Site-Specific Protein Synthesis in Liver Regeneration Determined by PET

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Site-specific regeneration of the liver after 70% partial hepatectomy was investigated noninvasively in terms of protein synthesis using PET with L-[methyl-^{11}C]methionine in a living animal. Protein synthesis in rat liver at 24 hr after partial hepatectomy did not occur uniformly in the whole liver but intensely in the middle part of the regenerating organ in comparison with the other parts. The activity was significantly low at the posterior aspect of the liver. On the other hand, site-specific protein synthesis was not observed in normal liver. These results were confirmed by bioimaging analyzer system (BAS) analysis, an invasive method that indicates radioactivities of precise intrahepatic sites. DNA synthesis in regenerating liver was also monitored with [2-^{14}C]thymidine and analyzed by BAS 24 hr after 70% hepatectomy. Site-specific DNA synthesis in regenerating liver corresponding to the protein synthesis was also observed by BAS analysis. These results indicate that liver regeneration occurs intensely in the middle part of the liver and that PET enables noninvasive in vivo biochemical analysis.

Key Words: positron emission tomography; carbon-11-methionine; liver regeneration; bioimaging analyzer system

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Liver is known to regenerate actively and recover its lost mass to compensate its specific functions after partial hepatectomy. It is not known, however, whether liver regeneration occurs homogeneously or site-specifically in vivo. As an approach to answering to this question, we employed PET, a procedure that enables the noninvasive study of protein synthesis in vivo. A marker of protein synthesis in regenerating liver, L-[methyl-^{11}C]methionine ([^{11}C]Met), was chosen because methionine is an essential amino acid important in protein synthesis and other reactions such as transmethylation. In fact, [^{11}C]Met has been successfully used for metabolic imaging of brain and tumors (*1,2*), non-Hodgkin's lymphoma (*3*), breast cancer (*4*) and head and neck cancer (*5,6*).

A PET study of liver regeneration using [2-^{11}C]thymidine was reported by Vander-Borght et al. (*7*), in which they suggested the usefulness of [2-^{11}C]thymidine for noninvasive measurement of cellular proliferation, namely, liver regeneration; although they did not show the site of the regeneration.

Although PET is noninvasive and can be performed on a living animal in real time, identifying the precise sites of synthesis is difficult. Thus, we determined site-specific protein and DNA syntheses precisely with a bioimaging analyzer system (BAS), which, although an invasive method, enables identification of precise sites.

MATERIALS AND METHODS

Preparation of Partially Hepatectomized Rats

Male Wistar rats weighing 150 to 170 g (Japan SLC. Hamamatsu, Japan) were 70% hepatectomized under anesthesia with ethyl ether according to the procedure of Higgins and Anderson (*8*).

PET Determination and Analysis

PET studies were performed with an animal PET camera with an effective slice aperture of 6.5 mm. A normal rat, to obtain the basal level of protein synthesis, a 70% partially hepatectomized animal one day after the operation, or a sham-operated rat was fixed in the PET apparatus under anesthesia with sodium pentobarbital. Before tracer injection, transmission scans were obtained by use of an 18.5 MBq ^{68}Ge/^{68}Ga ring source for attenuation correction. The emission scans were obtained over a 90-min period right after the injection of [^{11}C]Met (18.5MBq/rat) into a tail vein of the animal. The radioactivity in each scan was corrected for decay and attenuation, and [^{11}C]Met accumulation was calculated by mean pixel counts in the region of interest (ROI) on PET images where ROIs that covered the liver image were chosen. PET analysis was also performed on rats at 48 and 72 hr after surgery. Each experiment was repeated at least twice and similar results were obtained in repeat experiments, although each figure represents a typical result obtained from a single animal.

BAS Determination and Analysis

L-[methyl-^{14}C]methionine ([^{14}C]Met, Amersham 74 kBq/rat) was injected into a tail vein of a normal or hepatectomized rat 24 hr after surgery. The liver was perfused with saline and then removed at 30 min after injection. After removal, the organ was fixed with 4% sodium carboxymethylcellulose, frozen at −20°C and sliced at every 300 μm with a cryotome. Liver slices were exposed 3 days on an imaging plate and analyzed by a BAS. The ROI value was measured from the BAS images and presented as mean pixel counts (PSL/mm^2). BAS analysis was also performed using [2-^{14}C]thymidine ([^{14}C]TdR, Amersham, 74 kBq/rat) in a

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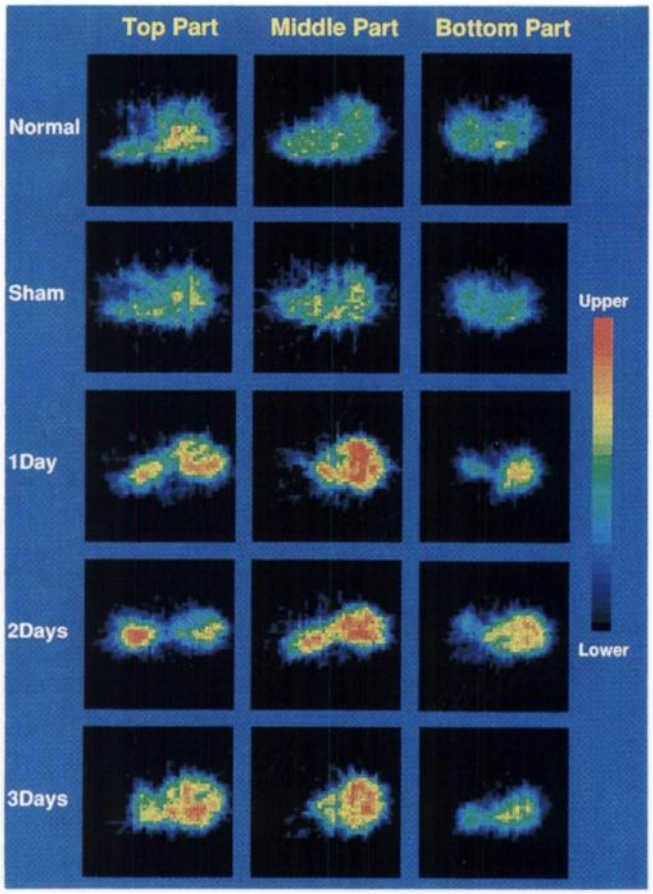

FIGURE 1. PET images of [^{14}C]Met accumulation in regenerating rat liver. The accumulation of [^{11}C]Met in liver was imaged by PET for 0–60 min. Similar results were obtained in a separate experiment.

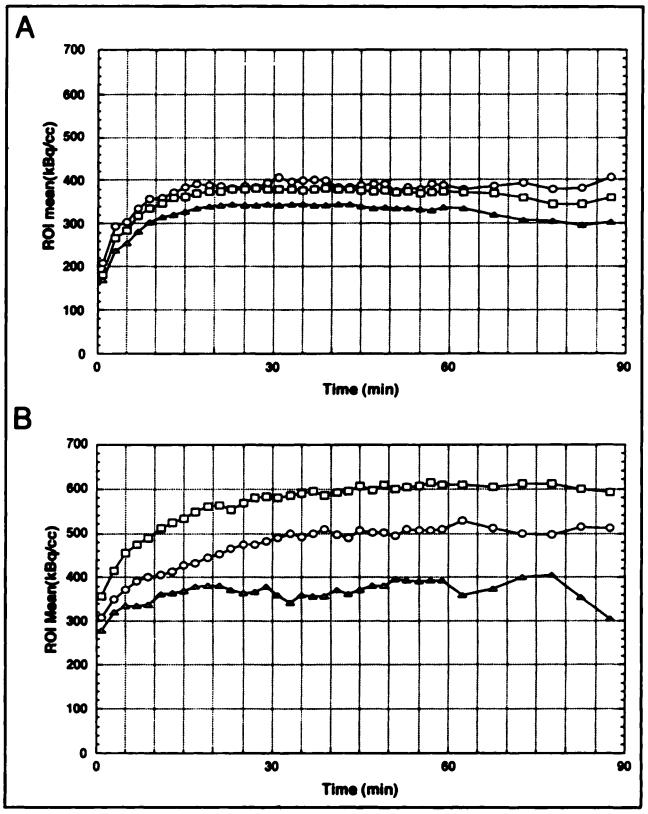

FIGURE 2. Time-activity curves of [^{11}C]Met taken up by regenerating rat liver. Normal (A) and 70% hepatectomized rats (B) were injected with [^{11}C]Met (18.5 MBq/rat). An ROI was chosen to cover the liver image, and the time-activity curve of ^{11}C in the top (○), middle (□) and bottom (△) parts of the liver was obtained.

similar procedure as used for [^{14}C]Met. Statistical analysis was done by Student's t-test. Differences were considered significant when the p value of comparison was less than 0.05.

Intrahepatic Distribution of [^{14}C]Met

Hepatectomized rats were injected under anesthesia with [^{14}C]Met (74 kBq/rat) at 24 hr after surgery and perfused with phosphate-buffered saline. The animals were then killed and their livers were removed and cut into 0.1-g pieces. Liver samples were weighed and homogenized with 1 ml of 0.25 *M* sucrose. Proteins were precipitated with ice-cold 10% trichloroacetic acid (TCA). After centrifugation at 3000 rpm × 10 min, the radioactivity in the supernatant fraction was measured in a liquid scintillation counter with a Hionic-fluor scintillation cocktail (Packard). The precipitated fraction was washed twice with 5% ice-cold TCA, solubilized overnight at 40°C with 1 ml of Solvable (NEN Research Products), and treated with 0.5 ml of 2-propanol and 0.5 ml of hydrogen peroxide. The radioactivity then was measured in a liquid scintillation counter with the Hionic-fluor scintillation cocktail. A standardized uptake value (SUV) of [^{14}C]Met was calculated from the following equation:

$$\text{SUV} = \frac{\text{tissue radioactivity (Bq)/tissue weight (g)}}{\text{injected dose (Bq)/body weight (g)}}. \qquad \text{Eq. 1}$$

RESULTS

PET Data

To determine the protein synthesis in regenerating liver in vivo, we injected 70% partially hepatectomized rats with [^{11}C]Met at 24, 48 or 72 hr after surgery. Figure 1 shows the PET image of liver slices at 0–60 min after the tracer injection. These images show high accumulation of [^{11}C]Met in regenerating liver compared with that in livers of normal or sham-operated animals. Furthermore, whereas the distribution of [^{11}C]Met in normal or sham-operated rat liver was homogeneous, an uneven distribution of [^{11}C]Met was observed in the hepatectomized liver. Carbon-11-Met accumulated more in the middle part of the partially hepatectomized liver compared with the other parts. Especially lower accumulation of the tracer was observed at the bottom or posterior part of the liver. Carbon-11-Met uptake decreased during liver regeneration, indicating that the protein synthesis is more active at 24 hr than at 48 and 72 hr after hepatectomy. Figure 2 shows the time-activity curve of [^{11}C]Met in normal and in 24-hr posthepatectomized rat liver. In both cases, [^{11}C]Met uptake occurred quite fast, with a half maximum uptake time of 1 min, and reached plateau in less than 30 min after injection of the tracer.

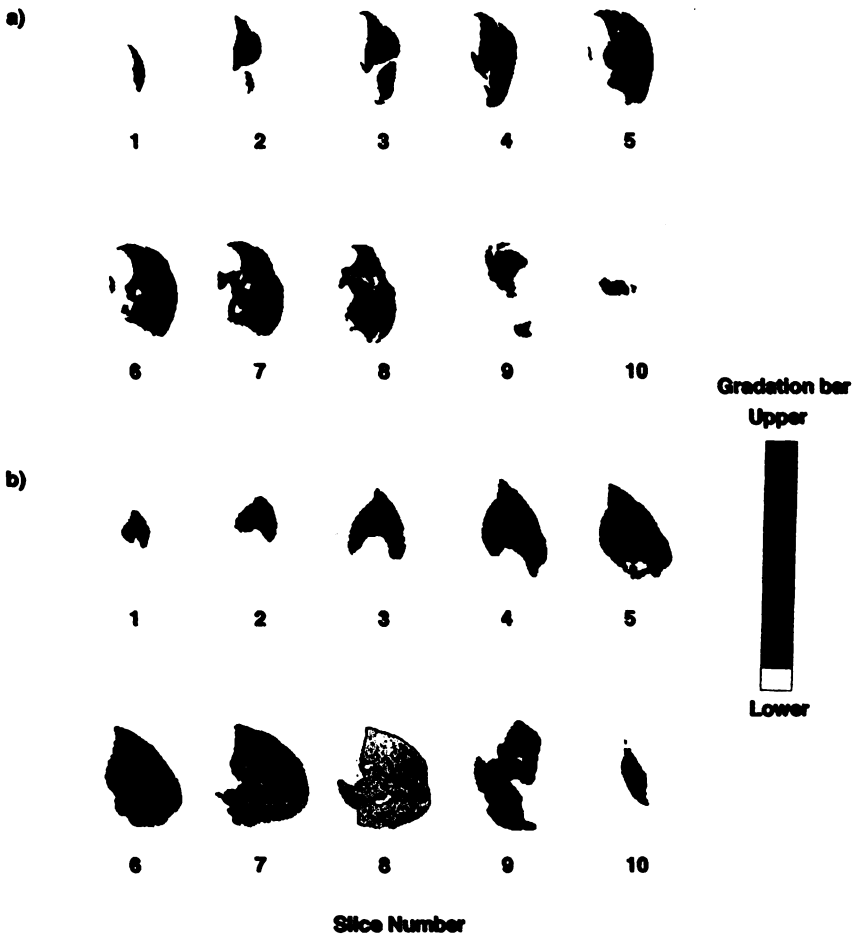

FIGURE 3. BAS images of [^{14}C]Met accumulation in regenerating rat liver. Normal (a) and 70% hepatectomized rats (b). Slices were taken every 2.4 mm from the top to the bottom of the liver. Similar results were obtained in a separate experiment.

BAS Data

Since the uptake of [^{11}C]Met reached a plateau in 30 min after injection, BAS analysis was performed at 30 min after injection of [^{14}C]Met. Figure 3 shows the distribution of [^{14}C]Met in normal and partially hepatectomized rat livers, as seen in BAS images of slices of 0.3 mm thickness taken every 2.4 mm from the top part to the bottom of the liver. In correlation with the PET images, [^{14}C]Met accumulation was intense in the middle part of partially hepatectomized liver and gradually decreased in the direction toward the bottom part, whereas horizontal heterogeneity was not observed in any of the slices. On the contrary, BAS images

FIGURE 4. BAS images of [2-^{14}C]thymidine accumulation in regenerating rat liver. Slice thickness was 300 μm and the slices shown were taken every 2.4 mm from the top to the bottom of the liver. Similar results were obtained in a separate experiment.

TABLE 1
Intrahepatic Distribution of [^{14}C]Methionine and [2-^{14}C]Thymidine in Regenerating Rat Liver

Rat	Tracer	Intrahepatic distribution (PSL/mm^2)		
		Top part	Middle part	Bottom part
Normal	[^{14}C]Met	200 ± 4	198 ± 4	194 ± 4
24 hr after 70% hepatectomy	[^{14}C]Met	570 ± 3	578 ± 30	418 ± 68*
24 hr after 70% hepatectomy	[^{14}C]TdR	128 ± 8	130 ± 8	75 ± 29*

*Significantly different from other parts.
Values represent mean ± s.d. (n = 5), where PSL is the relative radioactivity in the ROI on BAS images.

of normal liver showed that [^{14}C]Met was evenly taken up by the liver. BAS analysis was also performed to determine DNA synthesis using [^{14}C]TdR (Fig. 4). The distribution of [^{14}C]TdR in partially hepatectomized rat liver was similar to that of [^{14}C]Met, indicating active sites of both DNA synthesis and protein synthesis in the middle part of the partially hepatectomized liver. The ROI values of [^{14}C]Met and [^{14}C]TdR are summarized in Table 1. Carbon-14-Met uptake in the bottom part of the liver at 24 hr after partial hepatectomy was significantly lower than that in the middle part (72.4%, $p < 0.05$), as was [^{14}C]TdR uptake (57.3%, $p < 0.05$).

Carbon-14-Met Uptake in Acid-Insoluble Fraction

To determine that methionine uptake reflects protein synthesis, we examined radioactivity distribution in the acid-insoluble fraction prepared from liver removed at 30 min after tracer injection; most of the radioactivity was recovered in this fraction (Table 2). Furthermore, the SUVs in normal and partially hepatectomized rat liver supported the data from PET and BAS analyses.

DISCUSSION

PET can detect biochemical processes in vivo, such as protein synthesis and DNA synthesis. Here, we examined functional imaging of protein synthesis in regenerating liver by means of methionine uptake, and observed site-specific protein synthesis. Protein synthesis was more active in the middle part of the residual liver after partial hepatectomy than in the other parts, especially in the bottom part. On the other hand, radioactivity was evenly distributed in normal and sham-operated rat liver. According to the time-activity curve obtained from PET images, the uptake of [^{11}C]Met occurred quite fast and reached plateau in 30 min after injection of the tracer. PET analysis would also be effective to determine labeling time of the tissue by the tracer. Although the site-specific uptake of [^{11}C]Met was observed at 48 and 72 hr after partial hepatectomy, the activity was lower than that at 24 hr after surgery, suggesting that liver regeneration was more active at 24 hr after surgery. Here, we used L-[methyl-11C]methionine, although this compound is not the best to determine protein synthesis due to methionine metabolism other than protein synthesis, i.e., transmethylation, transsulfuration, and so on (*9*). Therefore, it is possible that the regenerating liver is not only active in protein synthesis but also active in methionine metabolism. The methionine uptake in specific liver sites observed here, however, may be reflecting protein synthesis, since enhanced DNA synthesis was also observed at the same sites.

Data from BAS analysis and the tissue distribution study supported the PET results. DNA synthesis was maximum at 24 hr after 70% partial hepatectomy when the division of liver parenchymal hepatocytes is not yet observed (*10*). Therefore, the intense uptake of [^{14}C]TdR in the middle part of the residual liver suggests that the parenchymal hepatocytes divide intensely in this region.

TABLE 2
L-[methyl-^{14}C]Methionine Uptake in Rat Liver 24 Hours after Partial Hepatectomy

	Intrahepatic distribution (SUV)		
	Top part	Middle part	Bottom part
Acid-insoluble fraction	8.25 ± 0.92	8.64 ± 0.67	7.47 ± 0.66*
Acid-soluble fraction	0.85 ± 0.12	0.98 ± 0.12	0.82 ± 0.07

*Significantly different from other parts.
Values represent mean ± s.d. (n = 5).

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