was no change in the expression of the basolateral iron-transport molecules Ireg1 and hephaestin. After FCA administration, hepatic hepcidin levels increased, confirming the inverse relationship between hepcidin and transporter expression observed in the dietary-switch experiment. In the case of APR, however, hepcidin expression changed more rapidly (peaking at 8 h following FCA treatment and returning to normal levels by 48 h) than the expression of iron transporters. The increase also preceded the decline in plasma transferrin saturation, suggesting that hepcidin activation may be a primary event.

Conclusions
Each of the studies described above demonstrates a close inverse relationship between the expression of hepcidin in the liver, iron transporters in the duodenum and iron absorption. The data also indicate that changes in each of these parameters can occur before any alteration in the level of hepatic storage iron or any overt changes in haematological parameters. The only plasma parameter that we measured that reflected these changes was the transferrin saturation, but whether it plays a causative role or simply reflects changes in iron supply to the plasma could not be determined. Our data provide strong circumstancial evidence in support of the proposal that hepcidin is a signalling molecule that acts as a negative regulator of iron absorption. They suggest that hepcidin activation is an early event and that the peptide subsequently acts on the intestinal epithelium to alter the expression of iron-transport molecules. However, the mechanism by which this might be achieved has yet to be resolved.

References

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Down-regulation of liver iron-regulatory protein 1 in haemochromatosis
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Abstract
Cellular iron homoeostasis is maintained by iron sensor proteins known as iron-regulatory proteins (IRPs), which act post-transcriptionally by binding RNA stem-loop structures, termed iron-responsive elements (IREs), present on the mRNAs of proteins involved in iron storage, utilization and transport. IRP1 is a bifunctional protein that can act either as a cytoplasmic aconitase or as an IRE-binding protein. The RNA-binding activity of IRP1 is regulated post-translationally by the insertion or extrusion of a 4Fe–4S cluster, without changes in the levels of protein. In hereditary haemochromatosis (HH) accumulation of iron in parenchymal tissues, including the liver, occurs, possibly through dysfunctional IRP1. Investigation of IRP1 expression in liver biopsies from HH patients showed that the protein is completely absent or markedly reduced in heavily iron-loaded HH patients. Real-time PCR was then conducted in an attempt to investi-
igate the mRNA levels and establish the underlying mechanism behind the disappearing act of IRPl.

The two possibilities are: transcriptional regulation (through the inhibition of transcription) or post-transcriptional regulation (either through increased turnover of protein or inhibition of translation) of IRPl. Preliminary data suggest that transcription of IRPl is not affected by chronic iron overload, and down-regulation may be attributable instead to degradation of the protein.

**Introduction**

In hereditary haemochromatosis (HH), a genetic condition of iron overload affecting approx. 1 in 300 individuals, with 1 in 8–10 being carriers, accumulation of iron in parenchymal tissues, including the liver, occurs [1–3]. The candidate gene for HH, the *HFE* gene, was identified in 1996 through a positional cloning approach and the most common mutation in patients with HH results in a cysteine-to-tyrosine substitution at amino acid 282 (C282Y) [4]. Six years after the discovery of the *HFE* gene, it still remains unclear how iron accumulates in the liver. Since expression of the transferrin receptor is decreased in that tissue, a dysfunctional iron-regulatory protein 1 (IRPl) might be the molecular clue to the iron overloading that characterizes the disease. IRPl is a bifunctional protein that can act either as a cytoplasmic aconitase or as an iron-responsive element (IRE)-binding protein [5]. The RNA-binding activity of IRPl is regulated post-translationally by the insertion or extrusion of a 4Fe–4S cluster, without changes in the levels of protein [6]. Investigation of IRPl expression in liver biopsies from HH patients, by immunohistochemistry and Western blotting, showed that the protein is completely absent or markedly reduced in heavily iron-loaded HH patients. The underlying mechanism behind the disappearing act of IRPl was investigated by real-time PCR. Preliminary data suggest that transcription of IRPl is not affected by chronic iron overload, and down-regulation may be attributable instead to degradation of the protein.

**Materials and methods**

The levels of IRPl were assessed by immunohistochemistry and Western blotting of liver biopsy specimens in both normal and heavily iron-loaded subjects using antisera that have been described previously [7,8]. Control liver samples were obtained from patients that had a hepatic iron concentration of less than 2000 μg of non-haem Fe/g of dry weight of liver and had undergone liver biopsy for other indications. Biopsies with a hepatic iron concentration in the range of 2000–8000 μg of non-haem Fe/g of dry weight of liver were considered mild iron-overload cases, while those in the range of 8000–30000 μg of non-haem Fe/g of dry weight of liver indicated heavy iron overload. Immunostaining was performed on 10 sections from five patients who had undergone venesection to restore iron levels to normal (two sections from each patient representing the before and after venesection states). Western blotting was carried out on five samples (two controls, one mild case and two heavily iron-loaded cases) and the results were reproduced several times. The levels of IRPl mRNA were assessed by real-time PCR in a total of 12 samples (five controls, five heavy cases and two mild) using IRPL-specific primers and expressed in ratios with human β-actin mRNA as a control.

**Results and discussion**

The results of immunohistochemistry (Figure 1) and Western blotting (Figure 2) are consistent
Western blotting of liver samples

Western blotting was performed with rabbit anti-peptide A of human IRP1 antibody against 2 μg of protein from liver samples from two control patients (lanes 1 and 2), one HH patient with mild iron loading (lane 3), and two HH patients with heavy iron loading (lanes 4 and 5).

Figure 2

both between themselves as well as with previous studies suggesting that there is a down-regulation of IRP1 in iron-loaded liver samples [9]. A reduction of IRP1 levels in heavily iron-loaded patients with HH requires rethinking of the predominantly post-translational, iron-sulphur switch mechanism proposed for the iron-mediated regulation of IRP1. The decrease in the levels of IRP1 under extreme iron-loading conditions could be the result of two possible mechanisms: transcriptional regulation (through the inhibition of transcription) or post-transcriptional regulation (either through increased turnover of protein or inhibition of translation) of IRP1. Real-time PCR was employed in the investigation of the above possible mechanisms of the down-regulation of IRP1, by quantifying IRP1 mRNA levels in HH patients and controls. Preliminary results are expressed as a ratio of IRP1 to β-actin mRNA and indicate that samples with a normal iron status have a ratio ranging from 0.76 x 10^{-4} to 1.96 x 10^{-4}, mild iron-loaded samples range from 1.35 x 10^{-4} to 2.69 x 10^{-4}, while heavily iron-loaded samples range from 0.71 x 10^{-4} to 1.81 x 10^{-4}. These values suggest that IRP1 mRNA levels remain relatively constant regardless of iron loading, indicating that transcription of this protein is not affected by iron status.

IRP1 is an RNA-binding protein that post-transcriptionally modulates the expression of mRNAs encoding proteins involved in iron homeostasis. It is well established that the RNA-binding activity of IRP1 is regulated post-translationally by the insertion or extrusion of a 4Fe-4S cluster, without changes in IRP1 levels. However, the possibility of an alternative mechanism of regulation of this protein is suggested from findings that under extreme iron-loading conditions, as in HH, the levels of the protein decrease. The results of the present study confirm a down-regulation of IRP1 under conditions of heavy iron loading and suggest control either at the level of translation or turnover of IRP1 protein. There is no known mechanism by which translation of IRP1 would be selectively inhibited under stress induced by iron overload. There have been reports of iron-induced degradation of IRP1, which is thought to involve haem [10]. However, the molecular mechanism by which iron can induce IRP1 breakdown remains unclear.

References


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