

## Imaging of intraperitoneal tumors with technetium-99m GSA

Zhengsheng YAO,\* Meili ZHANG,\* Harumi SAKAHARA,\* Tsuneo SAGA,\* Yuji NAKAMOTO,\*  
Noriko SATO,\* Songji ZHAO\*, Yasushi ARANO\*\* and Junji KONISHI\*

\*Department of Nuclear Medicine, Faculty of Medicine, Kyoto University

\*\*Department of Radiopharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Kyoto University

<sup>99m</sup>Tc labeled galactosyl serum albumin (GSA) has been used clinically as a receptor-binding agent for the assessment of liver function. The aim of this study was to investigate the usefulness of <sup>99m</sup>Tc-GSA in intraperitoneal (i.p.) tumor imaging. A tumor model was established by i.p. inoculating nude mice with human ovarian cancer cell SHIN-3, or colon cancer cell LS180. Radiolabels were i.p. injected into the tumor-bearing mice and the biodistribution of radioactivity was examined. After administration, <sup>99m</sup>Tc-GSA rapidly accumulated in the tumor. The tumor uptake was 5.82–8.46 %ID/g from 30 min to 6 h after the injection. Radioactivity in the blood was very low, less than 0.3 %ID/g, resulting in high tumor-to-blood ratio. Tumors could be clearly seen by scintigraphic imaging. Accumulation of i.p.-injected <sup>99m</sup>Tc labeled human serum albumin (HSA) in i.p. tumors was similar to that of <sup>99m</sup>Tc-GSA, but radioactivity of <sup>99m</sup>Tc-HSA in the circulation was high, resulting in a significantly lower tumor-to-blood ratio. In conclusion, <sup>99m</sup>Tc-GSA, when i.p. injected, accumulated in i.p. tumors and cleared from circulation rapidly, which would make it useful for the imaging of i.p. tumors.

**Key words:** tumor imaging, <sup>99m</sup>Tc-GSA, intraperitoneal xenografts

### INTRODUCTION

THE DEVELOPMENT of malignant ascites is a common event in ovarian and digestive cancers and often results in a serious condition.<sup>1</sup> Radiolabeled antitumor antibodies have been used in the intraperitoneal (i.p.) tumor imaging and radioimmunoguided surgery, but slow clearance of the antibody from the circulation is a problem.<sup>2</sup>

<sup>99m</sup>Tc-galactosyl human serum albumin (GSA) is a liver imaging agent which accumulates in the liver very rapidly through asialoglycoprotein receptor in the liver.<sup>3–5</sup> Radiolabeled human serum albumin (HSA) was found localizing to human cancer.<sup>6</sup> A recent study revealed that HSA and bovine serum albumin (BSA) bound to tumor cells through a characteristic protein present on the cell surface.<sup>7</sup> Because GSA is a galactosyl HSA, GSA

may also bind to tumor cells through the albumin binding protein.

In the present study, we administered <sup>99m</sup>Tc-GSA i.p. into nude mice bearing i.p. xenografted tumors to investigate the usefulness in i.p. tumor imaging. Because GSA accumulated to the liver and cleared from the circulation rapidly after being absorbed from the peritoneal cavity, clear imaging of the tumor could be obtained.

### MATERIALS AND METHODS

*Tumor cells and intraperitoneal tumors in nude mice*  
Human ovarian cancer cell line SHIN-3 and colon cancer cell line LS180 were grown in RPMI 1640 medium (Nissui Pharmaceutical Co., Tokyo, Japan) supplemented with 10% fetal calf serum (GIBCO Laboratories, Grand Island, NY, USA) and 0.03% L-glutamine. SHIN-3 cells were generously provided by Dr. Y. Kiyozuka (Nara Medical College, Nara, Japan) and LS180 cells were supplied by the American Type Culture Collection (Rockville, MD, USA). Subconfluent cells were harvested with calcium- and magnesium-free phosphate buffered saline (PBS) containing 0.02% ethylenedia-

Received September 29, 1997, revision accepted February 2, 1998.

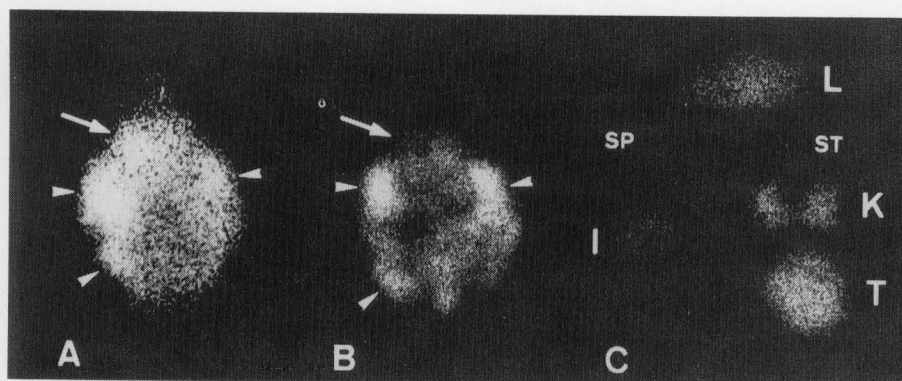
For reprint contact: Z. Yao, Department of Nuclear Medicine, Faculty of Medicine, Kyoto University, Shogoin, Sakyo-ku, Kyoto 606-8507, JAPAN.

E-mail: yao@kuhp.kyoto-u.ac.jp

**Table 1** Biodistribution of  $^{99m}\text{Tc}$  labeled HSA or GSA (20  $\mu\text{g}$ ) in mice bearing intraperitoneal SHIN-3 tumor xenografts

	HSA		GSA			
	Time (h)	2	6	0.5	2	6
Blood		8.70 $\pm$ 2.86	10.95 $\pm$ 5.38	0.21 $\pm$ 0.05	0.19 $\pm$ 0.11	0.12 $\pm$ 0.03
Liver		1.56 $\pm$ 0.56	3.12 $\pm$ 1.39	4.71 $\pm$ 3.13	5.80 $\pm$ 5.51	12.69 $\pm$ 5.54
Kidney		2.94 $\pm$ 0.97	5.71 $\pm$ 2.56	1.34 $\pm$ 0.11	2.11 $\pm$ 0.25	2.77 $\pm$ 0.59
Intestine		1.26 $\pm$ 1.38	3.03 $\pm$ 2.06	0.23 $\pm$ 0.05	1.71 $\pm$ 1.31	9.11 $\pm$ 2.69
Stomach		1.90 $\pm$ 2.58	0.69 $\pm$ 0.24	0.30 $\pm$ 0.07	0.63 $\pm$ 0.13	0.30 $\pm$ 0.09
Spleen		0.47 $\pm$ 0.18	1.14 $\pm$ 0.42	0.14 $\pm$ 0.02	0.15 $\pm$ 0.05	0.31 $\pm$ 0.15
Lung		1.18 $\pm$ 0.52	2.25 $\pm$ 1.02	0.19 $\pm$ 0.06	0.14 $\pm$ 0.03	0.23 $\pm$ 0.09
Muscle		0.09 $\pm$ 0.02	0.35 $\pm$ 0.12	0.05 $\pm$ 0.02	0.04 $\pm$ 0.02	0.06 $\pm$ 0.01
Bone		0.32 $\pm$ 0.13	0.81 $\pm$ 0.39	0.09 $\pm$ 0.04	0.07 $\pm$ 0.03	0.17 $\pm$ 0.03
Tumor		8.78 $\pm$ 3.49	8.99 $\pm$ 3.58	5.82 $\pm$ 0.68	6.85 $\pm$ 3.02	8.46 $\pm$ 3.10
Tumor/blood		1.05 $\pm$ 0.41	0.89 $\pm$ 0.19	29.60 $\pm$ 7.71	40.22 $\pm$ 14.03	67.84 $\pm$ 18.24

Mean  $\pm$  S.D. of % ID/g or tumor-to-blood ratio for 4–5 mice.



**Fig. 1** Scintigrams of mice bearing SHIN-3 intraperitoneal (i.p.) xenografts obtained 30 min after i.p. injection of  $^{99m}\text{Tc}$  labeled HSA (A) or GSA (B).  $^{99m}\text{Tc}$ -HSA showed high radioactivity level in blood pool (arrows indicating heart) while  $^{99m}\text{Tc}$ -GSA showed low background radioactivity and clear tumor image. Immediately after the imaging, the mouse was killed, distribution of tumor nodules was examined (arrowheads indicating clusters of tumor nodules). In mice receiving  $^{99m}\text{Tc}$ -GSA, the tumors (618 mg) were collected and imaged again together with other organs (T: tumor, L: liver, ST: stomach, K: kidneys, SP: spleen and I: intestine).

minetetraacetic acid (EDTA).  $1 \times 10^7$  of SHIN-3 or  $3 \times 10^6$  of LS180 cells in 0.2 ml PBS were injected i.p. into female BALB/c nude mice. Many tumor nodules were found in the peritoneal cavity 11–13 days postinjection. The combined tumor weight was 100–350 mg and the body weight of the mice was 17–21 g at the time of the experiment.

#### Biodistribution study

$^{99m}\text{Tc}$  labeled HSA and GSA were supplied by Nihon Medi-Physics Ltd. (Nishinomiya, Japan). The labeled HSA or GSA (370 KBq/20  $\mu\text{g}$  in 0.2 ml) was injected i.p. into the tumor-bearing mice. At 30 min, 2 h or 6 h, groups of 4–5 mice were killed and the biodistribution of radioactivity was examined. Statistical analysis was performed by Student's t-test.

#### Scintigraphy

To obtain scintigrams, 3.7 MBq of  $^{99m}\text{Tc}$  labeled HSA or GSA (20  $\mu\text{g}$ ) was administered i.p. to SHIN-3 tumor-bearing mice. Thirty minutes after the radiolabel injection, the mice were anesthetized and scintigrams of the whole body were obtained with a gamma camera equipped with a pinhole collimator. Immediately after obtaining the image, the mice were killed and the distribution of tumor nodules was examined. All of the tumor nodules were collected and an *ex vivo* image was done together with other organs in mice receiving  $^{99m}\text{Tc}$ -GSA.<sup>8</sup>

All procedures involving animal controls were carried out in accordance with the regulations for animal welfare in Japan.

## RESULTS

### *Biodistribution of <sup>99m</sup>Tc labeled HSA and GSA*

When i.p. injected, the labeled HSA localized in tumors (Table 1), but radioactivity in blood was very high, resulting in tumor-to-blood ratios of around 1, which made it difficult to get clear images early.

<sup>99m</sup>Tc-GSA had a different distribution pattern from HSA (Table 1). A similar tumor uptake of radioactivity was seen, and 5.82 %ID/g was found in SHIN-3 xenografts 30 minutes after the injection and the accumulation increased a little thereafter (Table 1), but radioactivity in blood was very low, resulting in significantly higher tumor-to-blood ratios ( $p < 0.005$ , Table 1). The radioactivity in the intestines increased with time, which suggested excretion of the radiolabel from the liver. The biodistribution of the labeled GSA in LS180 tumor-bearing mice was similar, with tumor uptake of radioactivity being  $5.90 \pm 2.22$  %ID/g 2 hours after the injection.

### *Scintigraphy*

Whole body images of SHIN-3 tumor-bearing mice 30 minutes after <sup>99m</sup>Tc labeled HSA or GSA i.p. injections are shown in Fig. 1. The image with <sup>99m</sup>Tc-HSA (A) demonstrated high radioactivity in the blood pool, as shown by the heart (arrow) and the background of the abdomen, but in mice receiving <sup>99m</sup>Tc-GSA (B), the background radioactivity was low and the tumors were clearly visualized. Apart from the clusters of tumor nodules indicated (arrow heads), there were many tumor nodules disseminated in the peritoneal cavity, so that *ex vivo* imaging was done in mice receiving <sup>99m</sup>Tc-GSA by separating all the tumor nodules from normal organs. As a result, a clear image of the tumor was obtained (C), which was consistent with the biodistribution data (Table 1).

## DISCUSSION

This study suggested a new application of <sup>99m</sup>Tc-GSA. When given i.p., the radiolabel accumulated in i.p. tumors and cleared from the circulation rapidly, resulting in very high tumor-to-blood ratios of radioactivity, which could make the i.p. tumor imaging a useful clinical practice.

The results of this study showed that the i.p.-injected <sup>99m</sup>Tc-GSA accumulated in the liver and cleared from the circulation rapidly, similar to i.v. administration both in mice and in man.<sup>3,5,9</sup> In mice, the radioactivity was then excreted into the intestines, which may interfere with the imaging of the i.p. tumor, but the interference could be minimized by taking an early image before the radioactivity reached the intestines. Because we have not seen much intestinal radioactivity in <sup>99m</sup>Tc-GSA scintigraphy in human subjects, there may not be a big problem in its clinical use in the tumor imaging.

The mechanisms related to GSA accumulation in tumors are not clear. Wang et al. reported that tumor cells

expressed albumin binding molecules on their surface, and that the binding of HSA or BSA to tumor cells was specific as it was saturable and selective.<sup>7</sup> The mechanism of GSA binding to tumor cells may be similar to that of HSA and BSA. On the other hand, GSA is a glycoprotein which accumulates in the liver through the asialoglycoprotein receptor. The presence of asialoglycoprotein receptor on SHIN-3 or LS180 tumor cells has not been demonstrated, but it has been reported that various lectins, including galactose-binding lectin, were expressed on the cell surface in many different tumor types.<sup>10,11</sup> Therefore, the carbohydrate residue of GSA may also relate to its binding to tumors. Clarification of the mechanism may improve the tumor targeting efficiency.

The pharmacokinetics of GSA after i.p. injection in human subjects needs to be investigated. The tumor to background contrast may be reduced in patients with liver dysfunction because of slower clearance of radiolabeled GSA from the circulation. In addition, ascites and the volume of injectate may affect the accessibility of i.p. injected GSA to the tumor and absorption into the circulation.

In conclusion, i.p.-injected radiolabeled GSA bound to i.p. tumor and cleared from the circulation rapidly, so that it could be used to diagnose i.p. dissemination of tumors.

## ACKNOWLEDGMENT

We are grateful to the Kato Asao International Scholarship Foundation for the kind help. <sup>99m</sup>Tc-HSA and -GSA were generously supplied by Nihon Medi-Physics Co. Ltd. This work was supported in part by a grant-in-aid for Scientific Research (08266230, 08671024) from the Ministry of Education, Science and Culture, and a grant-in-aid from the Sankyo Foundation of Life Science, Japan.

## REFERENCES

1. Lacy JH, Wieman TJ, Shively EH. Management of malignant ascites. *Surg Gynecol Obstet* 159: 397-412, 1984.
2. Chatal JF, Saccavini JC, Gestin JF, Thedrez P, Curtet C, Kremer M, et al. Biodistribution of indium-111-labeled OC 125 monoclonal antibody intraperitoneally injected into patients operated on for ovarian carcinomas. *Cancer Res* 49: 3087-3094, 1989.
3. Vera DR, Stadalnik RC, Krohn K. Technetium-99m galactosyl-neoglycoalbumin: preparation and preclinical studies. *J Nucl Med* 26: 1157-1167, 1985.
4. Kurtaran A, Li SR, Raderer M, Leimer M, Muller C, Pidlich J, et al. Technetium-99m-galactosyl-neoglycoalbumin combined with iodine-123-Tyr-(A14)-insulin visualizes human hepatocellular carcinomas. *J Nucl Med* 36: 1875-1881, 1995.
5. Ha-Kawa SK, Tanaka Y, Hasebe S, Kuniyasu Y, Koizumi K, Ishii Y, et al. Compartmental analysis of asialoglycoprotein receptor scintigraphy for quantitative measurement of liver function: a multicentre study. *Eur J Nucl Med* 24: 130-137, 1997.

6. Ohtake E, Matsui K. Lymphoscintigraphy in patients with lymphedema. A new approach using intradermal injections of technetium-99m human serum albumin. *Clin Nucl Med* 11: 474-478, 1986.
7. Wang J, Ueno H, Masuko T, Hashimoto Y. Binding of serum albumin on tumor cells and characterization of the albumin binding protein. *J Biochem* 115: 898-903, 1994.
8. Yao Z, Zhang M, Sakahara H, Saga T, Arano Y, Konishi J. Avidin targeting of intraperitoneal tumor xenografts. *J Natl Cancer Inst* 90: 25-29, 1998.
9. Arano Y, Mukai T, Uezono T, Wakisaka K, Motonari H, Akizawa H, et al. A biological method to evaluate bifunctional chelating agents to label antibodies with metallic radionuclides. *J Nucl Med* 35: 890-898, 1994.
10. Lotan R, Raz A. Lectins in cancer cells. *Ann NY Acad Sci* 551: 385-398, 1988.
11. Monsigny M, Roche AC, Midoux P. Endogenous lectins and drug targeting. *Ann NY Acad Sci* 551: 399-414, 1988.