The Entering of Indium-111 and Iron-59 into the Hepatocytes from Partially Hepatectomized Rats Differ from That of Gallium-67

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We recently suggested that the transferrin (Tf)–gallium-67 (\({\text{\textsuperscript{67}}\text{Ga}}\)) complex dissociated on the surface of the hepatocytes after partial hepatectomy and free \({\text{\textsuperscript{67}}\text{Ga}}\) bound to heparan sulfate in the extracellular matrix. In the present study, we investigated whether the entering of indium-111 (\({\text{\textsuperscript{111}}\text{In}}\)) and iron-59 (\({\text{\textsuperscript{59}}\text{Fe}}\)) with high affinity to transferrin differed from the entering of \({\text{\textsuperscript{67}}\text{Ga}}\) by the hepatocytes after partial hepatectomy. \({\text{\textsuperscript{111}}\text{In}}\) was almost taken by the plasma and little taken by the red blood cell. On the other hand, the uptake of \({\text{\textsuperscript{59}}\text{Fe}}\) by the red blood cell was higher than plasma. The uptake of \({\text{\textsuperscript{59}}\text{Fe}}\) by the bone marrow was significantly higher than that of \({\text{\textsuperscript{111}}\text{In}}\). The uptake of \({\text{\textsuperscript{111}}\text{In}}\) and \({\text{\textsuperscript{59}}\text{Fe}}\) by the liver tissue was reached a maximum 2 d after partial hepatectomy but the uptake ratio of \({\text{\textsuperscript{111}}\text{In}}\) was lower than that of \({\text{\textsuperscript{59}}\text{Fe}}\). We suspected that the uptake of \({\text{\textsuperscript{59}}\text{Fe}}\) by the liver tissue was the highest because of the high binding affinity of Tf–\({\text{\textsuperscript{59}}\text{Fe}}\) to Tf-receptor. The entering of \({\text{\textsuperscript{111}}\text{In}}\) and \({\text{\textsuperscript{59}}\text{Fe}}\) into the hepatocytes was also reached a maximum 2 d after partial hepatectomy but the ratio of \({\text{\textsuperscript{59}}\text{Fe}}\) was slightly lower than that of \({\text{\textsuperscript{111}}\text{In}}\). These results suggested that the binding affinity to Tf could have played a crucial role in the differences of the entering of \({\text{\textsuperscript{111}}\text{In}}\), \({\text{\textsuperscript{59}}\text{Fe}}\), and \({\text{\textsuperscript{67}}\text{Ga}}\) into the hepatocytes of partially hepatectomized rats.

Key words indium-111 (\({\text{\textsuperscript{111}}\text{In}}\)); iron-59 (\({\text{\textsuperscript{59}}\text{Fe}}\)); partial hepatectomy; liver tissue; hepatocyte

Gallium-67 (\({\text{\textsuperscript{67}}\text{Ga}}\)) has been used for the detection of various tumors and acute and chronic inflammations since the first observation of \({\text{\textsuperscript{67}}\text{Ga}}\) accumulation in tumors and inflammatory lesions. Although many hypotheses concerning the mechanism of \({\text{\textsuperscript{67}}\text{Ga}}\) uptake by tumors and inflammatory lesions have been proposed, consensus has not been reached.

It was reported that \({\text{\textsuperscript{67}}\text{Ga}}\) bound to transferrin (Tf) and was accumulated into tumor cells through Tf receptor. Conversely, it was reported that Tf was not involved in the uptake of \({\text{\textsuperscript{67}}\text{Ga}}\) by the tumor cells. We have reported that Tf was not involved in the uptake of \({\text{\textsuperscript{67}}\text{Ga}}\) by rat granuloma tissue, that was inflammatory tissue induced by turpentine oil and the hepatocytes of carbon tetrachloride-treated rats. Sohn M.-H. et al. suggested that the uptake of \({\text{\textsuperscript{67}}\text{Ga}}\) by normal liver was Tf-dependent process. We have also reported that Tf was involved in the uptake of \({\text{\textsuperscript{67}}\text{Ga}}\) by the liver of normal mice. Moreover, we recently reported that the entering pattern of \({\text{\textsuperscript{67}}\text{Ga}}\) into the hepatocytes after partial hepatectomy differed from the uptake of \({\text{\textsuperscript{67}}\text{Ga}}\) by the liver tissue. We suggested that the Tf–\({\text{\textsuperscript{67}}\text{Ga}}\) complex easily dissociated on the surface of hepatocytes after partial hepatectomy and free \({\text{\textsuperscript{67}}\text{Ga}}\) bound to heparan sulfate in the extracellular matrix. Hara reported that the binding affinity of Tf was indium-111 (\({\text{\textsuperscript{111}}\text{In}}\)) > iron-59 (\({\text{\textsuperscript{59}}\text{Fe}}\)) > \({\text{\textsuperscript{67}}\text{Ga}}\). Therefore, we thought that the weak binding affinity of \({\text{\textsuperscript{67}}\text{Ga}}\) to Tf was responsible for the dissociation of \({\text{\textsuperscript{67}}\text{Ga}}\) on surface of the hepatocytes. We expected that \({\text{\textsuperscript{111}}\text{In}}\) and \({\text{\textsuperscript{59}}\text{Fe}}\) with high binding affinity to Tf than \({\text{\textsuperscript{67}}\text{Ga}}\) could not be dissociated from Tf on the surface of the hepatocytes and it may be taken by hepatocytes.

In the present study, we investigated whether or not the dissociation of \({\text{\textsuperscript{67}}\text{Ga}}\) from Tf was responsible for the weak binding affinity of \({\text{\textsuperscript{67}}\text{Ga}}\) to Tf. For this purpose, we examined the entering of \({\text{\textsuperscript{111}}\text{In}}\) and \({\text{\textsuperscript{59}}\text{Fe}}\) with high binding affinity to Tf into the hepatocytes.

MATERIALS AND METHODS

Animals Male Wistar rats weighing 180—200 g (SLC, Hamamatsu, Japan) were kept under conditions of a 12-h light–dark cycle, 23±1°C and 55±5% humidity, and had free access to food and water. Animals were cared following the standard procedures indicated in the “Guide for the Care and Use of Laboratory Animals” published by the Tohoku Pharmaceutical University.

Chemicals Indium-111 chloride was purchased from Nilhon Mediphysics Co. Ltd. (Takarazuka, Japan). Iron-59 chloride was purchased from PerkinElmer Life Sciences, Inc. (Boston, MA, U.S.A.). Hanks’ balanced salt solution (HBSS) was purchased from Sigma (St. Louis, MO, U.S.A.). Collagenase was purchased from Nitta Gelatine (Osaka, Japan). All other reagents were purchased from Nacalai Tesque (Tokyo, Japan).

Partial Hepatectomy Two-thirds partial hepatectomy was performed according to the technique described by Higgins and Anderson. Briefly, the liver was exposed through a 1—2 cm midline abdominal incision and the two anterior lobes were exteriorized, the vascular pedicles were ligated, and the lobes were excised. Rats were sacrificed at 1, 2 and 4 d after partial hepatectomy.

Administration of \({\text{\textsuperscript{111}}\text{In}}\) and \({\text{\textsuperscript{59}}\text{Fe}}\) Indium-111 chloride was diluted with saline to 185 kBq/ml. Each rat was intravenously injected with \({\text{\textsuperscript{111}}\text{In}}\) in a dose of 37 kBq. Iron-59 chloride was diluted with saline to 92.5 kBq/ml. Each rat was intravenously injected with \({\text{\textsuperscript{59}}\text{Fe}}\) in a dose of 18.5 kBq. The rats were administered with \({\text{\textsuperscript{111}}\text{In}}\), \({\text{\textsuperscript{59}}\text{Fe}}\) under urethane anesthesia (1.5 g/kg, i.p.). After 4 h blood was obtained from the inferior vena cava and then immediately perfused with 0.9% NaCl solution. The liver tissue was then removed. Bone marrow was obtained from femur by flushing with 0.9% NaCl solution. Blood was centrifuged at 1500 × g for 20 min and plasma was obtained from supernatants. Also, red blood cells were obtained as pellets and washed twice with 0.9%
Preparation of Hepatocytes  The hepatocytes were prepared according to the method by Abe et al.17) as follows. Rat liver was perfused at 37 °C via the portal vein with 300 ml of calcium-free HBSS containing 5 mM EGTA and 10 mM N-hydroxyethylpiperadine-N'-2-ethansulfonate (Hepes, pH 7.4). The perfusion was continued with 200 ml of HBSS containing 5 mM CaCl₂, 0.05% (w/v) collagenase and 0.005% (w/v) trypsin inhibitor. After the perfusion, the digested liver was suspended in 30 ml of calcium-free HBSS containing 4 mM NaHCO₃ and filtered through coarse gauze. The filtrate was centrifuged for 3 min at 50×g at 4 °C and the pellet was washed once with calcium-free HBSS containing 4 mM NaHCO₃.

Acid-Resistance Assay  Acid-resistance assay was carried out according to Katoh et al.20) as follows. The hepatocytes were suspended with 1 ml of 0.5 M NaCl–0.2 M acetic acid at 4 °C for 5 min. The suspension was centrifuged for 3 min at 50×g at 4 °C and removed supernatants. The pellet was used for determining the radioactivity with a well-type NaI-scintillation counter (ARC-370M, Aloka, Tokyo, Japan).

Determination of Radioactivity  The amount of radioactive ¹¹¹In and ⁵⁹Fe was determined with a well-type NaI-scintillation counter. The uptake ratios of ¹¹¹In and ⁵⁹Fe in plasma, bone marrow, red blood cell, liver and hepatocyte were expressed using the following formula:

\[
\text{uptake ratio} = \frac{A}{B}
\]

\[A = \text{sample activity (cpm)/sample weight (g)}\]

\[B = \text{total activity administered (cpm)/rat body weight (g)}\]

RESULTS

The Uptake of ¹¹¹In and ⁵⁹Fe by the Plasma, Bone Marrow and Red Blood Cell after Partial Hepatectomy  The uptake ratio of ¹¹¹In and ⁵⁹Fe by the plasma, bone marrow and red blood cell at 1, 2, 4 d after partial hepatectomy were shown in Fig. 1. The uptake of ¹¹¹In by the plasma was remarkably higher than that of ⁵⁹Fe (Fig. 1a). The uptake of ⁵⁹Fe by the bone marrow was considerably higher than that of ¹¹¹In (Fig. 1b). The uptake of ⁵⁹Fe by the red blood cell increased immediately after partial hepatectomy and reached a maximum on the first day after operation (Fig. 1c). ¹¹¹In was slightly taken by the red blood cell compared to ⁵⁹Fe.

The Uptake of ¹¹¹In and ⁵⁹Fe by the Liver Tissue after Partial Hepatectomy  The uptake ratio of ¹¹¹In and ⁵⁹Fe by the liver tissue at 1, 2, 4 d after partial hepatectomy was shown in Fig. 2. The uptake of ¹¹¹In and ⁵⁹Fe by the liver tissue gradually increased and reached a maximum 2 d after partial hepatectomy (Fig. 2). The uptake ratio of ⁵⁹Fe by the liver tissue was higher than that of ¹¹¹In.

The Entering of ¹¹¹In and ⁵⁹Fe into the Hepatocytes after Partial Hepatectomy  The entering of ¹¹¹In and ⁵⁹Fe into the hepatocytes was similar (data not shown). To ascertain the net entering of ¹¹¹In and ⁵⁹Fe into the hepatocytes, we used by acid-resistance assay following purification of the hepatocytes (Fig. 3). The entering of ¹¹¹In and ⁵⁹Fe into the hepatocytes isolated from partially hepatectomized rats gradually increased and reached maximum 2 d after partial hepatectomy, but ratio was lower than that of liver tissue. The ratio of ¹¹¹In into the hepatocytes was slightly higher than

![Fig. 1. Time Course of the Uptake of ¹¹¹In and ⁵⁹Fe by the Plasma (a), the Bone Marrow (b) and the Red Blood Cell (c) after Partial Hepatectomy](image-url)

Each point represents the mean±S.E. of 4—6 rats. The data were analyzed by Student’s t test. *p<0.01 and **p<0.05 compared with normal rat about ¹¹¹In. #p<0.01 and ##p<0.05 compared with normal rat about ⁵⁹Fe.

![Fig. 2. Time Course of the Uptake of ¹¹¹In and ⁵⁹Fe by the Liver Tissue after Partial Hepatectomy](image-url)

Each point represents the mean±S.E. of 4—6 rats.
that of 59Fe.

DISCUSSION

It is well known that Tf is a carrier glycoprotein for iron in the blood. A major pathway for cellular iron uptake is through the internalization of the complex of iron-bound Tf and the Tf receptor.21) Many researchers had reported that 67Ga bound to Tf and was transported to various tissues by Tf in the blood.8—10)

First, we studied the difference of distribution of 111In and 59Fe on blood. 111In was almost taken by the plasma (Fig. 1a) and slightly taken by the red blood cell (Fig. 1c). These results showed that 111In almost existed in the Tf-bound form in the blood. On the other hand, the uptake of 59Fe by the red blood cell was higher than plasma and was remarkably higher than 111In. The uptake of 59Fe by the bone marrow was considerably higher than that of 111In (Fig. 1b). These results suggested that the Tf–59Fe complex was preferentially transferred to the bone marrow from blood and was immediately taken by the red blood cell. Therefore, the uptake of 59Fe by the plasma was smaller than that of 111In. The red blood cell containing 59Fe was released from the bone marrow to blood and increased in 1 d after partial hepatectomy. Our results suggested that the hematopoietic function was enhanced by partial hepatectomy and supplemented blood deprivation by the operation. Actually, 111In has been used for bone marrow scintigraphy. In fact, since the uptake of 111In by the bone marrow increased in 1 and 2 d after partial hepatectomy (Fig. 1b), these results showed that the hematopoietic function increased after partial hepatectomy.

On the other hand, although the binding affinity of 111In to Tf was higher than that of 59Fe, 111In was slightly taken by the bone marrow as compared with 59Fe. 67Ga was also slightly taken by the bone marrow (data not shown). These results showed that the uptake of 59Fe by the bone marrow was not related to the binding affinity to Tf. Therefore, these results suggested that Tf–59Fe complex was selectively recognized by reticuloocyte of the bone marrow.

Then we examined the uptake of 111In and 59Fe by the liver tissue. Kumagai et al. reported that Tf receptors increased in the hepatocytes during rat liver regeneration.22,23) Tei et al. reported that the binding of Tf to the hepatocytes began to increase 1 d after partial hepatectomy and reached maximum 2 d.24) Our results showed that the uptake of 111In and 59Fe by the liver tissue increased and reached maximum 2 d after partial hepatectomy (Fig. 2). This result was similar to the uptake pattern of 67Ga by the liver tissue after partial hepatectomy.17) Consequently, the uptake patterns of 67Ga, 111In and 59Fe were similar to the increases of Tf receptor. However, the uptake ratio of 59Fe by the liver tissue was the highest among 111In, 59Fe and 67Ga, i.e., the uptake ratio of 59Fe, 111In and 67Ga7) by the liver tissue of 2 d after partial hepatectomy were 6.13 ± 0.4, 4.68 ± 0.27 and 3.25 ± 0.9, respectively. Hara reported that the binding affinity of Tf was 111In> 59Fe> 67Ga.18) Therefore, the uptake of 59Fe by the liver tissue did not depend on the binding affinity to Tf. We suspected that the binding affinity of Tf–59Fe complex to Tf-receptor was the highest among Tf-111In and 59Fe and 67Ga.

Moreover, in order to ascertain the net entering of the Tf–111In and –59Fe complex into the hepatocytes, we carried out acid-resistance assay following purification of the hepatocytes (Fig. 3). Contrary to our expectation, the entering of 59Fe into the hepatocytes was slightly lower than that of 111In. In fact, Kojima et al. have reported that Fe3+ represented strong affinity to heparan sulfate.25) Therefore, we suspected that the Tf–59Fe complex was in part dissociated on the surface of hepatocytes since the binding affinity of 59Fe to Tf was lower than that of 111In, and then free 59Fe might bind to the extracellular matrix of the liver tissue. Because of a high binding affinity of 111In to Tf, we expected that Tf–111In did not dissociate on the surface of the hepatocytes and might be taken.

We recently reported that the entering pattern of 67Ga into the hepatocytes after partial hepatectomy differed from the uptake of 67Ga by the liver tissue.17) These results showed that the uptake of 67Ga by the liver tissue reached maximum 2 d after partial hepatectomy but the entering of 67Ga into the hepatocytes did not become maximum 2 d. The entering of 67Ga into the hepatocytes was not similar to the increases pattern of Tf receptor,17) but the entering of 111In and 59Fe into the hepatocytes was similar to the increases pattern of Tf receptor after partial hepatectomy (Fig. 3).24) Therefore, these results suggested that the entering of 111In, 59Fe and 67Ga into the hepatocytes was closely related to the binding affinity to Tf.

In the present study, we suggested that the binding affinity to Tf could have played a crucial role in the differences of the entering of 111In, 59Fe and 67Ga into the hepatocytes of partially hepatectomized rats, and the dissociation of 67Ga from Tf on the surface of hepatocytes was responsible for the weak binding affinity of 67Ga to Tf.

REFERENCES