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POSTER ABSTRACTS

102.IRON HOMEOSTASIS AND BIOLOGY

Iron Chelation Efficiency in Human Hepatocytes Is Enhanced By Exogenous Hepcidin

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Iron chelation is clinically inefficient, with only a small percentage of the applied chelator being excreted in the iron-bound form. Chelation is also slow because only a small proportion of storage iron is in the chelatable labile iron pool (LIP) at any given moment. In principle, by increasing the magnitude of the LIP within hepatocyte, the major site of body storage iron in conditions of iron overload, the magnitude and hence the efficiency of chelation may be increased. Recent work has shown that SLN124, a RNAi-therapeutic inhibitor of Tmprss6 gene expression, increases iron chelation by deferiprone in a murine model of thalassemia (b-thalassemic Hbb $^{th3/+}$ animals) (Vadolas et al Brit J Haematol 2021) and murine hereditary haemochromatosis (Altamura et al. HemaSphere 2019). We wished to determine whether the mechanism of increased chelation was consistent with the blocking of iron exit from hepatocyte through ferroportin channels by increased levels of hepcidin. Here we have examined whether application of exogenous hepcidin to a human hepatocyte cell line HepG2 can increase the chelation efficiency of deferiprone and whether this effect is also seen with other iron chelators such as deferasirox and deferoxamine. A model was developed from previous work (Vlachodimitropoulou et al. Blood 2017) where the net effect of iron uptake (from fetal bovine serum medium) versus intracellular iron removal by chelation, could be interrogated. The dose and time-dependent effect of exogenous hepcidin and its interaction with iron chelators on intracellular iron content was examined. Briefly, to load cells with iron, confluent HepG2 cells were incubated for 2h with 10% FBS followed by media change containing 10-30 uM iron binding equivalents (IBE) of therapeutic chelators, hepcidin, or control media. This loading method doubled the cellular iron; and subsequently increased cellular ferritin by at least 4-fold. Both effects could be completely inhibited with iron chelation.

Following a pulse with hepcidin (100nM-10μM), intracellular iron content of HepG2 cells increased in a dose dependent manner, by 30-100% over the next 4h, followed by a decrease in cellular iron at 8 hours (Fig. 1A). This transient effect on iron retention could be prolonged with serial addition of hepcidin to the media (at 2 and 4h, data not shown). Co-treatment with the exclusively extracellular chelator CP40 demonstrated that while a substantial fraction of the hepcidin effect on iron retention was dependent on iron uptake from media (Fig 1B), the remaining increase in iron retention was independent of this effect and consistent with inhibition of iron egress from . Compared with control cells (without chelator but with or without hepcidin), a 50-200% enhancement of hepatocyte iron removal by 10uM IBE deferiprone was observed at 2 and 4 hours (Fig. 1C) with the addition of 300nM exogenous hepcidin. Correspondingly, cellular iron removal with deferoxamine (10-30 μM IBE) was enhanced by 250% at 4 h by exogenous hepcidin (Fig. 1D) whereas iron removal by deferasirox (30μM IBE) was enhanced by at least 50% at 2 and 4 h when hepcidin (300nM) was present (Fig. 1E), as compared to controls. Enhanced chelation with DFO was not observed until after 2h incubation, consistent with the known slower access of DFO to intracellular pools compared with deferiprone or deferasirox. Beyond 4h, the effect of hepcidin on enhanced chelation was not seen, suggesting that hepcidin is consumed or degraded in this culture system.

We conclude that enhanced removal of intracellular iron by chelation is demonstrable with all three clinically available chelators following the application of exogenous hepcidin. This iron would be destined for increased excretion of chelate complexes in vivo and hence enhanced decrements in body iron. Two mechanisms for enhanced chelation are possible in this model; the first is by increased iron loading of hepatocytes incubated with hepcidin and secondly through the blocking by hepcidin of labile intracellular iron egress through ferroportin channels, thereby increasing the magnitude of chelatable in-

tracellular iron pools. Future work in primary human hepatocytes will examine whether manipulation of intracellular hepcidin levels by SLN124 has similar chelation-enhancing effects.

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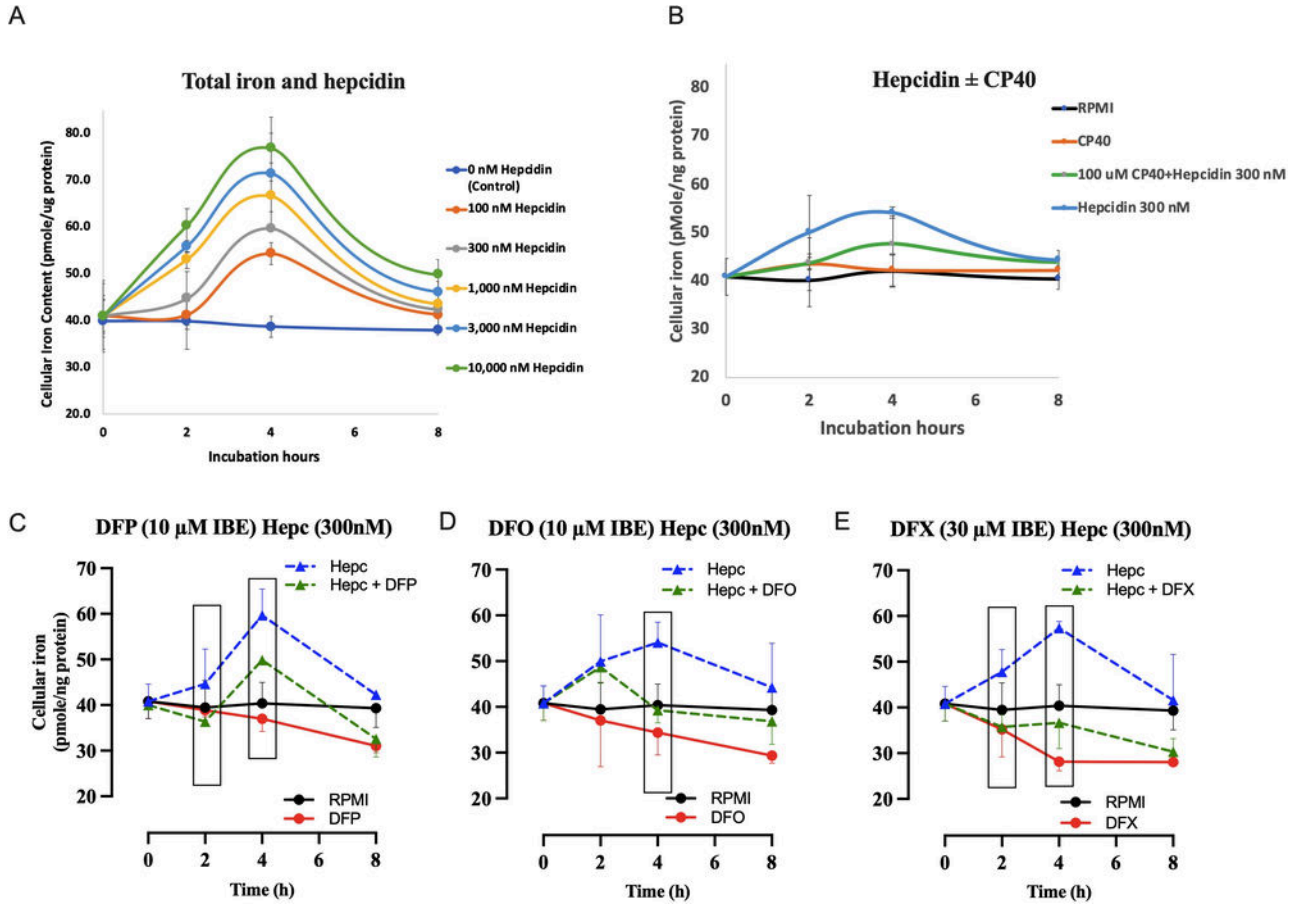

Figure 1

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