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^{67}Ga in transferrin-unbound form is taken up by inflamed liver of mouse treated with CCl_4

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In order to investigate whether or not transferrin is involved in the uptake of ^{67}Ga by inflamed liver (acute inflammatory tissues) the uptake of ^{67}Ga by the liver of mice treated with carbon tetrachloride (CCl_4) was studied. The serum GPT value reached its maximum on the 1st day after the CCl_4 treatment. The uptake of ^{67}Ga by the liver also reached its maximum on the 1st day after the CCl_4 treatment and the amount uptaken into inflamed liver was about 6 times that uptaken into normal liver. On the other hand, the uptake of ^{125}I-transferrin into inflamed liver on the 1st day after CCl_4 treatment was only about 1.6 times that into normal liver. Moreover, cold Fe^{3+} decreased the uptake of ^{67}Ga by normal liver but increased the uptake of ^{67}Ga by inflamed liver. These results show that transferrin plays an important role in the uptake of ^{67}Ga by normal liver but not by inflamed liver, i.e. ^{67}Ga in the transferrin-unbound form is preferentially taken up by inflamed liver.

Key words: ^{67}Ga uptake, CCl_4 treatment, mouse damaged-liver, transferrin

INTRODUCTION

SINCE the first observation of ^{67}Ga accumulation in tumors[1] and inflammatory lesions,[2,3] ^{67}Ga has been used for the detection of various tumors[4] and acute and chronic inflammation.[5,6] Many hypotheses concerning the mechanism of uptake of ^{67}Ga into tumors and the inflammatory lesions have been proposed[5,7–15] but a consensus has not yet been reached. It is well known that almost all ^{67}Ga is bound to transferrin in the blood.[16,18] It has been reported that transferrin plays a major role in the uptake of ^{67}Ga into tumors.[19–22] On the other hand, it has been shown that transferrin transports ^{67}Ga to tumor tissues but that the uptake of ^{67}Ga into tumor cells occurs in an unbound form.[10,23–25] Concerning whether transferrin is involved in the uptake of ^{67}Ga into tumors or not, a final conclusion cannot now be drawn. The involvement of transferrin in the uptake of ^{67}Ga into the inflammatory tissues also has not been conclusively demonstrated. It has been reported that the uptake of ^{67}Ga 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 ^{67}Ga into the liver and spleen occurred in a transferrin-bound form.[18,26] Hayes et al.[9] reported that the initial entry of ^{67}Ga into the inflammatory lesions, such as abcess 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 ^{67}Ga 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 ^{67}Ga into CCl_4-damaged liver (acute inflammatory tissues).

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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 CCl_4
A dose of 0.1 m*l* of 10% CCl_4 in olive oil per 10 g body weight was given intraperitoneally. Control mice were treated similarily with equivalent amounts of olive oil alone.

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

Administration of ^{125}I-labeled transferrin
Labeling of transferrin with ^{125}I was carried out by essentially the same method as that of Markwell.[27] Fifty μl of Na ^{125}I 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 radioiodide. 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 ^{125}I-Tf.

Administration of ^{59}Fe
Radioactive ferric chloride solution ($^{59}FeCl_3$, 1.3 GBq/mg Fe, The Japan Radioisotope Association, Tokyo, Japan) was diluted with saline to 37 kBq/m*l*. Each mouse was intravenously injected with ^{59}Fe in a dose of 3.7 kBq (100 μl).

Administration of cold $FeSO_4$
Each mouse was intragastrically injected with cold $FeSO_4$ (6.25–25 μmole/m*l* saline) in a dose of 100 μl per 10 g body weight 30 sec before the administration of ^{67}Ga citrate solution, ^{125}I-Tf solution, or ^{59}Fe chloride solution. Control mice were intragastrically injected with saline instead of cold $FeSO_4$ solution.

Removal of various tissues
At 2 h after the administration of ^{67}Ga citrate solution, ^{125}I-Tf solution, or ^{59}Fe chloride solution, mice were anesthetized with urethane (1.5 g /kg, i.p.) and the blood (1 m*l*) 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 Frankel[28], and expressed as Karmen units (KU) per m*l* of serum.

Determination of radioactivity
The radioactivities of ^{67}Ga, ^{125}I, and ^{59}Fe were determined with a well-type NaI-scintillation counter (Aloka, ARC-300).

The uptake ratios of ^{67}Ga ^{125}I, and ^{59}Fe 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 administered (cpm)/mouse body weight (g).

RESULTS

Time course of serum GPT activity after CCl_4 treatment
The serum GPT value increased immediately after CCl_4 treatment, reaching its maximum on the 1st day after CCl_4 treatment, and then on the 3rd day after CCl_4 treatment the value decreased to that of the control mice (Table 1).

Time course of the uptake of ^{67}Ga into various tissues after CCl_4 treatment
Concerning the uptake of ^{67}Ga by the blood and

Table 1 Time course of serum GPT activity after CCl_4 treatment.
Each value represents the mean and SEM for six mice

	Days after CCl_4 treatment			
	0*	1	2	3
GPT activity (KU/m*l*)	20.7±14.9	1,772.5±155.2	1,036±335.3	28.6±3.0

* Control mice

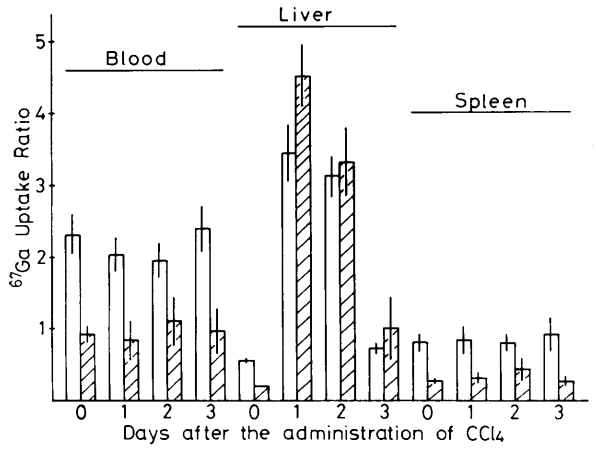

Fig. 1 Time course of the uptake of ^{67}Ga by the blood, liver, and spleen and the effect of cold $FeSO_4$ on the uptake. Each mouse was intragastrically preinjected with saline (□) or cold $FeSO_4$ sulution (▨) 30 sec before the administration of ^{67}Ga. Each point represents the mean and SEM for six mice. Mice on 0 days after the administration of CCl_4 are control mice.

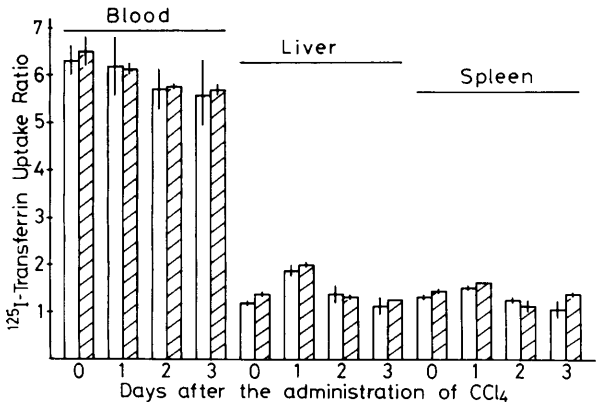

Fig. 2 Time course of the uptake of ^{125}I-Tf by the blood, liver, and spleen and the effect of $FeSO_4$ on the uptake. Each mouse was intragastrically preinjected with saline (□) or cold $FeSO_4$ (▨) 30 sec before the administration of ^{125}I-labeled transferrin. Each point represents the mean and 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.

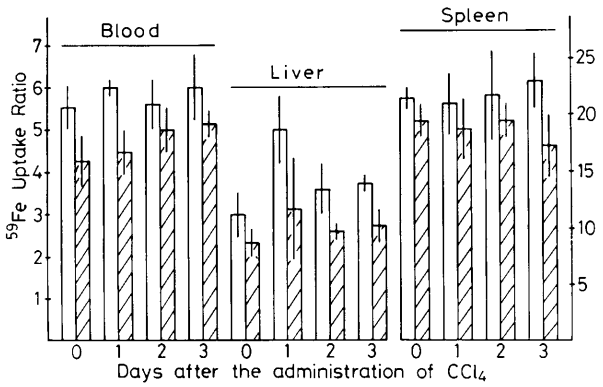

Fig. 3 Time course of the uptake of ^{59}Fe by the blood, liver, spleen and the effect of cold $FeSO_4$ on the uptake. Each mouse was intragastrically preinjected with saline (□) or cold $FeSO_4$ (▨) 30 sec before the administration of ^{59}Fe. Each point represents the mean and SEM for six mice. Mice 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 $FeSO_4$ on the liver-to-blood ratio of ^{67}Ga, ^{125}I-Tf, or ^{59}Fe uptake. Each value represents the ratio of the mean ^{67}Ga, ^{125}I-Tf, or ^{59}Fe uptake ratio into the liver to that into the blood

	Preinjection with	Days after CCl_4 treatment			
		*0	1	2	3
^{67}Ga	saline	0.33	1.69	1.44	0.47
	cold $FeSO_4$	0.22	5.38	3.07	0.86
^{125}I-Tf	saline	0.29	0.30	0.24	0.20
	cold $FeSO_4$	0.20	0.33	0.23	0.22
^{59}Fe	saline	0.55	0.84	0.64	0.63
	cold $FeSO_4$	0.55	0.70	0.52	0.53

* Control mice

the liver or spleen. Concerning the uptake of ^{67}Ga into normal liver and spleen, our results recently reported[18,26] agree with those reported by Hayes et al. Hayes et al.[9] also reported that the initial entry of ^{67}Ga into the inflammatory lesions might occur by endocytosis in which transferrin is involved, while the uptake of ^{67}Ga into tumors occurs mainly by the diffusion of the unbound form. On the other hand, have suggested that the uptake of ^{67}Ga into the inflammatory tissues such as granuloma occurs in an unbound form.[18] Therefore, in the present study we have attempted to investigate whether or not transferrin is involved in the initial entry of ^{67}Ga into inflamed liver. In order to clarify this point, the uptake of ^{67}Ga, ^{125}I-Tf and ^{59}Fe into the liver of CCl_4-treated mouse at 2 h after the administration of these radioisotopes and the effect of Fe^{3+} on the uptake of ^{67}Ga, ^{125}I-Tf and ^{59}Fe by the blood, liver, and spleen was studied. It is well known that transferrin is a carrier glycoprotein of iron in the blood Hara[29] reported that the binding affinity of iron for transferrin was stronger than that of gallium, i.e. Fe^{3+} can inhibit the binding of ^{67}Ga to transferrin. Since intragastrically injected Fe^{2+} is changed to Fe^{3+} in the blood, $FeSO_4$ was used as an inhibitior for the binding of ^{67}Ga to transferrin in the blood. Concerning the time courses of serum GPT activity and ^{67}Ga uptake in the liver after CCl_4 treatment, Kojima et al.[30] reported that the increase in serum GPT and the increase in ^{67}Ga uptake were not concurrent, i.e. serum GPT activity reached its maximum on the 2nd day, but ^{67}Ga uptake had not reached its maximum by the 3rd day after CCl_4 treatment. In the present study, however, the peak of serum GPT activity was consistent with the peak of ^{67}Ga uptake by inflamed liver, moreover these peaks reached the maximum on the 1st day after CCl_4 treatment, i.e. the peak of inflammation was consistent with the peak of ^{67}Ga 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 ^{67}Ga by inflamed liver was completely different from the uptake of ^{125}I-Tf and ^{55}Fe. The uptake of ^{67}Ga by inflamed liver was 6 times that by normal liver. On the other hand, the uptake of ^{125}I-Tf and ^{59}Fe by inflamed liver was 1.6 and 1.7 times that by normal liver, respectively. Therefore, ^{59}Fe must be taken up together with transferrin by inflamed liver. Moreover, when the binding of ^{67}Ga to transferrin in the blood was inhibited by Fe^{3+}, the uptake of ^{67}Ga by inflamed liver was increased slightly but the uptake of ^{59}Fe decreased. On the other hand, cold Fe^{3+} did not influence the uptake of ^{125}I-Tf by inflamed liver. Cold Fe^{3+} also decrease the uptake of ^{67}Ga by normal liver and spleen, none-inflamed tissues. These results show that ^{67}Ga is not taken up together with transferrin by the inflammatory tissues (CCl_4-damaged liver) but is taken up together with transferrin by normal tissues such as normal liver and spleen. Many workers have expressed confidence that ^{67}Ga is exclusively bound to and transported to various tissues by transferrin in the blood. Therefore, at the inflammatory site, ^{67}Ga 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 ^{67}Ga 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 producted 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 ^{67}Ga is transported to the inflammatory sites either in a transferrin bound form or in an unbound form and ^{67}Ga in unbound form directly enters the inflammatory tissues, whereas ^{67}Ga 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.

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