Enhancement of Hepatic Gallium-67 Uptake by Asialo-Transferrin

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A significant fraction of intravenously injected ^{67}Ga localizes in the liver. The mechanism of ^{67}Ga uptake by the liver is not clear. Hepatocyte membranes contain galactose-specific receptors which selectively remove asialo-glycoproteins from the circulation by way of endocytosis. In this investigation, we examined whether endocytic uptake of asialo-transferrin would involve ^{67}Ga transport into hepatocytes. We demonstrated that asialo-transferrin bound ^{67}Ga as effectively as apotransferrin. Human asialo-transferrin markedly enhanced ^{67}Ga uptake by isolated, perfused rat livers. Human asialo-orosomucoid, but not orosomucoid competitively inhibited hepatic uptake of the ^{67}Ga asialo-transferrin complex, indicating that hepatic ^{67}Ga uptake in the presence of asialo-transferrin occurred by way of galactose-specific receptors. Our results point to a novel pathway for metal ion transport into hepatocytes by way of galactose receptor mediated endocytosis.

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After i.v. injection of carrier-free gallium-67 (^{67}Ga) citrate, the tracer is almost exclusively bound to serum transferrin (*1*). In normal individuals, ~5–10% of the injected ^{67}Ga accumulates in the liver (*2*). We have recently studied the hepatic uptake of ^{67}Ga using isolated rat hepatocytes and perfused livers (*3*). We demonstrated that (a) in the absence of transferrin, the liver was able to take up ^{67}Ga and secrete it into the bile, (b) transferrin inhibited hepatic uptake of ^{67}Ga and its biliary secretion, and (c) iron-deficiency markedly enhanced hepatic uptake of ^{67}Ga. On the other hand, Hayes et al. (*4*) reported enhancement of ^{67}Ga uptake by rat livers after i.v. injection of relatively large doses (140 mg/kg) of transferrin.

The plasma membrane of hepatocytes contains galactose-specific receptors that selectively remove asialo-glycoproteins from the circulation by way of receptor mediated endocytosis (*5*). Regoeczi et al. (*6,7*) have shown that in the rabbit, human asialo-transferrin is prematurely catabolized because of preferential uptake by the liver and the bone marrow. Binding of asialo-transferrin to asialo-glycoprotein receptors on hepatocytes has been reported by Tolleshaug et al. (*8*) and by Young et al. (*9*). Whether or not the catabolic pathway of asialo-transferrin involves metal (gallium, iron) transport into the liver is not known.

In the current investigation we studied ^{67}Ga uptake by the liver by way of the asialo-glycoprotein (asialo-transferrin) mechanism. Our results indicate that (a) desialylation of apotransferrin does not affect its binding of ^{67}Ga, (b) human asialo-transferrin enhances ^{67}Ga uptake by the isolated, perfused rat liver, and (c) human asialo-orosomucoid, but not the unmodified orosomucoid, competitively inhibits hepatic uptake of the ^{67}Ga asialo-transferrin complex. The data suggest that in the presence of asialo-transferrin, gallium is taken up by the liver by way of galactose receptor mediated endocytosis.

MATERIALS AND METHODS

Human apotransferrin, human α_1 acid glycoprotein (orosomucoid) and neuraminidase (from Clostridium perfringens, type VI-A, E.C.3.2.1.18) attached to agarose beads were obtained commercially.* Carrier-free [^{67}Ga]citrate was also obtained commercially.† Asialo-transferrin was prepared by incubating 100 mg of apotransferrin with one unit of neuraminidase in 10 ml of 0.05*M* Tris-phosphate buffer pH 6.0, for 18 hr at 37°C. After centrifugation to remove the neuraminidase agarose beads, transferrin was saturated with iron by

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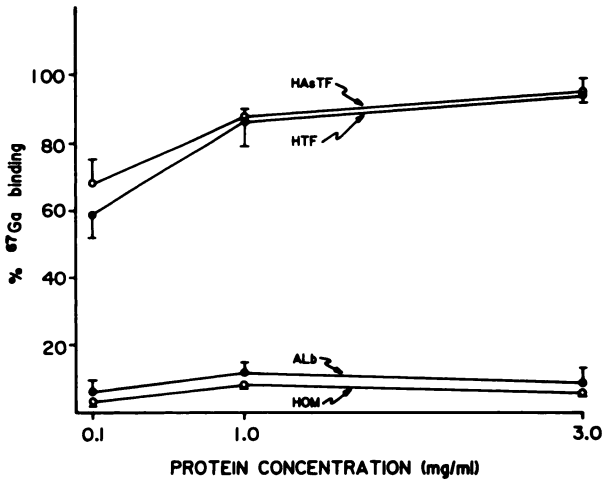

FIGURE 1
Effect of desialylation on binding of ^{67}Ga by human transferrin. Protein-binding of ^{67}Ga was determined by radioactivity retained on filter membrane after ultrafiltration. When ^{67}Ga in saline was filtered, 8.9 ± 3.1% (n = 6) of radioactivity was retained by membrane. Results are mean ± 1 s.d. of 3–5 experiments. HAsTF = human asialo-transferrin; HTF = Human apotransferrin; ALb = Bovine albumin; HOM = Human orosomucoid

addition of 1 mg ferric ammonium citrate. It was then dialyzed overnight at 4°C. Residual neuraminidase was removed by DEAE cellulose chromatography according to Regoeczi et al. (*10*). The isolated asialo-transferrin was concentrated with Centriflo Ultrafiltration membrane cones‡ and the iron removed by repeated washes with acetate-citrate buffer pH 4.5 (*4*), followed by distilled water. The final preparation was lyophilized and was free of sialic acid and neuraminidase activity as determined by the method of Aminoff (*11*). Asialo-orosomucoid was prepared according to Regoeczi et al. (*10*).

Gallium-67 protein binding was determined as described previously (*12*) using Centriflo Ultrafiltration membrane cones with a >95% retention for molecules with molecular weight above 25,000 dalton. Gallium-67 citrate (1 μCi/ml) was incubated for 2 hr at room temperature with saline, bovine albumin, orosomucoid, apotransferrin or asialo-transferrin in the presence of 1.5 m*M* $NaHCO_3$. The mixture was then centrifuged in a membrane cone at 950 *g* for 10 min. The radioactivity in 0.1 ml of the filtrate was determined and the amount of ^{67}Ga retained by the cone was then calculated (*12*).

Hepatic uptake and biliary secretion of ^{67}Ga was determined as described previously (*3*) using a recirculating isolated, perfused rat liver preparation. After the liver was isolated, it was perfused for 1 hr at 37°C to equilibrate the system. Gallium-67 citrate (5 μCi) was then added to the perfusate. Apo-transferrin, asialo-transferrin, or orosomucoid, was introduced into the system 1 min before the addition of [^{67}Ga]citrate. In competitive inhibition studies, asialo-orosomucoid was added to the perfusate 2 min before the addition of asialo-transferrin. The liver was then perfused for 2 hr, after which time the original perfusate was disconnected and the liver flushed with 100 ml of fresh medium to remove intravascular ^{67}Ga. The radioactivity in the perfusate, wash medium, bile, and liver was determined as described previously (*3*). The results were expressed as percent of the dose originally introduced into the perfusion medium.

TABLE 1
Effect of Asialo-Transferrin on Hepatic Uptake and Biliary Secretion of ^{67}Ga*

Item	^{67}Ga hepatic uptake (% dose/g liver)	^{67}Ga biliary secretion (% dose/g liver)
Control	1.41 ± 0.29 (5)	0.55 ± 0.20 (5)
HTF (0.1 mg/ml)	1.02 ± 0.36 (5)	0.27 ± 0.11 (5)†
HAsTF (0.1 mg/ml)	2.64 ± 0.39 (5)†	0.40 ± 0.07 (5)

* Isolated rat livers were perfused with ^{67}Ga (5 μCi) in presence or absence of human apotransferrin (HTF) or human asialo-transferrin (HAsTF) for 2 hr. At end of perfusion, radioactivities in liver and bile were determined. Results are expressed as % dose/g liver (mean ± 1 s.d.). Numbers in parenthesis are number of experiments.

† Significantly different from control values ($p < 0.025$, or less).

RESULTS

As shown in Fig. 1, asialo-transferrin bound ^{67}Ga effectively. At three different concentrations (0.1 mg, 1.0 mg, and 3.0 mg/ml) asialo-transferrin bound comparable amounts of ^{67}Ga as apotransferrin. In contrast, at similar concentrations, albumin or orosomucoid showed little or no binding of ^{67}Ga. Thus, desialylation of transferrin does not affect its binding of ^{67}Ga.

Table 1 shows the effect of apotransferrin and asialo-transferrin on the hepatic uptake and biliary secretion of ^{67}Ga. Apotransferrin at 0.1 mg/ml perfusion medium had no effect on hepatic uptake of ^{67}Ga, while it inhibited biliary secretion of ^{67}Ga. These results are similar to those reported previously (*3*). In contrast, asialo-transferrin at a similar concentration markedly enhanced hepatic uptake of ^{67}Ga, while the biliary secretion of ^{67}Ga was not affected.

To determine whether the effect of asialo-transferrin on the hepatic uptake of ^{67}Ga was due to galactose receptor-mediated endocytosis, we studied the effect of asialo-orosomucoid. If the uptake of the asialo-transferrin ^{67}Ga complex was due to this mechanism, then it should be inhibited by asialo-orosomucoid as a result of competition for the receptors (*5,10*). As shown in Fig. 2, orosomucoid had no effect on hepatic uptake of ^{67}Ga. In contrast, asialo-orosomucoid but not orosomucoid, markedly inhibited the effect of asialo-transferrin on the hepatic uptake of ^{67}Ga.

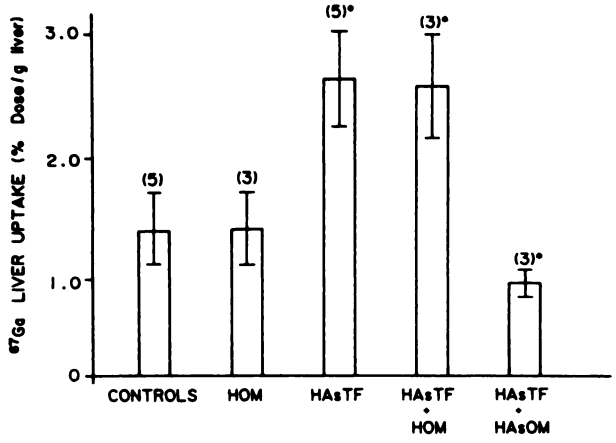

FIGURE 2
Effect of asialo-orosomucoid on enhancement of hepatic ^{67}Ga uptake by asialo-transferrin. Isolated rat livers were perfused with ^{67}Ga for 2 hr in absence of transferrin (controls) and in presence of human orosomucoid (HOM), human asialo-transferrin (HAsTF), HAsTF + HOM and HAsTF + HAsOM (human asialo-orosomucoid). For all added proteins concentration in perfusate was 0.1 mg/ml. Gallium-67 uptake is expressed as percent dose/liver (mean ± 1 s.d.). Number of experiments is in parenthesis. *Significantly different from controls ($p < 0.05$, or less)

Further support for the concept of receptor-mediated uptake of asialo-transferrin-^{67}Ga was provided by our observation that hepatic uptake of ^{67}Ga in the presence of 1.0 mg/ml asialo-transferrin was lower than in the presence of 0.1 mg/ml asialo-transferrin (1.59 ± 0.09 % dose/g liver compared with 2.64 ± 0.39 % dose/g liver). This is presumably due to the competition of the higher concentration of asialo-transferrin for the receptors.

DISCUSSION

The results presented in this study suggest that in the presence of asialo-transferrin, ^{67}Ga can be taken up by hepatocytes by way of galactose-receptor mediated endocytosis of the asialo-transferrin-^{67}Ga complex. This conclusion is based on the following observations.

1). Desialylation of apo-transferrin did not affect its binding of ^{67}Ga.

2). The desialylated transferrin enhanced hepatic uptake of ^{67}Ga.

3). The uptake of ^{67}Ga in the presence of asialo-transferrin could be inhibited by asialo-orosomucoid but not by orosomucoid. The biliary secretion of ^{67}Ga in the presence of asialo-transferrin remained unchanged, though the hepatic uptake was markedly stimulated. This suggests that ^{67}Ga taken up by way of galactose receptor-mediated endocytosis is not accessible for immediate biliary secretion.

In the circulation, ^{67}Ga is mainly bound to plasma transferrin (*1*). Elevation of plasma transferrin levels by either injection of transferrin or in the iron deficient state leads to significant increase in hepatic ^{67}Ga uptake (*4,13*). Based on this observation, Hayes et al. (*4*) hypothesized that ^{67}Ga enters into the liver in the form of a ^{67}Ga transferrin complex. However, recent in vitro studies in isolated rat hepatocytes and perfused rat livers (*3*) have indicated that transferrin at low concentrations (0.002–0.25 mg/ml) has no effect on ^{67}Ga uptake, whereas at higher concentration (0.5–1.0 mg/ml) it inhibits hepatic ^{67}Ga accumulation.

The catabolic pathway of transferrin is not completely understood. Unlike other glycoproteins, homologous asialo-transferrin is cleared from the circulation only slightly faster (15–29%) than the intact apotransferrin (*6*). However, when heterologous asialo-transferrin is used, the catabolic rate increases markedly (*6*). Depending on the number of exposed galactosyl residues, human asialo-transferrin is taken up chiefly by the rabbit liver and/or the bone marrow (*7*). Our finding that ^{67}Ga uptake by the isolated perfused liver could be enhanced by asialo-transferrin points to a novel pathway for metal ion transport into hepatocytes. Its role in the hepatic uptake of ^{67}Ga under normal circumstances remains to be determined.

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FOOTNOTES

* Sigma Diagnostics, St. Louis, MO.
† Medi-Physics, Inc., Richmond, CA.
‡ Amicon Corp., Danver, MA.

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