We investigated the effects of the iron regulatory peptide hepcidin on iron transport by the human intestinal epithelial Caco-2 cell line. Caco-2 cells were exposed to hepcidin for 24 hours prior to the measurement of both iron transport and transporter protein and mRNA expression. Incubation with hepcidin significantly decreased apical iron uptake by Caco-2 cells. This was accompanied by a decrease in both the protein and the mRNA expression of the iron-response element containing variant of the divalent metal transporter (DMT1[+IRE]). In contrast, iron efflux and iron-regulated gene 1 (IREG1) expression were unaffected by hepcidin. Hepcidin interacts directly with a model intestinal epithelium. The primary effect of this regulatory peptide is to modulate the apical membrane uptake machinery, thereby controlling the amount of iron absorbed from the diet. (Blood. 2004;104:2178-2180)
In parallel sets of cells we studied the effects of hepcidin on iron transporter expression. Following hepcidin treatment, there was a significant decrease \((P < .01)\) in the membrane expression of DMT1 (+IRE) protein (Figure 1) but no effect on the non-IRE isoform. Protein expression of the efflux transporter IREG1 was also unaffected by hepcidin. Similarly, there was no effect of hepcidin on the expression of the housekeeper protein, villin. The observed changes in iron transporter protein expression were paralleled by alterations in transporter mRNA levels following hepcidin treatment. DMT1 (+IRE) mRNA was significantly decreased \((P < .05)\), whereas DMT1 (non-IRE) and IREG1 expression were unaltered by hepcidin (Figure 2).

A major role for hepcidin in the regulation of iron metabolism was established in studies with knockout mice,\(^ {12,13} \) in which deletion of the hepcidin gene led to the development of a severe iron overload similar to that observed in human haemochromatosis in which injection of hepcidin into mice had no effect on mucosal iron transfer.\(^ {7} \) Thus it is possible that other humoral mediators in the present study we did not observe any effect of hepcidin on IREG1 expression following hepcidin treatment. Quantitative measurements for each transporter mRNA were derived from a standard curve constructed from known amounts of PCR product. Data have been normalized to the expression of the housekeeper gene HPRT and are expressed as means ± SEM from 6 separate experiments.

In the present study we did not observe any effect of hepcidin on IREG1 expression or function and this is supported by our previous work in which injection of hepcidin into mice had no effect on mucosal iron transfer.\(^ {7} \) Thus it is possible that other humoral mediators in addition to hepcidin are required to regulate IREG1 expression.

The prevailing serum hepcidin concentration is unclear. Extrapolations from urinary measurements suggested that circulating hepcidin was within the nanomolar range\(^ {3} \) and was positively correlated with the reduced liver hepcidin mRNA levels correlated with increased intestinal iron absorption and elevated expression of the intestinal iron transporter, DMT1 (+IRE) protein. In our own studies, direct injection of hepcidin into mice decreased specifically the apical uptake step of duodenal iron absorption.\(^ {7} \) In light of these findings, the aim of our present study was to determine whether hepcidin elicited its effects by interacting directly with intestinal epithelial cells. The data presented here demonstrate that hepcidin specifically decreases iron uptake across the apical surface of the Caco-2 epithelial layer, which is consistent with our previous findings.\(^ {7} \) At the molecular level these changes in iron transport are explained by a reduction in DMT1 (+IRE) transporter expression following hepcidin treatment. Interestingly, Frazer et al\(^ {16} \) also showed a correlation between decreased hepcidin and elevated IREG1 expression. In our present study we did not observe any effect of hepcidin on IREG1 expression or function and this is supported by our previous work in which injection of hepcidin into mice had no effect on mucosal iron transfer.\(^ {7} \) Thus it is possible that other humoral mediators in addition to hepcidin are required to regulate IREG1 expression.
correlated with serum ferritin levels. However, a recent study indicated that serum levels could be much higher, in the mid- to high-micromolar range. The concentration of human synthetic hepcidin used in our study (10 \(\mu\)M) sits between these 2 extremes. As noted previously, our synthetic hepcidin contains a mixture of species including one or more active forms. At present the nature of the circulating active form of hepcidin remains unclear. The effects of hepcidin observed in the present study are unlikely to be due to toxicity since there was no effect of peptide incubation on monolayer transepithelial resistance or paracellular permeability (data not shown).

In conclusion, our data provide the first direct evidence that hepcidin can regulate intestinal iron absorption by interacting with a model intestinal epithelial cell line. The primary effect of hepcidin is to modulate the apical membrane uptake machinery, thereby controlling the amount of iron absorbed from the diet.

References

Inhibition of iron transport across human intestinal epithelial cells by hepcidin

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