Rat liver storage iron and plasma ferritin during D-galactosamine–HCl-induced hepatitis

Laboratory of Experimental Medicine, University of Amsterdam, and *Department of Nuclear Medicine, University of Amsterdam, Wilhelmina Gasthuis, Amsterdam, The Netherlands

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Summary

1. D-Galactosamine–HCl induces toxic hepatitis in the rat and was used as a model to study some aspects of iron metabolism during liver cell damage. Some changes in iron metabolism were similar to those encountered in human acute viral hepatitis.

2. During the first 3 days of liver cell damage induced by galactosamine, liver depot iron and especially ferritin iron decreased by approximately 20%. Plasma ferritin rose, with a peak mean value which was approximately 20 times the concentration measured in normal rats.

3. During the acute phase, plasma ferritin did not accurately reflect the change in the level of liver depot iron.

4. During and after the acute phase, liver depot iron increased after an initial decrease. The non-ferritin depot iron fraction was elevated approximately 75% compared with the value in normal rats. This increase in non-ferritin iron was probably caused by increased erythrocyte catabolism in the liver and recapture followed by catabolism of liver ferritin that had leaked into the blood.

Key words: iron, induced hepatitis, liver, plasma ferritin.

Introduction

It is known that during liver cell damage and necrosis (e.g. that encountered in viral hepatitis) the serum iron concentration (Peterson, 1952) and the serum ferritin concentration (Prieto, Barry & Sherlock, 1975) are often elevated. This is probably caused by release of iron and ferritin from disintegrated liver cells. The release of these compounds indicates that liver depot iron might decrease. In viral hepatitis in man a low depot iron concentration is sometimes found (Hengeveld, Zuyderhoudt & Van Gool, 1978; Zuyderhoudt, Hengeveld, Van Gool & Jörning, 1978a). However, we could not compare these results with values from a normal population because liver biopsy specimens from healthy people would be required. Accordingly, we have studied liver depot iron in rats, both during the acute phase of the toxic hepatitis and the following repair phase. To induce liver cell damage we used D-galactosamine–HCl. Galactosamine is known to influence several hepatic functions (Van Gool, Boers & De Nie, 1978). Histological examination of liver slices revealed liver cell damage to be quite comparable with the kind of liver cell damage seen in human viral hepatitis (Medline, Schaffner & Popper, 1970). We have also studied liver iron fractions and some indices of iron metabolism in the blood during 14 days after induction of the hepatitis in male rats.

Methods

Liver ferritin protein was measured by using a
radial immunodiffusion method (Zuyderhoudt, 1975).

Ferritin iron and total tissue iron were measured after wet ashing (Zuyderhoudt, 1975; Zuyderhoudt, Van Gool & Jörning, 1977). Haem iron was measured as the pyridine haemochromogen (Zuyderhoudt et al., 1977).

This iron fraction, measured as haem, represents the haem from intracellular haem compounds in the liver. In rats with intrahepatic bleeding, haemoglobin-derived haem is included in the measured liver haem iron content.

The difference between total liver iron and the sum of haem iron and total ferritin iron represents the non-ferritin depot iron fraction (Zuyderhoudt, Jörning & Van Gool, 1978b). Total liver depot iron is calculated as the difference between total liver iron and liver haem iron.

Plasma ferritin was measured by an enzyme-linked immuno-assay (Zuyderhoudt, Boers, Linthorst, Jörning & Hengeveld, 1978c).

Haemoglobin, packed cell volume, plasma iron, the total iron-binding capacity of plasma and plasma alanine aminotransferase (EC 2.6.1.2) were measured by standard clinical chemical methods.

Male Wistar rats (270–300 g) received 375 mg of d-galactosamine-HCl in NaCl solution (154 mmol/l) intraperitoneally to induce acute hepatitis. Before the rats were killed they were kept overnight without food. To prevent hypoglycaemia during the fasting period the drinking water contained d-glucose (5 g/100 ml). During the first phase of the hepatitis liver swelling occurred. Therefore, instead of tissue concentrations, compounds are presented as amounts/whole liver. To remove the blood, livers were perfused in situ through the portal vein with NaCl solution (154 mmol/l). A part of the median lobe was used for histological identification of depot iron granules (haemosiderin). Liver slices were stained for iron with Perls stain.

Rats were killed at 0 and 6 h, and 1, 3, 7 and 14 days after galactosamine administration. Rats killed at 0 h were normal rats that had received NaCl solution (154 mmol/l) instead of galactosamine intraperitoneally.

Erythrocyte haemoglobin was labelled with $^{59}$Fe in donor rats by intravenous administration of 20–50 μCi of $^{59}$Fe ([$^{59}$Fe]ferric citrate; The Radiochemical Centre, Amersham, Bucks., U.K.). After 7 days the erythrocytes were labelled in vitro (ICSH-panel, 1971) with $^{51}$Cr (sodium [$^{51}$Cr]-chromate; The Radiochemical Centre). Ten normal rats were given these doubly labelled erythrocytes in an amount corresponding to 1 ml of rat blood. Subsequently six rats received galactosamine intraperitoneally and all were killed 3 days later.

In a following experiment four donor rats received intravenously 20–50 μCi of $^{59}$Fe ferric citrate. After 4 days two of the donor rats received galactosamine in NaCl solution (154 mmol/l) intraperitoneally and the other two donor rats received NaCl solution (154 mmol/l) intraperitoneally. Three days later the rats' erythrocytes were obtained after bleeding and separately labelled with approximately 50 μCi of sodium $^{51}$Cr. Erythrocytes, corresponding to 1 ml of rat blood, from one rat were used to transfuse three normal rats, so six normal rats had received erythrocytes from two donors with galactosamine-induced hepatitis and six normal rats had received labelled erythrocytes from two normal donors.

Because of the small differences in liver accumulation of the radionuclides in the recipient rats, mean values were calculated per six rats with transfused 'normal' erythrocytes and per six rats with transfused 'hepatitis' erythrocytes. Of all 22 recipient rats 3 ml of liver homogenates and 0.5 ml of blood samples (diluted to 3 ml) were measured for their $^{54}$Cr and $^{59}$Fe contents in a 5 inch well-type NaI(TI) crystal.

The energy windows of the spectrum analyser were adjusted in such a way as to exclude the $^{51}$Cr photons from the $^{59}$Fe channel. The radioactivity counts collected in the $^{51}$Cr channel were corrected for the contribution of the $^{59}$Fe photons. Standard deviation of the measurements was approximately 2%.

All points in the Figures are mean values with the SEM. Significances are calculated by use of Student's t-test (non-paired values).

**Results**

Total liver iron (Fig. 1) and the ferritin iron part of this fraction (Fig. 2a) decreased in the first 3 days, the acute phase, of the toxic hepatitis. After 1 week the ferritin iron fraction had recovered, reaching maximal values that were not significantly different from the mean value in the normal rats ($P > 0.05$). We denote the period after the acute phase as the repair phase of the hepatitis.

After an initial decrease, the non-ferritin depot iron fraction increased between 1 and 3 days after galactosamine administration (Fig. 2b). On day 7 this fraction was significantly higher ($P < 0.001$) than in the normal rats. The rapid decrease of liver depot iron could have been accompanied by loss of
Liver iron and plasma ferritin in hepatitis

Fig. 1. Total liver iron during 14 days after galactosamine-induced hepatitis. Figures in the graph indicate the numbers of observations.

Fig. 2. (a) Total liver ferritin iron during 14 days after galactosamine-induced hepatitis. (b) Total liver non-ferritin depot iron 14 days after galactosamine-induced hepatitis. Figures in the graphs indicate the numbers of observations.

Iron via the urine and the bile. However, 6 h after galactosamine, during the period of the lowest depot iron content of the liver, iron was not increased in the urine or the bile. The bile flow was not influenced by the hepatitis.

The changes of the plasma ferritin protein concentration and of the liver ferritin protein content are shown in Fig. 3a and 3b. The plasma ferritin concentration paralleled plasma alanine aminotransferase activity. Three days after galactosamine the values had almost returned to normal.

The plasma ferritin concentration and the alanine aminotransferase activity at 24 h after galactosamine are very much higher than the values measured at 6 h. The liver ferritin protein content at 24 h was already increasing compared with the value at 6 h after galactosamine administration. The mean iron content of liver ferritin did not change significantly over 14 days.

Fig. 4 shows that the plasma iron concentration and the percentage of transferrin saturation increased during the acute phase and remained increased until day 3. Then plasma alanine aminotransferase activities had reached almost normal values (Fig. 3a).

Compared with the blood-haemoglobin concentration in the normal rats (9.0 mmol/l; SEM 0.2 mmol/l, n = 8), the haemoglobin concentration on day 14 after galactosamine administration had decreased slightly but significantly \( P < 0.05 \) to 8.4 mmol/l (SEM 0.2 mmol/l, n = 10).

In the acute phase haem iron in the liver
Discussion

During the first 24 h of galactosamine-induced hepatitis, liver cell damage and leakage led to an increased plasma transaminase activity and raised plasma ferritin protein concentration (Fig. 3a).

Because of ferritin release from the liver (Fig. 2a and Fig. 3b), liver depot iron decreased during the first 6 h after galactosamine administration (Fig. 1). The mean iron content of the plasma ferritin during liver cell leakage was in the same range as in liver ferritin (preliminary results). The iron content of plasma ferritin during the acute phase of the hepatitis, combined with the similar changes of the plasma transaminase activity and the plasma ferritin concentration during these experiments (Fig. 3a) indicated that the transient increase of the plasma ferritin concentration was caused by liver cell leakage.

About 6 h after galactosamine administration the iron concentrations in the bile and the urine were equal to those in normal rats. So in this period there was no increased iron release from the body.

From 6 to 24 h after galactosamine administration the situation became complex. Liver depot iron and liver ferritin started to increase (Figs. 1, 2a and 3b), and plasma ferritin concentration increased as well (Fig. 3a). In this period changes in the plasma ferritin concentration did not reflect the change in liver depot iron.

The increased liver haem concentration, and the erythrocyte sequestration (Table 1), indicated that haemolysis had occurred. The increased erythrocyte sequestration was not caused by galactosamine-induced erythrocyte damage followed by liver macrophage capture, but by small intrahepatic haemorrhage. During the first 24 h of the toxic hepatitis, petechiae on the liver surface and in the liver could be seen regularly.

After an initial decrease, liver depot iron fractions started to increase (Figs. 1 and 2). Part of the
Iron of the intrahepatic sequestered haemoglobin was incorporated into ferritin. On day 3 after galactosamine administration and transfusion of the labelled erythrocytes, the specific $^{59}$Fe radioactivity of liver ferritin increased to three times the value of that for control rats. Increased haemoglobin catabolism also results in increased liver ferritin concentration (Zuyderhoudt et al., 1977).

Liver ferritin is known to be taken up from the plasma by the liver. After uptake into the parenchymal cells ferritin is catabolized rapidly. Part of this iron is incorporated in ferritin synthesized de novo (Unger & Hershko, 1974). Thus we conclude that catabolism of the extravasated blood, and recapture in the liver of ferritin that had leaked to the plasma, were the primary causes of the increase in the liver depot iron fractions.

During the repair phase of the toxic hepatitis, total liver depot iron increased; the increase was less than 25%. This grade of siderosis is minor compared with the accumulation of iron in the livers of patients with profound iron-loading diseases (Barry & Sherlock, 1971). The increase of liver depot iron was primarily caused by the increase of the non-ferritin depot iron fraction. As we have shown earlier, haemoglobin catabolism as well as intrahepatic catabolized liver ferritin sequestered from the plasma can provide non-ferritin iron (Zuyderhoudt et al., 1977, 1978b). The low grade of iron loading was confirmed by histological examination of liver slices.

During the repair phase, liver macrophages contained haemosiderin granules, suggesting a relationship between haemosiderin and the non-ferritin depot iron, although these fractions are not identical (Zuyderhoudt et al., 1978b).

In man, spotty deposition of iron-positive material in the liver during the repair phase of acute viral hepatitis has been observed (Wepler & Opitz, 1958).

Serum iron remained elevated during the repair phase just as in human hepatitis, whereas plasma ferritin had already declined to normal concentrations. It seems likely that part of the newly formed depot iron, built up in the first 3 days, is transferred gradually from the liver to the blood, contributing to the raised plasma iron concentration (Fig. 4). Possibly the high plasma iron concentration reflected catabolism of extravasated blood.

The decrease of liver depot iron during the acute phase of the toxic hepatitis was soon followed by an increase, though the grade of haemosiderosis was low. The non-ferritin depot iron fraction accounted for the greatest part of this increase and was primarily due to minor intrahepatic bleeding and recapture followed by catabolism of liver ferritin that had leaked into the blood. During the acute phase, plasma ferritin did not accurately reflect the change in the amount of liver depot iron.

In conclusion, galactosamine hepatitis in the rat is a model that can be used to study iron metabolism during acute liver cell damage and necrosis.

References


