Studies on Tc-99m-Labeled Liver Ferritin: Hepatobiliary Behavior in Rats: Concise Communication

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Ferritin was isolated from the livers of iron-loaded rats and labeled with Tc-99m. The radionuclide was found to be firmly bound to ferritin and essentially free of colloids. When injected into rats, about 65% of administered dose was found to be concentrated in the liver within 30 min. The blood activity declined rapidly. Liver activity appeared to be excreted slowly through the hepatobiliary route, though there was 5 to 9% constant excretion through the kidneys. The results suggest that this labeled protein may prove useful as a hepatobiliary radiopharmaceutical.

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A host of new radiopharmaceuticals for hepatobiliary imaging have been introduced in recent years. All of them—even the conventional rose bengal—are labeled exogenous chemicals, none being a physiological substance. Lately some interest has been directed toward the use of labeled endogenous serum glycoproteins which, at least in the sialic acid-deficient form, appear to be localized in the liver (*1–3*). But the experimental evidence for subsequent biliary clearance of radioactivity has not been conclusively shown in these studies, the emphasis being on liver imaging only.

Ferritin is the major iron-storage glycoprotein of mammalian tissues, found mainly in liver and spleen. Ferritin labeled with Fe-59 and I-131 has been investigated already for its biological behavior (*4, 5*), and it was shown that more than 85% of the intravenously administered radioactivity was localized in the parenchymal cells of the liver. This finding prompted us to study the behavior of Tc-99m-labeled rat-liver ferritin and to explore its development as a possible radiopharmaceutical for hepatobiliary imaging.

This communication describes a method for the isolation and purification of rat-liver ferritin and its successful labeling with Tc-99m. The biodistribution of the labeled product in rats at different time intervals reveals that, subsequent to uptake by the liver, the radioactivity is excreted largely through the hepatobiliary route.

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MATERIALS AND METHODS

Isolation and purification of ferritin. Female Wistar rats (350–400 g) were fed hydroxy-iron(III) citrate polymer to induce ferritin synthesis in the liver (*6*). The crude ferritin was isolated by the method of Drysdale and Munro (*7*), and further purified by a slight modification of the method described by Massover (*8*), which uses successive gel-filtration chromatography on Sephadex G-200 and Sepharose 4-B (*8*). The fractions containing ferritin were pooled and concentrated with the help of Lyphogel. The purity of the product was ascertained by polyacrylamide gel electrophoresis.

Labeling with Tc-99m. Ferritin was labeled with Tc-99m using the $SnCl_2$ technique of Eckelman et al. (*9*). About 200 μg of an aqueous solution (1.0 ml) of ferritin was treated with 20 μg (20 μl) of $SnCl_2$ solution in 0.05 *N* HCl, followed by the addition of $^{99m}TcO_4^-$ (~2 mCi in 3.0 ml of physiological saline obtained by the solvent-extraction method). During this procedure, care was taken to avoid sudden and drastic changes in the pH, which may affect the physicochemical properties of the protein. The final pH was 4 to 5. The purity of labeled

TABLE 1. BIODISTRIBUTION OF Tc-99m-LABELED RAT-LIVER FERRITIN*

Organs	15 min	30 min	4 hr	24 hr
Liver	55.61 ± 7.70	61.51 ± 7.04	28.98 ± 5.63	11.78 ± 1.84
Intestines (small + large)	2.52 ± 0.52	6.39 ± 2.11	37.59 ± 5.90	8.26 ± 2.68
Kidneys	6.63 ± 1.90	8.69 ± 2.67	9.46 ± 1.28	5.12 ± 0.89
Blood	15.07 ± 1.62	13.06 ± 2.50	3.85 ± 1.19	0.62 ± 0.42
Spleen	0.30 ± 0.11	0.34 ± 0.04	0.33 ± 0.10	0.10 ± 0.01
Thyroid	0.44 ± 0.001	0.06 ± 0.001	0.04 ± 0.004	0

* Values represent mean ± s.d. for percent administered dose in 6–8 rats. Organs showing less than 1% uptake are not included in the table.

product was checked by ascending paper chromatography (Whatman Grade I) in methanol:water (85:15) and physiological saline systems. The labeled product was tested by polyacrylamide gel electrophoresis and ammonium sulphate precipitation, with carrier protein to rule out the presence of colloids.

Biodistribution. The biological behavior of Tc-99m ferritin in male Wistar rats (400–500 g) was determined by injecting the labeled product (30–50 μCi in 0.2 ml) into the dorsalis penis vein of anesthetized rats, which were then killed at intervals of 15 and 30 min and at 4 and 24 hr after the injection. Before sacrifice, as much blood as possible was removed by cardiac puncture. Tissues excised for counting included spleen, liver, stomach, kidneys, heart, lungs, pancreas, thyroid, muscle, blood, bone with marrow, and gut, consisting of small and large intestine, including contents. Counting was done in a "broad" well scintillation counter designed for animal tissues. The carcass and site of injection were also counted to rule out anomalous injection. In a few instances, animals were also killed at time periods of 30-60 sec, 2-2.5 min, and at 5, 45, 60, 120, 160, 360, and 480 min after injection. The 24-hr excreta (feces plus urine) was also collected and counted.

A few experiments were also carried out in Swiss mice (20–30 g) by injecting similarly prepared mouse-liver ferritin into the tail vein. In these experiments the gallbladder was also excised and counted in addition to the above-mentioned organs.

RESULTS

Radiochemical purity of the labeled liver ferritin. The rat-liver ferritin obtained by the above isolation and purification procedure showed the presence of typical ferritin subunit bands on polyacrylamide gel electrophoresis (*10*). The labeled product showed $R_f = 0.0$ in both the chromatographic solvent systems. The amount of free pertechnetate was <1.5% as determined by chromatography.

The tissue distribution data (Table 1) afforded indirect biological evidence for the absence of free pertechnetate. The biological behavior of a solution of the labeled product filtered through a 0.22-μm membrane was exactly similar to that of unfiltered material when injected intravenously into rats. Furthermore, there was

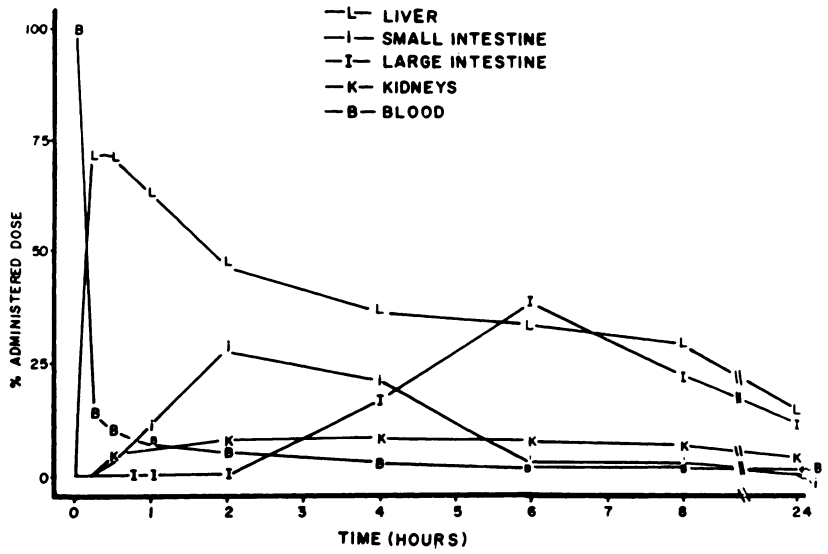

FIG. 1. Kinetics of Tc-99m ferritin in selected organs of rats, based on results obtained in one set of experiments where one or two animals were used per data point.

less than 0.35% uptake in the spleen, thereby indicating indirectly the absence of any particulate matter in the preparation. Upon polyacrylamide gel electrophoresis, the radioactivity was found to be associated with the protein band. Likewise all the activity was precipitated with ammonium sulfate.

Biological distribution of Tc-99m ferritin. Figure 1 shows the biological uptake of the labeled product in a few selected rat tissues, expressed as percent of administered dose at various time intervals. During the initial experiments it was observed that the excretion of the labeled material is slow; hence two early (15 and 30 min) and two late (4 and 24 hr) time intervals were chosen for sacrifice. The pharmacokinetic data obtained from these studies are presented in Table 1. The organs not listed in the table did not show any significant accumulation of Tc-99m. Even spleen, thyroid, stomach, and lungs showed uptake of less than 1% of the administered dose. The pattern of biodistribution in mice was similar to that in rats.

From the data it is clear that the liver avidly picks up the activity from the blood, and then slowly excretes it into the gut. The peak liver activity is seen within 25–35 min after injection. The gut content increased very slowly at the start and reached a maximum of about 40% at 4 hr. The activity in the small and large intestines peaked at about 2 and 6 hr, respectively. The excretion from the liver to the gut appeared to be through the biliary tract, as was shown by the activity found in the bile collected (0.5 ml/hr) from rats whose common bile duct was cannulated, and also by the presence of Tc-99m in the gallbladder of mice. The kidneys were found to exhibit a constant amount of the activity. It was not possible to collect urine from individual rats at hourly intervals, but in a few instances the completely filled bladder was removed with its contents and proved to be radioactive. The contribution of the bladder was similar to that of the kidneys—at least during the first 4 hr—thereby indicating a minor urinary route of excretion. Chromatography of the urine showed no free pertechnetate. At 24 hr the liver, intestines, and kidneys still retained some activity whereas the blood was practically devoid of it. The combined urine and feces collected at 24 hr accounted for approximately 60–70% of the activity, thereby showing that both routes are involved in the excretion of the labeled product. The data in the table and figure show, however, that the hepatobiliary route appears to be of major importance.

CONCLUSION

It is evident from the findings that in rats the labeled material is cleared to a large extent by the hepatobiliary pathway. The presence of an enterohepatic circulation cannot be completely excluded, especially since some liver activity persists even up to 24 hr. Technetium-99m ferritin displays a slow hepatobiliary excretion pattern, in contrast to the comparatively rapid clearance of currently used hepatobiliary agents, such as Tc-99m HIDA, Tc-99m diethyl-IDA, Tc-99m diisopropyl-IDA, etc. In rats the kidney/bladder contribution is not negligible, however, although it appears minor in comparison with gut (Fig. 1). All the same, the high liver uptake and subsequent biliary clearance of Tc-99m ferritin merits attention, especially since it parallels the reported biological behavior of ferritin labeled with I-131 or Fe-59 (*4, 5*). The rather slow excretion of Tc-99m ferritin through the liver has some advantages in imaging procedures, especially when one is evaluating obstructive jaundice, for which very reason I-131 rose bengal still continues to be used for hepatobiliary imaging. Slower excretion may allow imaging of the liver in multiple views for the detection of space-occupying lesions, in addition to the functional study of excretion through biliary passages.

The liver ferritin isolated and purified as above appears to consist of a mixed population of native, apo-, and asialo-protein and the kinetics of this mixed ferritin may be related to its carbohydrate content (*5*).

The study reported here is confined to rats and mice. If these results are to be extrapolated to humans, one may have to use human ferritin, since ferritin is antigenic in nature and appears to be species-specific. Further work will be necessary to ascertain the behavior of heterologous ferritin.

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