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MINI-REVIEW

Hepatic iron deposition in human disease and animal models

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Iron deposition occurs in parenchymal cells of the liver in two major defects in human subjects (i) in primary iron overload (genetic haemochromatosis) and (ii) secondary to anaemias in which erythropolesis is increased (thalassaemia). Transfusional iron overload results in excessive storage primarily in cells of the reticule endothelial system. The storage patterns in these situations are quite characteristic. Excessive iron storage, particularly in parenchymal cells eventually results in fibrosis and cirrhosis. There is no animal model or iron overload which completely mimics genetics haemochromatosis but dietary iron loading with carbonyl iron or ferrocene does produce excessive parenchymal iron stores in the rat. Such models have been used to study iron toxicity and the action of iron chelators in the effective removal of excessive iron stores.

Keywords: iron, liver, human, animal

Introduction

In normal human subjects approximately one-third of the iron in the body is present as iron stores and approximately one-third of these stores are present in the liver. The level of iron stores is maintained in normal human subjects by regulation of iron absorption as there is a limited capacity for iron excretion (around 1 mg day^{-1}) in humans. Two major types of defect result in excessive iron-deposition in the parenchymal cells of the liver and other organs: (i) primary iron overload such as occurs in genetic haemochromatosis (HC), an inherited defect of iron metabolism resulting in an inappropriate life-long increase in iron absorption, and (ii) iron loading secondary to anaemias in which erythropoiesis is increased, such as thalassemia. In these latter conditions iron absorption is increased in response to increased erythropoiesis as a result of excessive red cell destruction. Transfusions necessary to maintain haemoglobin levels in such patients also contribute to the excessive iron, load which again is stored primarily in the liver. Genetic HC is inherited as an autosomal recessive trait and occurs in 1 in 300 of the Australian population (Leggett *et al.* 1990), while thalassemia is extremely common in people of Mediterranean origin and in some areas of South east Asia such as Thailand. Both diseases are major causes of morbidity and mortality (Powell *et al.* 1992, Halliday & Powell 1992).

The histological picture of iron overload in a liver biopsy from a patient with 'genetic' or hereditary HC is quite characteristic (Searle *et al.* 1994). In early cases it consists of periportal deposition of iron (Figure 1) that with time gradually extends towards the centrilobular vein until the parenchymal cells of the whole lobule are heavily loaded with iron. Such iron is in the form of ferritin and haemosiderin, and is readily visible using a Perls' Prussian blue stain and by electron microscopy. In some instances discrete iron-free foci are seen in such heavily iron-loaded livers (Figure 2). The significance of these foci is unknown, but the suggestion has been made that they are areas of cellular proliferation.

Eventually iron stores become excessive and fibrosis begins to dissect the lobules. If untreated, cirrhosis follows (Figure 3), although the mechnanism by which this occurs is still unknown. Once such a patient becomes cirrhotic there is a 200-fold increase in the relative risk of the development of a hepatocellular carcinoma (Figure 4) (Bradbear *et al.* 1985, Niederau *et al.* 1985). The subject of iron toxicity and its role in carcinogenesis is still controversial, but some well-recognized chemical reactions which are known to result in free-radical formation may contribute to cellular damage.

In β-thalassemia major, where iron absorption is increased, blood transfusion further contributes to the excessive iron stores. Although the pattern of iron deposition may eventually resemble that seen in genetic HC, there is much more iron in Kupffer cells even in the early stages of iron accumulation (Figure 5).

In the 1960s it was considered by some investigators that all HC was due to excessive alcohol intake. The discovery by Marcel Simon in France that HC was closely linked to

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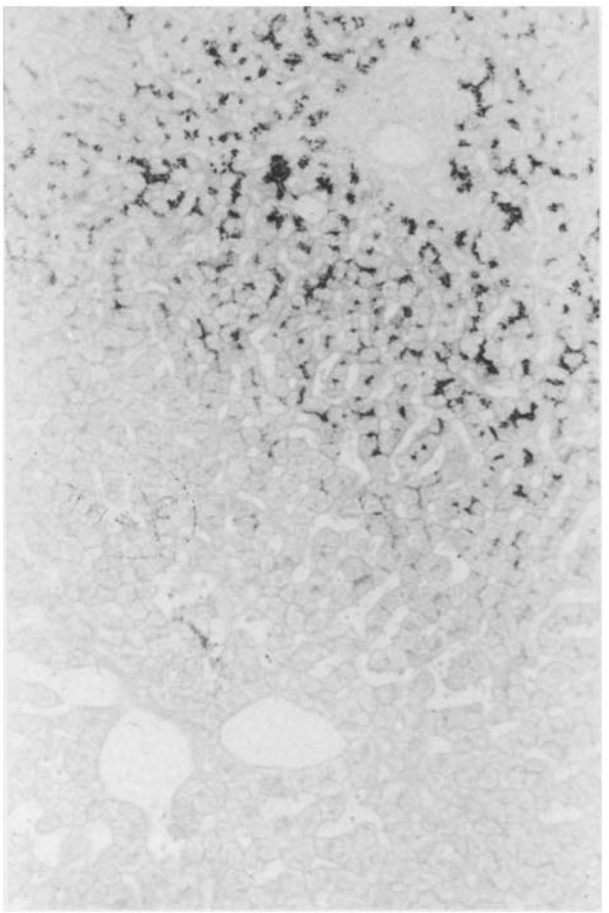

Figure 1. Early homozygous genetic HC in a woman aged 26. Haemosiderin is present in periportal and outer zone hepatocytes with sparing of the centrilobular zone. Perls' stain × 140.

the HLA region (HLA-A) on chromosome 6 established the fact that it was an inherited disease and showed that it was inherited as an autosomal recessive trait (Powell *et al.* 1992). Excessive alcohol intake does occur in approximately 25% of males who are symptomatic at presentation and such subjects are frequently also cirrhotic. Alcohol certainly produces an exaggeration of symptoms and probably an acceleration of liver damage. The biopsy of a patient who drinks alcohol to excess and who is also homozygous for the disease is usually distinguishable from that of a subject who does not imbibe to excess. There is often considerable fat and fibrosis or cirrhosis is associated with patchy parenchymal iron overload as well as clusters of macrophages laden with iron (Searle *et al.* 1994). The diagnosis of genetic HC is ultimately made on liver biopsy and the decision to undertake a biopsy is usually made on the basis of a raised plasma transferrin concentration and/or a raised plasma ferritin concentration. The chemical determination of hepatic iron concentration (HIC) is often diagnostic and its usefulness is enhanced by the calculation of an hepatic iron index (HIC÷age), in which homozygous HC is characterized by an index of 2 or more (Bassett *et al.* 1986, Summers *et al.* 1990).

Animal models of iron overload

In attempts to elucidate the pathways of iron metabolism and toxicity, animal models of iron overload have been used for many years. They have included both dietary and parenteral iron administration.

Parenteral administration of 'Imferon' (iron dextran) produces excessive iron stores which are very heavy in the Kupffer cells but which eventually 'rearrange' (possibly via the hepatic ferritin receptor) to produce heavy iron loading in parenchymal cells also. However, unlike the characteristic pattern seen in genetic HC, the Kupffer cell loading remains very heavy in the Imferon model (Figure 6).

Early studies using dietary administration of iron to animals using ferric or ferrous compounds (such as ferrous sulphate) did not readily produce excessive iron loading. Fe-NTA administration did produce heavy iron loading but was also shown to be very toxic. A major advance occurred with the finding of Bacon, Tavill and colleagues that the oral administration of carbonyl iron (very finely divided iron particles produced by 'sublimation') at a dietary concentration of 2.5% produced very heavy parenchymal iron loading in the rat (Figure 7) (Brittenham *et al.* 1981).

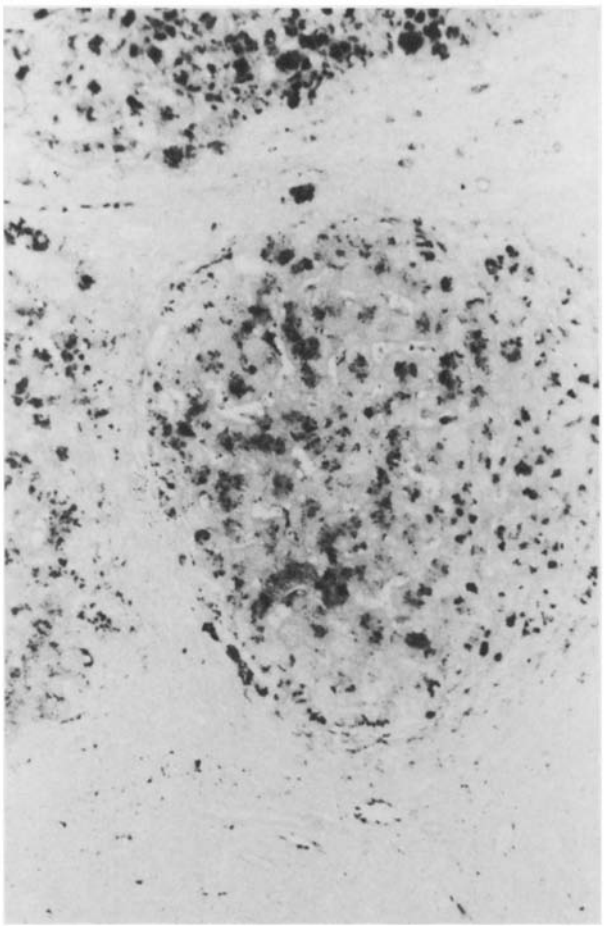

Figure 2. Iron-free focus in non-cirrhotic genetic HC. Some Kupffer cells within the focus contain haemosiderin, but not the hepatocytes. Perls' stain × 140.

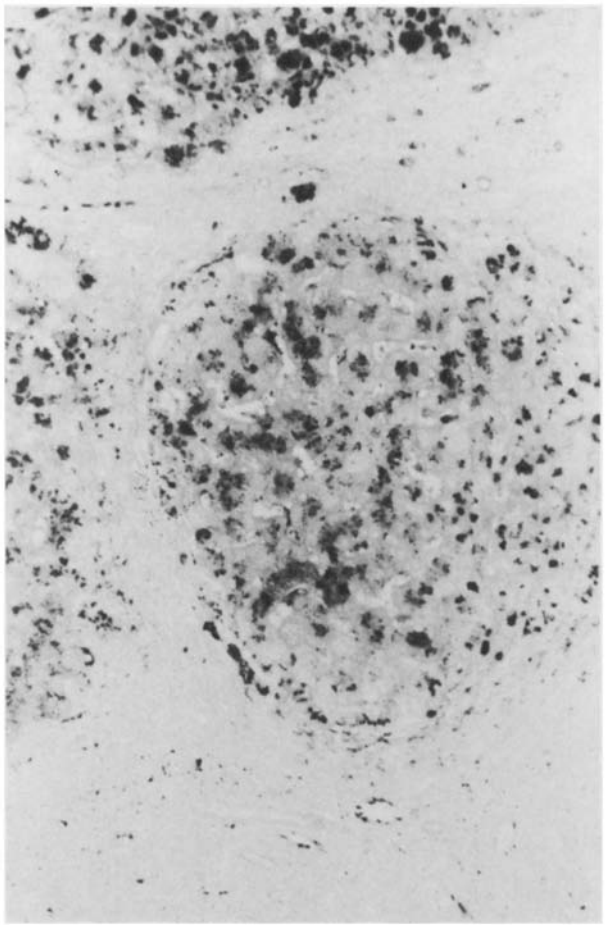

Figure 3. Micronodular cirrhosis in untreated HC. Parenchymal cells are heavily laden with iron, but the fibrous septa contain much less. Perls' stain × 140.

This model has subsequently been used to study iron toxicity, fibrosis and the pathology of iron overload.

Iron toxicity

Our studies in humans have shown that liver damage and fibrosis are frequently produced in the genetic disease when the hepatic iron concentration exceeds 400 μmol d^{-1} dry weight (Bassett *et al.* 1986). Others have suggested that liver damage can occur at a lower iron concentration than this (Sallie *et al.* 1991) and this is certainly true in the presence of other hepatotoxic agents such as alcohol, when fibrosis often *precedes* heavy iron loading.

The mechanism of iron toxicity has been the subject of many studies, notably those of Bacon *et al.* (Bacon *et al.* 1981, 1982, Fletcher *et al.* 1989, Bacon & Britton 1990, Britton *et al.* 1990). Iron is capable of existing in oxidized and reduced forms, and during these interconversions it can act as a potent agent in the production of free-radicals particularly the hydroxyl ($OH^{\bullet}$) radical (Fletcher *et al.* 1989, Bacon & Britton 1990). It has been suggested that iron in close proximity to lipids of organelle membranes results in the production of hydroxyl radicals ($OH^{\bullet}$) which in turn produce lipid peroxidation of organelle membranes. Other free radicals have also been suggested to play a role in iron toxicity.

The development of fibrosis and collagen deposition in areas associated with dense parenchymal cell iron deposits and the impressive clinical, biochemical and histological improvement which follows iron removal has led to the suggestion that iron is a causative agent of fibrosis, and there is now convincing evidence that the presence of iron in parenchymal cells over many years is an important aetiological factor in determining tissue damage, although a causal relation between iron-induced lipid peroxidation and fibrosis has not been firmly established. However, other hitherto undefined factors may also be important in determining the extent of hepatic damage and the particular organs which are affected. It has been suggested that fibrosis and cirrhosis in genetic HC (an iron storage disorder) may result from a direct effect of excess iron on collagen synthesis in the liver (Pietrangelo *et al.* 1990). In a study designed to investigate this in our laboratories, procollagen mRNA levels (types I, III and IV) have been measured in the livers of rats in which chronic iron overload was produced by

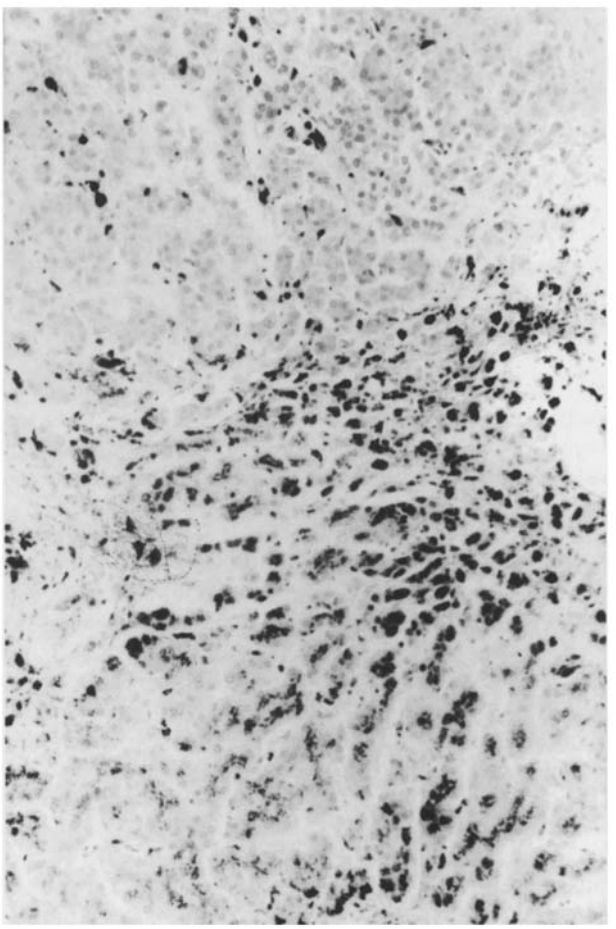

Figure 4. Hepatocellular carcinoma (top) compressing iron-laden liver cells (below) in genetic HC. Some macrophages within the tumour are laden with iron, but the carcinoma cells themselves show no such storage. Perls' stain × 140.

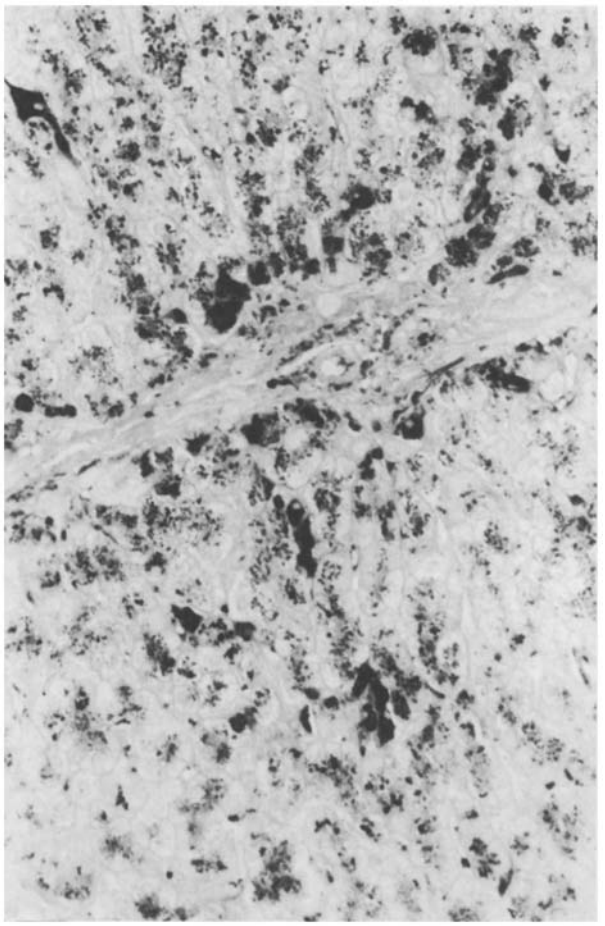

Figure 5. Teenage patient with β-thalassaemia major. Hepatocytes contain haemosiderin, and in addition clusters of macrophages and individual Kupffer cells are also heavily laden with iron. Perls' stain × 210.

feeding dietary carbonyl iron (2.5% w/w) for periods up to 18 months. This resulted in predominantly parenchymal iron deposition in a periportal distribution, similar to that seen in genetic HC. Increased amounts of collagen fibrils in a periportal distribution were observed in most iron-loaded livers under electron microscopy. Although very high hepatic iron concentrations (range 340–1100 μmol g^{-1} dry weight) were achieved in the carbonyl iron-loaded rats, there was no significant difference between steady-state mRNA levels for procollagen types I, III and IV in control and iron-loaded livers examined at five different time points up to 18 months. mRNA levels of the cytokine, transforming growth factor-β1, which has been implicated as playing a major role in the production of extracellular matrix proteins, were also measured in these rat livers. No significant differences were observed between chronically iron-loaded and control livers. These results suggest that excess parenchymal iron does not have a direct effect on the expression of either the procollagens or transforming growth factor-β1 genes in iron-loaded livers and that factors other than, or in addition to iron, appear to be required for fibrosis to occur.

The possibility that haemosiderin is responsible for cell damage has been addressed in numerous of studies by a number of groups (Williams *et al.* 1986, Anderson *et al.* 1988, Mann *et al.* 1988, O'Connell *et al.* 1988, Ward *et al.* 1988, 1989). Another potential source of toxic iron is the postulated intracellular labile iron pool. Many of the metabolic low molecular weight forms of iron suggested to be in this pool are capable of catalysing free radical formation.

The structure of the iron cores in ferritin and haemosiderin produced under different conditions has been the subject of much recent study, and may provide some information as to the mechanism of iron toxicity and also to the mechanisms of iron deposition within cells.

The treatment of iron overload varies with the disease underlying the iron excess. Phlebotomy is the most effective method of removing iron in the genetic disease but chelating agents are required in the iron-loading anaemias. The development of cheap, effective oral iron chelators is currently a subject of high priority and the relative rate of

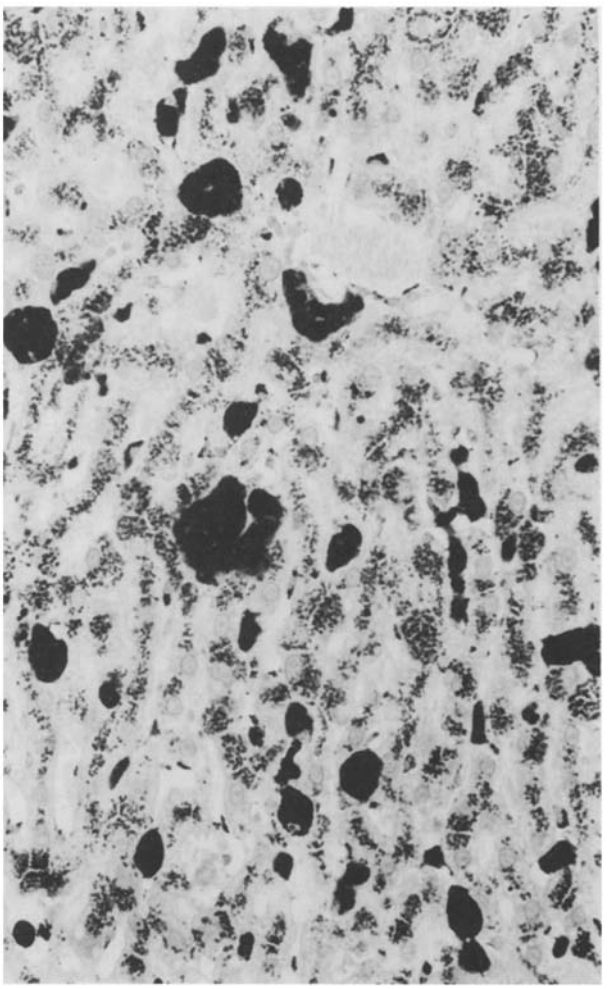

Figure 6. Liver of a rat given repeated intramuscular injections of iron–dextran ('Imferon'). Kupffer cells (singly and in clusters) are heavily laden with haemosiderin, while hepatocytes although significantly iron-loaded, contain much less. Perls' stain × 210.

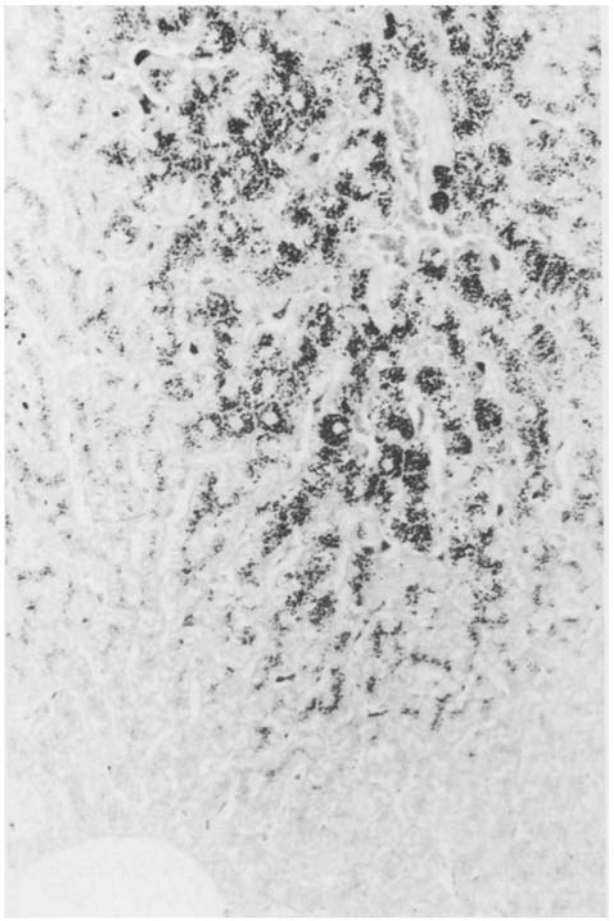

Figure 7. Liver of a rat fed a diet of 2.5% cabonyl iron for 2 months. Periportal and mid-zonal hepatocytes are heavily laden with haemosiderin, with very little in Kupffer cells. Perls' stain × 180.

iron removal from ferritin and haemosiderin stores by such chelators is highly relevant (O'Connell *et al.* 1989).

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