Localization of Radioiodinated Antibody to α-Fetoprotein in Hepatoma Transplanted in Rats and a Case Report of α-Fetoprotein Antibody Treatment of a Hepatoma Patient


The First Department of Internal Medicine, Nagasaki University School of Medicine, Nagasaki, Japan [T. K., N. I., T. M., Y. K., S. N., A. T.], and Department of Biochemistry, Hokkaido University, School of Medicine, Sapporo, Hokkaido, Japan [A. H., K. K., Y. T., S. N., H. H.]

Abstract

The localization of radioactive iodine-labeled anti-α-fetoprotein (AFP) antibody in transplanted rat hepatomas was examined by total body scintigraphy, subcellular distribution of radioactivity, and microautoradiography of tissues. The specific antibody was isolated from anti-rat AFP horse antiserum by immunoadsorbent that was coupled with rat AFP and radioiodinated with $^{125}$I. F(ab')$_2$ was isolated from peptic digests of the specific antibody. Normal horse IgG was taken as control. Scintigrams taken 48 to 168 hr after injection of the radioiodinated antibody or its F(ab')$_2$ showed a remarkable localization in the tumors. Normal immunoglobulin G was also taken up in the tumor to some extent.

The tumor/blood radioactivity ratio determined 7 days after the injection was about 4 times higher in the experimental group than was that in the control group, and the ratio on Day 7 was over 1.0. This indicated the active accumulation of the radioiodinated antibody in the tumor.

Tumor tissues were homogenized and fractionated into subcellular fractions. About 30 to 60% of the total radioactivity in the homogenate was found in a fraction which contained cell membrane and nucleus. Microautoradiograms of the tissue sections showed the specific localization of radioactivity in the tumor but not in noncancerous tissues. Normal immunoglobulin G did not show significant localization in the tumor. These experimental results indicated the specific uptake of the radioiodinated antibody to AFP into AFP-producing tumor cells.

A patient with an inoperable hepatoma was given 450 mg of purified anti-human AFP horse antibody i.v. Serum AFP level decreased in 2 days to the normal level (< 10 ng/ml), and this low level has been maintained continuously for over 4 months. To date, the clinical effect of the antibody to tumor has not been exhibited. No side effect of the horse antibody was observed.

Introduction

It has been demonstrated clearly that histochemically the antibody to AFP binds specifically to AFP-producing cells. The membrane-staining technique could demonstrate the presence of AFP on the surface of AFP-producing viable cells (9). Goldenberg et al. (1, 2) have shown the specific localization of radioiodinated antibody to CEA by photoscan in human colonic tumors, xenografted in hamster or in patients.

An experimental study was carried out in our laboratory to learn whether the radioiodinated anti-AFP antibody localizes specifically in AFP-producing tumors, and some positive results were obtained by photoscan or determination of radioactivity distributed in tumor tissues. The present paper describes the results of this animal experiment.

The cytotoxic and growth-inhibitory effects of the antibody to AFP upon AFP-producing tumor cells were demonstrated experimentally either in vitro or in vivo (3). The clinical study of therapeutic application of the antibody commenced in our laboratory. A case of a hepatoma patient is reported in this paper.

Materials and Methods

Preparation of Specific Antibody to Rat or Human AFP. Horses were immunized with purified rat or human AFP. The specific antibody to AFP was purified by affinity chromatography with immunoadsorbents coupled with purified AFP. F(ab')$_2$ was obtained by papain digestion of the specific antibody. One mg of the specific antibody bound about 100 to 150 μg of the antigen. F(ab')$_2$ showed about the same activity. Normal horse IgG was purified by DEAE-cellulose chromatography.

Iodination. The anti-rat AFP horse antibody, F(ab')$_2$, and normal horse IgG were labeled with $^{125}$I by the chloramine-T method. The specific radioactivity was approximately 1 mCi/100 μg.

Animals. Male 4-week-old Donryu rats (Nihon Rat Co., Ltd.) were used throughout the in vivo experiments. Yoshida ascites hepatoma AH-7974 cells (5 x 10$^6$), which are known to produce AFP, were inoculated s.c. in the lateral part of the thigh. After 10 days, the serum levels of AFP were elevated to 100 to 700 ng/ml, and the tumor grew to a diameter of approximately 1 cm. One hundred to 120 μCi of $^{125}$I-labeled anti-rat AFP horse antibody or its F(ab')$_2$ were given to each rat in the experimental group. The same doses of $^{125}$I-labeled normal horse IgG or F(ab')$_2$ were given to rats of the control group. Animals were given potassium iodide in their drinking water to block the uptake of radioactive iodine by the thyroid.

Total body scintigrams were obtained by γ-scintillation camera (Aloka Rev-207) at various time intervals (1 hr to 7 days).

After scintigraphy, the rats were sacrificed and tissue/blood radioactivity ratios were determined (cpm/g of tissue divided by cpm/g of blood).

Subcellular Distribution of Radioactivity in the Tumor Tissue. The rats were treated i.v. with about 100 μCi of $^{125}$I-labeled...
anti-AFP or normal horse IgG. They were sacrificed on Days 1, 3, 5, 7, and 10 after injection and the whole body was thoroughly perfused with 0.9% NaCl solution to remove the blood as far as possible. Tumor tissues were removed, fractionated by the method of Hogeboom (5) into subfractions and counted.

**Autoradiography.** Tissues removed from the rats on Day 7 after injection of $^{125}$I-specific antibody or $^{129}$I-normal horse IgG were fixed with a buffered formol solution, embedded in paraffin, and cut into 5-μm-thick sections. The sections were deparaffinated and coated with Sakura NR-M2 nuclear emulsion. Four weeks later, the exposed slides were developed, and the sections were stained with hematoxylin and eosin. The frozen section of each tissue was also examined in the same manner.

The treatment of a hepatoma patient with anti-AFP antibody is described in the following section.

**Results**

**Total Body Scintigraphy.** In the experimental group, a considerable radioactivity was detected in the thoracic and abdominal areas 24 hr after injection of $^{129}$I-antibody. After 48 hr, the background activity became feeble, and the photoscan detected a considerably intense imaged area corresponding to the tumor site. After 120 and 168 hr, images were still quite clear in the tumor. In one rat, the photoscan 7 days after injection gave the 4 radioactive areas, as shown in Fig. 1. The hot area on the left (arrow) was the original s.c. tumor. Two hot areas in the middle portion of the photoscan were confirmed as i.p. metastatic tumors, and the remaining one on the right was presumed to be the spleen. No differences were observed between F(ab')$_2$ and the specific antibody in the intensity of images through 7 days after injection. The radiolabeled normal horse IgG also gave some tumor images, although its intensity was generally less than that made by the radiolabeled antibody.

**Tissue/Blood Radioactivity Ratio.** In the experimental group, the ratios in the tumor, spleen, and kidney were slightly higher than those in the control group on Day 3 after injection of $^{125}$I-antibody or $^{129}$I-IGG. On Day 7 after injection, a remarkable increase in the ratio exceeding 1.5 was observed in the tumor tissue of the experimental group, which was nearly 4 times higher than was that of the control group (Chart 1). There were no significant changes in the ratio in the other tissues of either the control or experimental groups except in the spleen and kidney. These data indicated an active accumulation of radioactive antibody in the tumor tissues. It is also noteworthy that the spleen in the experimental group showed a high ratio on Day 10. This phenomenon might be caused by trapping antigen-antibody complexes in the spleen.

**Subcellular Distribution of Radioactivity in the Tumor Tissue.** About 30 to 60% of the total radioactivity was found in a fraction consisting of a cell membrane and a nucleus, 10 to 15% were found in the mitochondrial and microsomal fractions, and the rest was in the soluble fraction. The distribution of radioactivities of $^{129}$I-antibody increased gradually for 10 days in the cell membrane plus nucleus fraction, whereas the change in distribution in the microsomal or mitochondrial fractions was not significant. The distribution of antibody in the membrane plus nucleus fraction was always 10 to 20% higher than that of the normal IgG.

**Autoradiography.** In some fixed sections, radioactive antibody was demonstrated as black grains localizing on the surface of the tumor cells. Only a small number of grains were observed in the spleen, and practically no deposits were observed in the liver and kidney.
showed clearly that \(^{125}\text{I}}\)-antibody was localized on the surface of the tumor cells.

The nonspecific uptake of the normal horse IgG in the tumor was also demonstrated, although the uptake was faint compared to the antibody uptake.

The administration of the anti-human AFP antibody to a hepatoma patient caused an immediate suppression of a raised serum AFP level. This can be explained by the removal of serum AFP by the antibody. However, this low AFP level has been maintained for at least 5 months. This phenomenon is explained only by suppression of the production of AFP, because the antibody administered does not remain in the blood for such a long period. Presumably, the antibody attacked and overwhelmed some hepatoma cells which produce AFP. The hepatoma consists of both AFP-producing and nonproducing cells; in this patient, the fraction of AFP-producing cells is considered to be small because of a relatively low serum AFP (7). The antibody may attack probably only AFP-producing cells. If so, the AFP antibody therapy is hopeless. However, as far as the animal experiment is concerned, rat hepatoma which also consists of AFP-producing and nonproducing cells could be cured by the antibody treatment (6, 10). One possible explanation is that the antibody attacks the AFP-producing hepatoma cells. The cells that are killed or coated with antibody will be a good immunogen to the host which produces some immunity to hepatoma cells in relation to their tumor-specific antigens.

Although our patient is under investigation at present (6 months after the antibody administration), we have no evidence yet for the improvement of the disease.

A fairly large amount of the antibody (450 mg), which is a foreign protein to humans, was given to the patient, but no unfavorable side effects occurred.

References
Localization of Radioiodinated Antibody to $\alpha$-Fetoprotein in Hepatoma Transplanted in Rats and a Case Report of $\alpha$-Fetoprotein Antibody Treatment of a Hepatoma Patient

T. Koji, N. Ishii, T. Munehisa, et al.