• 2014 Aug;28(4):671-81
• 2014 Aug;28(4):671-81
• 2014 Aug;28(4):671-81

EHA EUROPEAN HEMATOLOGY ASSOCIATION

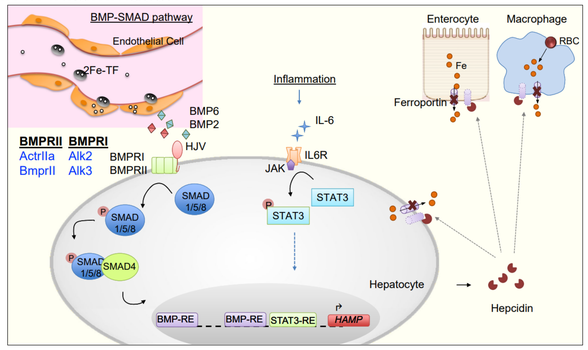
ALK2 INHIBITION LOWERED HEPCIDIN AND LIBERATED SPLEEN IRON FOR ERYTHROPOIESIS IN ANEMIA OF INFLAMMATION

KEROS THERAPEUTICS

C. Wang, N. Medeiros, E. Melgar-Bermudez, T. Nurse, E. Lema, C. Tseng, FM. Fisher, J. Seehra, J. Lachey, Keros Therapeutics, Lexington, MA, USA

INTRODUCTION

Persistent inflammation in chronic disease induces the iron homeostasis regulator hepcidin to retain tissue iron and limit circulating iron, leading to anemia, which is known as anemia of inflammation[1] (AI). Iron and inflammation regulate hepcidin via the bone morphogenetic protein (BMP)-SMAD and IL6-STAT3 pathway, respectively[2]. ALK2 is one of the BMP type I receptors which function collaboratively with the type II receptors and coreceptor HJV to govern hepcidin production[3]. We developed two investigational neutralizing antibodies against ALK2, KTI-m216 (m216) and KTI-m218 (m218), with unique variable regions, but observed to have similar affinity for ALK2 and effect on hepcidin suppression in mice and monkeys. Here, we examined their capability and mechanism of action in suppressing hepcidin in inflammation.



OBJECTIVE

To investigate the mechanism of ALK2 inhibition-mediated hepcidin suppression in acute inflammation and chronic AI mouse models.

METHODS

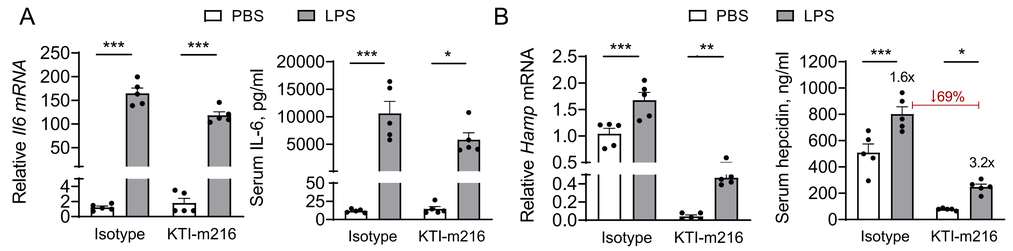
Acute: C57BL/6 mice at 7 weeks were subcutaneously (SQ) dosed with 3 mg/kg m216 or isotype (Ctrl) for 1 h, followed by an intraperitoneal injection of 1 mg/kg lipopolysaccharide (LPS) or PBS for 6 h.

Chronic AI: Modified AIN-93G diet containing 0.2% adenine and 40 ppm iron was used to induce chronic kidney disease (CKD) in C57BL/6 mice in 6-7 weeks. After disease induction, mice were SQ treated with 3 mg/kg m218 or isotype twice per week and 1) continued on adenine diet with 40 ppm iron for 3 weeks or 2) switched to adenine diet with 3 ppm iron for 9 days.

RESULTS

LPS induced liver *Il6* mRNA expression and IL-6 secretion in control mice and mice treated with m216.

Serum hepcidin had a similar fold increase in mice receiving isotype-LPS and m216-LPS, compared to the respective PBS controls, but m216-LPS mice had a 69% reduction in absolute hepcidin compared to isotype-LPS controls.



LPS increased pSTAT3, but not pSMAD5, indicating that LPS induced hepcidin via IL6-STAT3 and not BMP-SMAD pathway. By contrast, pSMAD5 was decreased in mice receiving KTI-m216, suggesting that m216-mediated hepcidin suppression is driven by inhibiting the SMAD signaling.

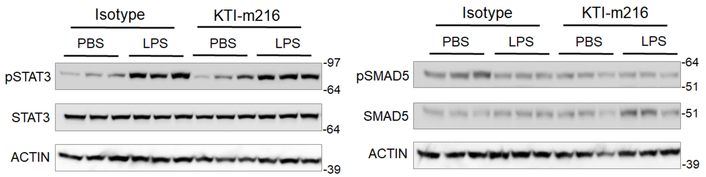


Figure 1. Tissues from mice that underwent acute inflammation (n=5 per group) were collected to examine (A-B) liver mRNA expression and serum levels of IL-6 (A) and hepcidin (B). Transcripts were quantified by qRT-PCR and normalized to an internal control *Rpl19*. (C) Liver phosphorylated proteins STAT3 and SMAD5 were determined by Western blots. Values represent mean ± SEM. **P*<0.05, ***P*<0.01, ****P*<0.001 relative to the respective control by two-way ANOVA with Sidak's posthoc test.

CKD mice developed characteristics of AI, including anemia, low-grade inflammation, increased serum hepcidin, hypoferremia, and tissue iron retention.

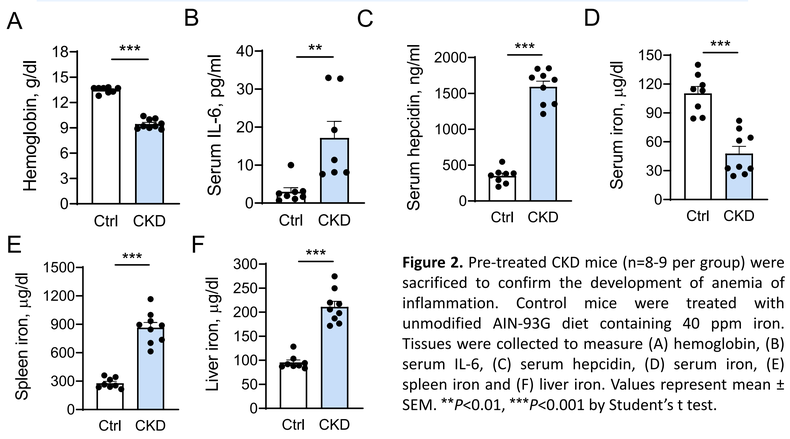
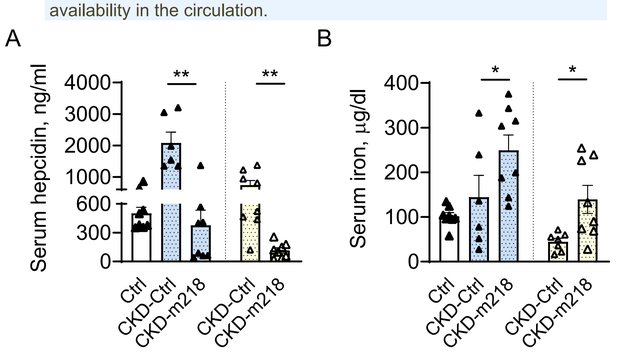


Figure 2. Pre-treated CKD mice (n=8-9 per group) were sacrificed to confirm the development of anemia of inflammation. Control mice were treated with unmodified AIN-93G diet containing 40 ppm iron. Tissues were collected to measure (A) hemoglobin, (B) serum IL-6, (C) serum hepcidin, (D) serum iron, (E) spleen iron and (F) liver iron. Values represent mean ± SEM. ***P*<0.01, ****P*<0.001 by Student's t test.

CONCLUSIONS

1. KTI-m216 and KTI-m218 at 3 mg/kg reduced serum hepcidin in healthy control animals and/or mice with acute and chronic inflammation.
2. In inflammation, m216 did not inhibit IL6-STAT3 signaling, but inhibited BMP-SMAD signaling to lower overall serum hepcidin production.
3. Data suggest that ALK2 inhibition-mediated hepcidin suppression was sufficient to improve erythropoiesis by liberating iron from the recycling pathway in a mouse model of anemia of inflammation.

KTI-m218 supported erythropoiesis in the CKD mice by lowering hepcidin production to liberate spleen iron and increase iron availability in the circulation.



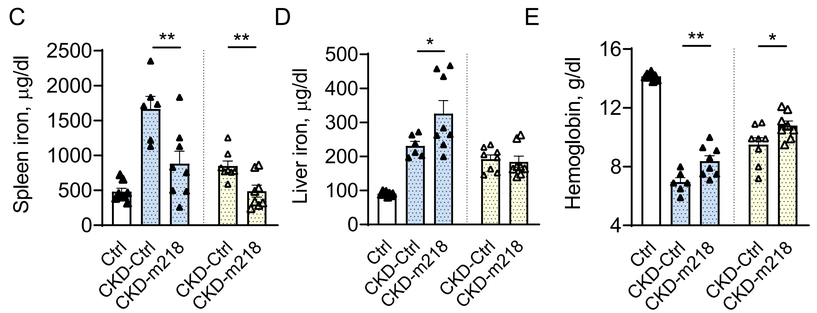


Figure 3. CKD mice (n=6-8 per group) were treated with KTI-m218 or isotype control for 3 weeks on a 0.2% adenine 40 ppm iron diet (blue bars). In a separate experiment, mice were treated for 9 days on a 0.2% adenine low 3 ppm iron diet (yellow bars). (A) Serum hepcidin, (B) serum iron, (C) spleen iron, (D) liver iron, and (E) hemoglobin levels were examined. Values represent mean ± SEM. **P*<0.05, ***P*<0.01 relative to the respective control by one-way ANOVA with Tukey's posthoc test or Student's t test.

REFERENCES

1. Nemeth E, Ganz T. Anemia of inflammation. *Hematol Oncol Clin North Am*. 2014 Aug;28(4):671-81.
2. Wang CY, Babitt, JL. Hepcidin regulation in the anemia of inflammation. *Curr Opin Hematol*. 2016 May;23(3):189-97.
3. Steinbicker AU, Bartnikas TB, Lohmeyer LK, et al. Perturbation of hepcidin expression by BMP type I receptor deletion induces iron overload in mice. *Blood*. 2011;118(15):4224-4230.

CONTACT INFORMATION

Justin Frantz
SOLEBURY TROUT
jfrantz@soleburytrout.com
+1 (617) 221- 9100