67Ga in transferrin-unbound form is taken up by inflamed liver of mouse treated with CCl4

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In order to investigate whether or not transferrin is involved in the uptake of 67Ga by inflamed liver (acute inflammatory tissues) the uptake of 67Ga by the liver of mice treated with carbon tetrachloride (CCl4) was studied. The serum GPT value reached its maximum on the 1st day after the CCl4 treatment. The uptake of 67Ga by the liver also reached its maximum on the 1st day after the CCl4 treatment and the amount uptaken into inflamed liver was about 6 times that uptaken into normal liver. On the other hand, the uptake of 125I-transferrin into inflamed liver on the 1st day after CCl4 treatment was only about 1.6 times that into normal liver. Moreover, cold Fe5+ decreased the uptake of 67Ga by normal liver but increased the uptake of 67Ga by inflamed liver. These results show that transferrin plays an important role in the uptake of 67Ga by normal liver but not by inflamed liver, i.e. 67Ga in the transferrin-unbound form is preferentially taken up by inflamed liver.

Key words: 67Ga uptake, CCl4 treatment, mouse damaged-liver, transferrin

INTRODUCTION

Since the first observation of 67Ga accumulation in tumors3 and inflammatory lesions,5,8 67Ga has been used for the detection of various tumors4 and acute and chronic inflammation.5,6 Many hypotheses concerning the mechanism of uptake of 67Ga into tumors and the inflammatory lesions have been proposed5,7-15 but a consensus has not yet been reached. It is well known that almost all 67Ga is bound to transferrin in the blood.16,18 It has been reported that transferrin plays a major role in the uptake of 67Ga into tumors.19-22 On the other hand, it has been shown that transferrin transports 67Ga to tumor tissues but that the uptake of 67Ga into tumor cells occurs in an unbound form.10,23-25 Concerning whether transferrin is involved in the uptake of 67Ga into tumors or not, a final conclusion cannot now be drawn. The involvement of transferrin in the uptake of 67Ga into the inflammatory tissues also has not been conclusively demonstrated. It has been reported that the uptake of 67Ga into normal soft tissues, such as the liver and spleen, occurred to a major extent by endocytosis in which transferrin is involved.9 We have also reported that the uptake of 67Ga into the liver and spleen occurred in a transferrin-bound form.18,26 Hayes et al.9 reported that the initial entry of 67Ga into the inflammatory lesions, such as abscess tissues, might occur in the same way as that into normal soft tissues. On the other hand, we have recently proposed that transferrin is not involved in the uptake of 67Ga into the inflammatory tissues, such as granuloma,18,26 Therefore, in the present study we have undertaken to clarify whether or not transferrin is involved in the uptake of 67Ga into CCl4-damaged liver (acute inflammatory tissues).

MATERIALS AND METHODS

Animals

Male mice weighing 18-22 g were purchased from Shizuoka Laboratory Animal Center (Hamamatsu,
Japan), and were housed in wire mesh cages at a room temperature of 23±1°C and relative humidity of 55±5%.

Administration of CCl₄
A dose of 0.1 ml of 10% CCl₄ in olive oil per 10 g body weight was given intraperitoneally. Control mice were treated similarly with equivalent amounts of olive oil alone.

Administration of ⁶⁷Ga
Carrier free ⁶⁷Ga citrate solution (kindly supplied by Daiichi Radioisotope Laboratory Ltd., Tokyo, Japan) was diluted with saline to 74 kBq/ml. Each mouse was subcutaneously injected with ⁶⁷Ga citrate solution in a dose of 7.4 kBq (100 µl).

Administration of ¹²⁵I-labeled transferrin
Labeling of transferrin with ¹²⁵I was carried out by essentially the same method as that of Markwell.²⁷ Fifty µl of Na¹²⁵I Tris-HCl buffer solution (37 MBq) was added to 100 µl Tris-HCl buffer solution containing 3 Iodo-beads (Pierce Chem. Com., U.S.A.). The mixed solution was incubated for 5 min and then after 100 µl of Tris-HCl buffer solution containing mouse transferrin was added, it was incubated for another 15 min. The iodinated mouse transferrin was then transferred with a glass Pasteur pipet to a second tube, leaving the Iodo-bead in the reaction tube. The iodinated mouse transferrin solution was applied to a column of Sephadex G-75 for the removal of unreacted radioidide. One hundred µl (350000 cpm) of the eluate containing iodinated mouse transferrin was injected into a mouse tail vein. The labeled transferrin is abbreviated to ¹²⁵I-Tf.

Administration of ⁵⁹Fe
Radioactive ferric chloride solution (⁵⁹FeCl₃, 1.3 GBq/mg Fe, The Japan Radioisotope Association, Tokyo, Japan) was diluted with saline to 37 kBq/ml. Each mouse was intravenously injected with ⁵⁹Fe in a dose of 3.7 kBq (100 µl).

Administration of cold FeSO₄
Each mouse was intragastrically injected with cold FeSO₄ (6.25-25 µmole/ml saline) in a dose of 100 µl per 10 g body weight 30 sec before the administration of ⁶⁷Ga citrate solution, ¹²³I-Tf solution, or ⁵⁹Fe chloride solution. Control mice were intragastrically injected with saline instead of cold FeSO₄ solution.

Removal of various tissues
At 2 h after the administration of ⁶⁷Ga citrate solution, ¹²³I-Tf solution, or ⁵⁹Fe chloride solution, mice were anesthetized with urethane (1.5 g /kg, i.p.) and the blood (1 ml) was taken for the determination of serum GPT activity. Then, the whole liver and spleen were removed.

Determination of serum GPT activity
Serum glutamic pyruvic transaminase (GPT) activity was determined by the method of Reitman and Frankel²⁸, and expressed as Karmen units (KU) per ml of serum.

Determination of radioactivity
The radioactivities of ⁶⁷Ga, ¹²³I, and ⁵⁹Fe were determined with a well-type NaI-scintillation counter (Aloka, ARC-300).

The uptake ratios of ⁶⁷Ga ¹²³I, and ⁵⁹Fe in blood, liver, and spleen were expressed in the following formula:

Uptake ratio = A/B
A: sample activity (cpm)/sample weight (g)
B: total activity administrated (cpm)/mouse body weight (g).

RESULTS

Time course of serum GPT activity after CCl₄ treatment
The serum GPT value increased immediately after CCl₄ treatment, reaching its maximum on the 1st day after CCl₄ treatment, and then on the 3rd day after CCl₄ treatment the value decreased to that of the control mice (Table 1).

Time course of the uptake of ⁶⁷Ga into various tissues after CCl₄ treatment
Concerning the uptake of ⁶⁷Ga by the blood and

<table>
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<th>Days after CCl₄ treatment</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
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</thead>
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<tr>
<td>GPT activity (KU/ml)</td>
<td>20.7±14.9</td>
<td>1,772.5±155.2</td>
<td>1,036±335.3</td>
<td>28.6±3.0</td>
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</table>

* Control mice

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Fig. 1 Time course of the uptake of $^{67}$Ga by the blood, liver, and spleen and the effect of cold FeSO$_4$ on the uptake. Each mouse was intragastrically preinjected with saline (■) or cold FeSO$_4$ solution (□) 30 sec before the administration of $^{67}$Ga. Each point represents the mean and SEM for six mice. Mice on 0 days after the administration of CCl$_4$ are control mice.

Fig. 2 Time course of the uptake of $^{125}$I-Tf by the blood, liver, and spleen and the effect of FeSO$_4$ on the uptake. Each mouse was intragastrically preinjected with saline (■) or cold FeSO$_4$ solution (□) 30 sec before the administration of $^{125}$I-labeled transferrin. Each point represents the mean and SEM for six mice. Mice on 0 days after the administration of CCl$_4$ are control mice.

spleen, there was no difference between normal and CCl$_4$-treated mice (Fig. 1). On the other hand, the uptake of $^{67}$Ga by inflamed liver reached its maximum on the 1st day after CCl$_4$ treatment and the uptake ratio into inflamed liver was about 6 times that into normal liver. The administration of cold FeSO$_4$ remarkably decreased the uptake of $^{67}$Ga by both the blood and spleen (Fig. 1). On the other hand, the uptake of $^{67}$Ga into inflamed liver was enhanced by the administration of cold FeSO$_4$, while the uptake into normal liver was decreased by the administration.

Fig. 3 Time course of the uptake of $^{59}$Fe by the blood, liver, spleen and the effect of cold FeSO$_4$ on the uptake. Each mouse was intragastrically preinjected with saline (■) or cold FeSO$_4$ solution (□) 30 sec before the administration of $^{59}$Fe. Each point represents the mean and SEM for six mice. Mice on 0 days after the administration of CCl$_4$ are control mice.

**Time course of the uptake of $^{125}$I-Tf into various tissues after CCl$_4$ treatment**

The uptake of $^{125}$I-Tf into both the blood and spleen of CCl$_4$-treated mice differed little from that of normal mice (Fig. 2). On the other hand, the uptake of $^{125}$I-Tf by inflamed liver on the 1st day after CCl$_4$ treatment increased as compared with that by normal liver. The uptake into inflamed liver was about 1.6 times that into normal liver. The administration of cold FeSO$_4$ did not affect the uptake of $^{125}$I-Tf by the blood, liver, and spleen of both normal and CCl$_4$-treated mice (Fig. 2).

**Time course of the uptake of $^{59}$Fe into various tissues after CCl$_4$ treatment**

Concerning the uptake of $^{59}$Fe by the blood and spleen, there was no difference between normal and CCl$_4$-treated mice (Fig. 3). On the other hand, the uptake of $^{59}$Fe into the liver on the 1st day after CCl$_4$ treatment was 1.7 times that into normal liver. The administration of cold FeSO$_4$ decreased the uptake of $^{59}$Fe into the blood, liver, and spleen in all cases (Fig. 3).

Table 2 shows the effect of cold FeSO$_4$ on liver-to-blood ratios of $^{67}$Ga, $^{125}$I-Tf, and $^{59}$Fe uptake. Cold FeSO$_4$ remarkably increased the liver-to-blood ratio of $^{67}$Ga on the 1st day after CCl$_4$ treatment, whereas it decreased that of $^{59}$Fe. On the other hand, cold FeSO$_4$ did not influence the liver-to-blood ratio of $^{125}$I-Tf.

**DISCUSSION**

Hayes et al.\(^9\) reported that transferrin is involved in the uptake of $^{67}$Ga into normal soft tissues such as
Table 2  Effect of cold FeSO₄ on the liver-to-blood ratio of ⁶⁷Ga, ¹²⁵I-Tf, or ⁵⁹Fe uptake. Each value represents the ratio of the mean ⁶⁷Ga, ¹²⁵I-Tf, or ⁵⁹Fe uptake ratio into the liver to that into the blood.

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<td>⁵⁹Fe</td>
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<td>cold FeSO₄</td>
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</tr>
</tbody>
</table>

* Control mice

the liver or spleen. Concerning the uptake of ⁶⁷Ga into normal liver and spleen, our results recently reported²⁸,²⁶ agree with those reported by Hayes et al. Hayes et al.⁹ also reported that the initial entry of ⁶⁷Ga into the inflammatory lesions might occur by endocytosis in which transferrin is involved, while the uptake of ⁶⁷Ga into tumors occurs mainly by the diffusion of the unbound form. On the other hand, they have suggested that the uptake of ⁶⁷Ga into the inflammatory tissues such as granuloma occurs in an unbound form.²⁸ Therefore, in the present study we have attempted to investigate whether or not transferrin is involved in the initial entry of ⁶⁷Ga into inflamed liver. In order to clarify this point, the uptake of ⁶⁷Ga, ¹²⁵I-Tf and ⁵⁹Fe into the liver of CCl₄-treated mice at 2 h after the administration of these radioisotopes and the effect of Fe²⁺ on the uptake of ⁶⁷Ga, ¹²⁵I-Tf and ⁵⁹Fe by the blood, liver, and spleen was studied. It is well known that transferrin is a carrier glycoprotein of iron in the blood Harrah⁹ reported that the binding affinity of iron for transferrin was stronger than that of gallium, i.e., Fe²⁺ can inhibit the binding of ⁶⁷Ga to transferrin. Since intragastrically injected Fe²⁺ is changed to Fe³⁺ in the blood, FeSO₄ was used as an inhibitor for the binding of ⁶⁷Ga to transferrin in the blood. Concerning the time courses of serum GPT activity and ⁶⁷Ga uptake in the liver after CCl₄ treatment, Kojima et al.²⁰ reported that the increase in serum GPT and the increase in ⁶⁷Ga uptake were not concurrent, i.e., serum GPT activity reached its maximum on the 2nd day, but ⁶⁷Ga uptake had not reached its maximum by the 3rd day after CCl₄ treatment. In the present study, however, the peak of serum GPT activity was consistent with the peak of ⁶⁷Ga uptake by inflamed liver, moreover these peaks reached the maximum on the 1st day after CCl₄ treatment, i.e., the peak of inflammation was consistent with the peak of ⁶⁷Ga uptake. This difference may be because the animals employed by Kojima et al were rats but those employed by us were mice. The results of the present study showed that the uptake of ⁶⁷Ga by inflamed liver was completely different from the uptake of ¹²⁵I-Tf and ⁵⁹Fe. The uptake of ⁶⁷Ga by inflamed liver was 6 times that by normal liver. On the other hand, the uptake of ¹²⁵I-Tf and ⁵⁹Fe by inflamed liver was 1.6 and 1.7 times that by normal liver, respectively. Therefore, ⁵⁹Fe must be taken up together with transferrin by inflamed liver. Moreover, when the binding of ⁶⁷Ga to transferrin in the blood was inhibited by Fe³⁺, the uptake of ⁶⁷Ga by inflamed liver was increased slightly but the uptake of ⁵⁹Fe decreased. On the other hand, cold Fe²⁺ did not influence the uptake of ¹²⁵I-Tf by inflamed liver. Cold Fe²⁺ also decrease the uptake of ⁶⁷Ga by normal liver and spleen, none-inflamed tissues. These results show that ⁶⁷Ga is not taken up together with transferrin by the inflammatory tissues (CCl₄-damaged liver) but is taken up together with transferrin by normal tissues such as normal liver and spleen. Many workers have expressed confidence that ⁶⁷Ga is exclusively bound to and transported to various tissues by transferrin in the blood. Therefore, at the inflammatory site, ⁶⁷Ga might be then dissociated from the transferrin complex. Vallabhajosula et al.²⁵,³¹ reported that acidic pH at the tumor site might be one of the factors involved in ⁶⁷Ga localization in tumors. Moreover, reduction of the pH to 6.8–7.0, below that of normal tissue (7.4), might be due to the accumulation of lactic acid produced by anaerobic glycolysis. Therefore, we think that the pH at the site of the inflammatory lesions must be reduced also by anaerobic glycolysis. Additionally we think that acid glycosaminoglycans also reduce the pH at the site of the inflammatory lesions. We think that ⁶⁷Ga is transported to the inflammatory sites either in a transferrin bound form or in an unbound form and ⁶⁷Ga in unbound form directly enters the inflammatory tissues, whereas ⁶⁷Ga in bound form is dissociated under acidic conditions at the inflammatory...
site and then enters the inflammatory tissues. We conclude that transferrin is not involved in the uptake of $^{67}$Ga by CCl$_4$-damaged liver (acute inflammatory tissues) but it is involved in normal liver.

REFERENCES