal cancer using polyclonal antibodies for carcinoembryonic antigen,\(^\text{2}\) the use of radiolabeled antibodies has shown promise as a clinically useful modality for the diagnosis and treatment of colorectal cancer.\(^\text{3}\)

There is little data on absolute hepatic metastasis and normal tissue uptake of radiolabeled mAb uptake because there are considerable limitations in obtaining suitable hepatic metastasis samples and normal tissue in humans. Recently, we have reported a murine model with hepatic metastasis from human colon cancer, which appears to be useful for the evaluation of localization of radiolabeled mAbs to hepatic metastasis.\(^\text{4}\)

Using this model we are able to determine the utility of intraperitoneal (IP) administration of radiolabeled mAb for hepatic metastasis from human colon cancer.

Materials and Methods

Establishment of Hepatic Metastasis in Nude Mice

Specific pathogen-free athymic BALB/c female mice 3 to 4 weeks of age were cared for at the animal facility. The mice were kept under sterile conditions in a laminar flow room in cages with filter bonnets and were fed sterilized mouse diet and sterilized water.

The human colon cancer cell line HT-29 LMM, a metastatic variant of the HT-29 cell line,\(^\text{5}\) was gener-
Macroscopic findings of hepatic metastases in a splenic xenograft. The hepatic metastases appeared as multiple irregular gray-white nodules of varying size and were evenly distributed in both liver lobes. ×2

Radiolabeling of Monoclonal Antibody (mAb)

HT-29-15, which was developed by Dr. J. Sakamoto at Memorial Sloan-Kettering Cancer Center in New York, is a murine IgG1 mAb reacting with a neuraminidase-sensitive cell-surface antigenic determinant (200Kd) present on the HT-29 human colon cancer cell line. It reacts with more than 60 per cent of primary and metastatic colorectal cancers in immunohistopathology. A study on colorectal cancer patients with hepatic metastasis demonstrated that radiolabeled HT-29-15 localized to tumor tissue.7

HT-29-15 mAb was labeled with iodine-125 sodium iodide by a modified chloramine-T method.8 The radiolabeled mAb was purified by exclusion chromatography on a Sephadex G-25 column (Pharmacia, U.S.A.). Per cent labeling was 80 per cent. The fraction corresponding to the radiolabeled mAb typically had more than 95 per cent of radioactive iodine bound to protein, as determined by trichloroacetic acid precipitation. Immunoreactivity was tested by a modification of the method of Lindmo et al.9 and was 55 per cent for HT-29-15 mAb.

Biodistribution

One μg/2μCi of labeled HT-29-15 in 0.5ml was injected intraperineally in 11 mice with hepatic metastasis, and additionally an IP administration of the same dose of I-125 labeled HT-29-15 in 2.0ml was given to 12 mice. An intravenous (IV) administration of dose quantity of HT-29-15 was also performed in 9 mice. Blood samples were obtained from the tail vein at 1, 3, 5 hours. Bleeding was obtained by nicking the tail vein with a scalpel, and 3–5 drops were collected into preweighed tubes. Data were expressed in terms of per cent injected dose per gram of blood (cpm/g of blood)/(total injected dose).

Three or 4 animals were sacrificed at days 1, 3, and 5. Normal tissue (liver, spleen, heart, lung, kidney, large intestine, thyroid, muscle, and brain), blood, splenic tumor, and all hepatic metastasis were removed, weighed and their radioactivity counted in a gamma well counter. Per cent injected dose per gram of tissue; (cpm/g of tissue)/(total injected dose) and tissue/blood ratios were calculated. A statistical analysis was performed by the Student's t-test.

Results

Efflux from Peritoneal Cavity to Blood

As shown in Fig. 3, with an injection volume of 0.5 ml, mAb was transferred from the peritoneal cavity to the