Triple-labeled Whole-body Autoradiography of Tumor Bearing Rat using 18F-FDG, 67Ga-Citrate and 75Se-Selenomethionine

III. 15. Triple-labeled Whole-body Autoradiography of Tumor Bearing Rat using ^{18}F-FDG, ^{67}Ga-Citrate and ^{75}Se-Selenomethionine

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Introduction

Double-tracer technique for whole body autoradiography has been described using two isotopes with different half-life and energy spectrum. This technique enables us to evaluate two different information from the same section. We reported the glucose and amino acid metabolism in tumor bearing rat using whole body autoradiography with a short-lived positron emitter and a long-lived β emitter [1]. We used ^{18}F-FDG with a half life of 110 min and ^{14}C-methionine with a half life of 5730 years.

The ^{67}Ga scintigram is widely used in clinical study of human cancers not only determine the extent of disease but to monitor the effectiveness of treatment. Since the exact mechanism of the uptake of ^{67}Ga by malignant cells is still obscure, it is thought to be meaningful if we can compare the uptake of ^{67}Ga in tumor with glucose and amino acid uptake of tumor in various experimental conditions using whole body autoradiography. The present report shows the method to obtain the triple-labeled autoradiographic images of tumor bearing rat using ^{18}F-FDG, ^{67}Ga-citrate and ^{75}Se-selenomethionine.

Materials and Methods

The synthesis of ^{18}F-FDG was according to the method described by Shiue et al.[2]. ^{75}Se-selenomethionine and ^{67}Ga-citrate were purchased commercially from Daiichi Isotope Laboratory(Tokyo).

Male Donryu rat weighing about 120g was used. Tumor cell suspension of ascitic hepatoma AH109A was injected subcutaneously into the back of the rat. Six days later, 0.2 ml of turpentine oil was injected subcutaneously to produce chemical inflammation. Seven days after implantation of tumor, the animal was received 62.5 μCi of ^{67}Ga-citrate via the tail vein. The rat was also received 3.46 mCi of ^{18}F-FDG 50 min before death and 5.9 μCi of

^{75}Se-selenomethionine 30 min before death. Autoradiography was performed 9 days after implantation.

After injection of three tracers and allowing time for distribution, the rat was killed by inhalation of chloroform. The body was frozen by immersion in a mixture of acetone and dry ice at -78°C and attached to a pre-cooled microtome stage by means of a carboxymethyl cellulose gel. The frozen stage-mounted rat was placed in the cutting position on a cryomicrotome (NA200, Nakagawa, Tokyo). Whole body sections were taken at -20°C in the sagittal plane at a thickness of 40 μm. The sections were placed immediately onto a x-ray film (MARG Tritium type, Konica, Tokyo) without amplification screen. After 70 min exposure, the film was removed and developed. Two days after the first exposure, the second exposure was carried out for 2 days to produce a ^{67}Ga-image. Then 4 weeks after the second exposure, the third exposure was done for 2 weeks to produce a ^{75}Se-image.

Results and Discussion

The purpose of the present approach is the differentiation of three isotopes in such a way that three single-tracer autoradiograms with minimal contamination by the other tracers are obtained. The following isotopes were used in this study: ^{18}F-FDG for the assessment of glucose metabolism, ^{75}Se-selenomethionine with a half life of 120.4 days for the assessment of amino acid metabolism, and ^{67}Ga-citrate with a half life of 77.9 hours for comparison with other two isotopes. ^{18}F-FDG and ^{67}Ga-citrate are differentiated as follows (Fig. 1). Specific activity of ^{18}F-FDG is so high that the short-time exposure to an X-ray film for 70 min results in blackening of the film by ^{18}F without significant contamination by ^{67}Ga-citrate. After 2 days ^{18}F has decayed. Autoradiogram obtained from a second exposure to a film for 2 days represents ^{67}Ga radioactivity because specific activity of ^{67}Ga-citrate is enough high that the exposure is carried out without significant contamination by ^{75}Se-selenomethionine. Then after 4 weeks ^{67}Ga has decayed. A third exposure of the same section for 2 weeks to a film allows the selective visualization of incorporated ^{75}Se-selenomethionine.

Figure 2 shows three autoradiograms of the rat from the same section. The distribution pattern was apparently different in the three exposures. In ^{18}F-FDG image, high radioactivity was observed in the tumor and inflammation of the back as well as heart and brain. The inflammation showed some uptake of ^{67}Ga-citrate but showed little distribution of ^{75}Se-selenomethionine. Selenomethionine distributed mainly in the tumor, liver and pancreas representing high amino acid metabolism.

The linear blackenings in the liver caused by accidental warming of the section can be seen in the ^{75}Se-selenomethionine image. It proved essential to avoid warming the non-dehydrated sections when placing them against films.

The time aspect is the most difficult problem in using short-lived nuclides. Although the killed animal was frozen just long enough to ensure that the inner portions had solidified and the sections obtained were immediately placed on the film without any time-consuming

dehydration, it had taken 3.7 hours when the contact started, and ^{18}F-FDG already decayed from 3.46 mCi to 0.86 mCi. That means the ^{18}F/^{67}Ga ratio of radioactivity decreases from 55 to 13. Contamination of short-lived isotope by long-lived isotope was, however, considered to be negligible in our study according to the three images.

References

1) Sato T., Fujiwara T., Abe Y., et al., RADIOISOTOPES 38(1989)7.
2) Shiue C-Y., Salvadori P. A., Wolf A. P., et al., J. Nucl. Med. 23(1982)899.
3) Higashi T., Nakayama Y., Murata A., et al., J. Nucl. Med. 13(1972)196.

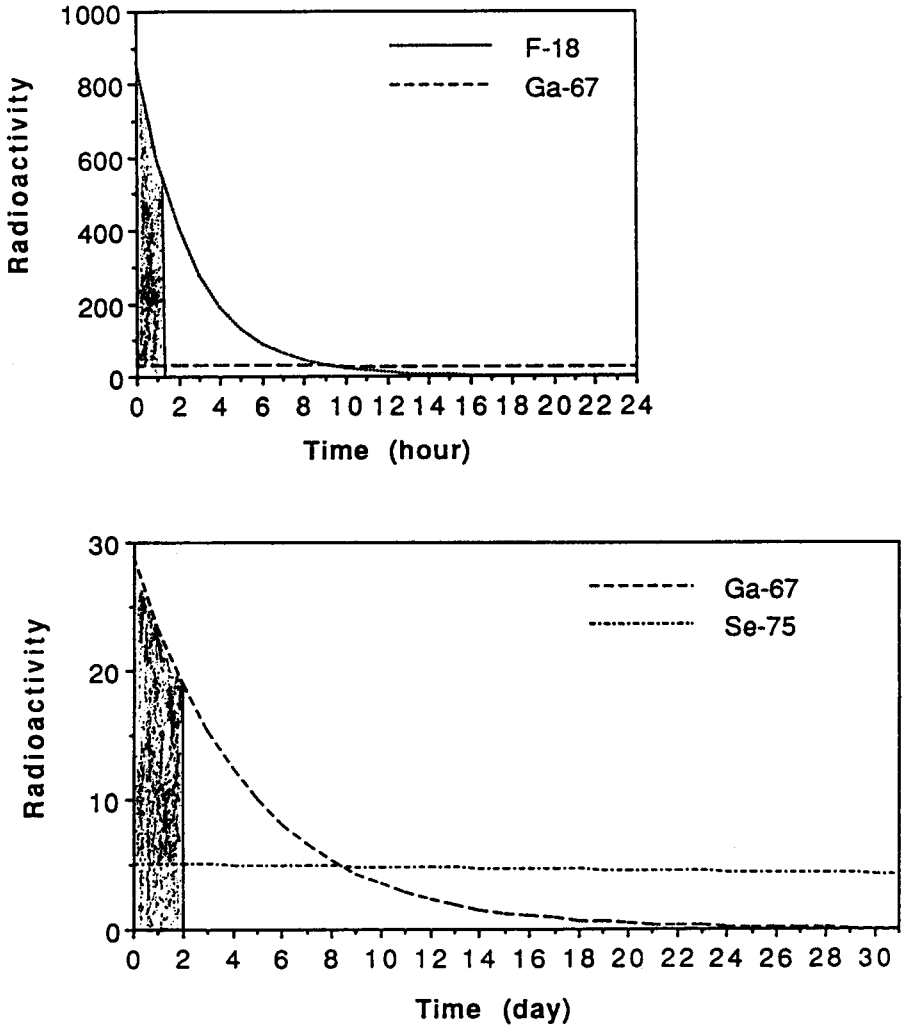

Fig. 1. Differentiation of three isotopes. Activity of ^{18}F-FDG is so high that the short-time exposure to an X-ray film results in blackening of the film by ^{18}F without significant contamination by ^{67}Ga-citrate. After ^{18}F has decayed, autoradiogram obtained from a second exposure to a film for 2 days represents ^{67}Ga radioactivity because specific activity of ^{67}Ga-citrate is enough high that the exposure is carried out without significant contamination by ^{75}Se-selenomethionine.

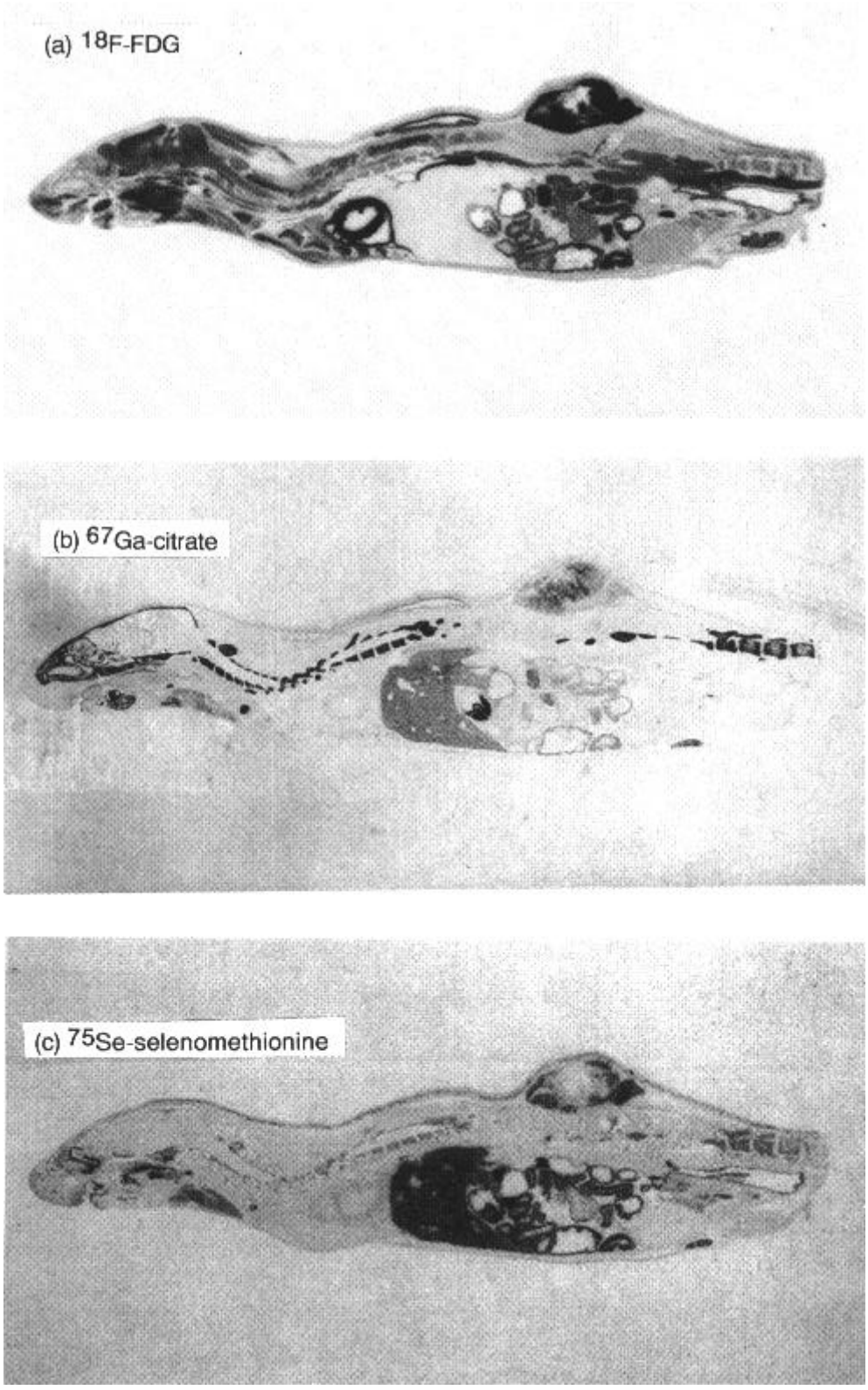

Fig. 2. Autoradiograms from a tumor bearing rat showing (a) the distribution of ^{18}F-FDG (50 min after injection), (b) ^{67}Ga-citrate (2 days after injection) and (c) ^{75}Se-selenomethionine (30 min after injection) in the same section. The distribution pattern is apparently different in the three exposures.