Abstract: S283

Title: THE NON-ANTICOAGULANT HEPARINOID COMPOUND, SEVUPARIN, STRONGLY REDUCES HEPCIDIN EXPRESSION IN CELLS, IN MICE AND IN HEALTHY VOLUNTEERS

Abstract Type: Oral Presentation

Session Title: Iron pathophysiology in hematological disorders - Part 1

Background:

Hepcidin is the master regulator of systemic iron, and its levels in the bloodstream directly reflect the iron availability due to the potency by which hepcidin controls the iron exporter ferroportin. High levels of hepcidin induce the degradation of ferroportin, inhibiting iron release from enterocytes and macrophages resulting in iron deficiency and subsequently the onset of anaemia commonly seen in inflammatory and chronic diseases. Normalising hepcidin levels is therefore a key element of treating this type of anaemia.Previous data have shown that heparinoids are potent suppressors of hepcidin but are limited in their use on account of their anticoagulant properties. Sevuparin is a novel, clinical stage, low-molecular weight heparinoid with substantially reduced anticoagulant activity making it a promising candidate drug to control hepcidin expression in patients.

Aims:

The aim of the present work was to investigate the effect of sevuparin on hepcidin *in vitro* and *in vivo* both in mice and healthy volunteers

Methods:

The effect of sevuparin was analysed *in vitro* in HepG2 cells, with dose- and time-dependent experiments and in mice to evaluate the levels of hepcidin and SMAD phosphorylation. A randomized double-blind, placebo-controlled single escalating dose study was conducted in healthy volunteers in which the effects on hepcidin levels were assessed.

Results:

Sevuparin strongly suppressed basal and BMP6-induced hepcidin expression in HepG2 cells, in a dose- and time-dependent manner. The strong inhibition of hepcidin level by sevuparin 3.6 ug/mL was evident starting from 2h post treatment (with a reduction of about 50 %) reaching a maximum effect after 6h (90% reduction) and still present after 16h (Figure 1A). Its mechanism of action seems to interfere with the BMP/SMAD pathway as suggested by the reduction of basal and BMP6-induced SMAD phosphorylation. The hepcidin lowering effect of sevuparin was confirmed also in mice with a 70% reduction of hepcidin mRNA after 6h. Interestingly, in HepG2 cells, sevuparin also suppressed the upregulation of hepcidin due to IL6 and the Jak/STAT3 pathway. IL6 increased the hepcidin level about 2.8-fold and sevuparin completely suppressed this stimulus, dampening IL6-induced hepcidin under the basal level by interfering selectively with the BMP/SMAD pathway without affecting the pSTAT3 induction. Furthermore, in healthy volunteers, subcutaneously administered sevuparin caused a significant reduction of about 80% (9 mg/kg, Figure 1B) and 50% (3 mg/kg) of serum hepcidin after 12 and 24 h, respectively, with human *in vivo* IC_{50} values reflecting those in cells, providing a robust translational context to the sevuparin effects on hepcidin levels.

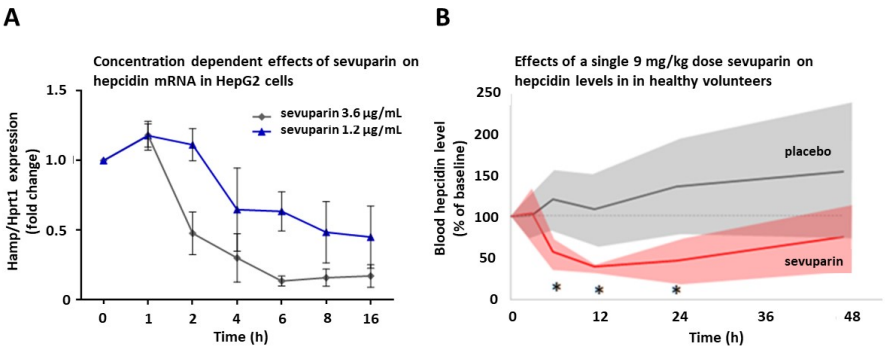

Figure 1: A HepG2 cells were treated with 1.2 or 3.6 µg/mL sevuparin at different time of exposure (1–2–4-6-8-16 h). The level of hepcidin mRNA was evaluated by qRT-PCR normalized to HPRT1 mRNA. The values are expressed as fold change over the untreated cells (0). Data are shown as means and SD of 3 different experiments (n=3). **B** Mean change from baseline in circulating hepcidin levels following a single subcutaneous dose of sevuparin at 9 mg/kg in healthy human volunteers. All data normalized to baseline; the solid line represents mean values, while the shaded areas represent 95% confidence intervals; * denotes $p<0.05$ (two-tailed t-test, unequal variance).

Summary/Conclusion:

The results are promising and support further mechanistic and clinical studies to elucidate a therapeutic role for sevuparin as a hepcidin inhibitor in anaemia of inflammatory and/or chronic diseases.

Keywords: Iron metabolism, Hepcidin, Heparin, Anemia