Effect of the antihepcidin Spiegelmer lexaptepid on inflammation-induced decrease in serum iron in humans

Lucas T. van Eijk,1,2 Aaron S. E. John,1,2 Frank Schwoebel,3 Luciana Summo,3 Stéphanie Vauléon,3 Stefan Zöllner,3 Coby M. Laarakkers,2,4,5 Matthijs Kox,1,2,6 Johannes G. van der Hoeven,1,2 Dorine W. Swinkels,2,4,5 Kai Riecke,3 and Peter Pickkers1,2

1Department of Intensive Care Medicine, and 2Radboud Institute for Infectious Diseases, Radboud University Medical Center, Nijmegen, The Netherlands; 3NOXXON Pharma AG, Berlin, Germany; 4Department of Laboratory Medicine, Laboratory of Genetic, Endocrine and Metabolic Diseases, Radboud University Medical Center, Nijmegen, The Netherlands; 5Hepcidinanalysis.com, Nijmegen, The Netherlands; and 6Department of Anesthesiology, Radboud University Medical Center, Nijmegen, The Netherlands

Key Points

- Lexaptepid modulates the inflammation-induced decrease in serum iron during experimental human endotoxemia.
- Hepcidin targeting with the novel compound lexaptepid may be a viable approach to the treatment of anemia of inflammation in humans.

Introduction

Anemia of inflammation is the most prevalent form of anemia in a hospital setting, occurring in a variety of acute and chronic inflammatory diseases. The dysregulation of iron homeostasis is central to the pathogenesis of anemia of inflammation. A redistribution of iron to the cells of the reticuloendothelial system occurs, with hepcidin as a central mediator. Hepcidin induces internalization and degradation of the iron-exporting channel ferroportin on duodenal enterocytes and iron-storing cells, thereby preventing the export of iron into the circulation and causing iron-restricted erythropoiesis. Hence, hepcidin inhibition may be an attractive therapeutic intervention to improve the utilization of iron from intracellular stores.

Lexaptepid (international nonproprietary name: lexaptepid pegol), a nonnatural mirror-image L-oligoribonucleotide modified at its 5′ end with a branched 40 kDa monomethoxy-polyethylene-glycol, inactivates hepcidin in a mode conceptually similar to antibodies. Lexaptepid, as a nonnatural mirror-image L-nucleotide modiﬁcation enabling investigations in the treatment of anemia of inflammation. This trial was registered at www.clinicaltrial.gov as #NCT01522794. (Blood. 2014;124(17):2643-2646)

Materials and methods

Experimental human endotoxemia

This double-blind, randomized, placebo-controlled trial was approved by the local ethics committee and carried out according to good clinical practice standards and the declaration of Helsinki, including current revisions. The study was registered at clinicaltrial.gov as #NCT01522794.

Experimental human endotoxemia is a generally accepted way of studying systemic inflammation in a controlled manner. Following written informed consent and screening, 24 healthy male subjects were prehydrated with 1.5 L NaCl 0.45%/glucose 2.5%. At T = 0 hours, 2 ng/kg Escherichia coli lipopolysaccharide was intravenously administered, followed by an intravenous injection of 1.2 mg/kg lexaptepid or placebo at T = 0.5 hours. The lipopolysaccharide-induced inflammatory response was similar in subjects treated with lexaptepid or placebo regarding clinical and biochemical parameters. At T = 9 hours, serum iron had increased by 15.9 ± 9.8 μmol/L from baseline in lexaptepid-treated subjects compared with a decrease of 8.3 ± 9.0 μmol/L in controls (P < .0001). This study delivers proof of concept that lexaptepid achieves clinically relevant hepcidin inhibition enabling investigations in the treatment of anemia of inflammation. The primary aim was to investigate the effectiveness of lexaptepid in preventing the serum iron decrease observed during systemic inflammation in healthy volunteers. The secondary objective was to assess the effects of lexaptepid on the innate immune response.
In animal models, including an IL-6-induced anemia model in cynomolgus monkeys. In the present study, we extend the concept of hepcidin inhibition to humans for the first time.

No differences in baseline parameters between the treatment groups were found (supplemental Table 1). Lexaptepid was well tolerated. Maximum lexaptepid plasma concentrations (Cmax) of 1.96 ± 0.19 μmol/L were measured at 0.7 ± 0.4 hours after administration (Figure 1A), after which levels declined in a biphasic manner (initial elimination half-life of 9.7 ± 3.6 hours, mean terminal elimination half-life of 22.5 ± 4.3 hours).

In placebo-treated subjects, LPS administration resulted in an increase in plasma hepcidin, peaking at T = 6 hours with maximum concentrations of 23.0 ± 5.2 nmol/L, followed by a gradual decrease reaching baseline levels at T = 24 hours (Figure 1B). In the lexaptepid-treated group, total hepcidin levels increased more rapidly and extensively (maximum levels: 547 ± 117 nmol/L, peak at T = 9 hours). This may largely be attributed to a reduced elimination of the lexaptepid-hepcidin complex through the kidney due to increased size and charge as also reported for an antihepcidin antibody. In a previous phase 1 study, constant concentration increases of hepcidin after increasing doses of lexaptepid from 0.3 to 4.8 mg/kg indicated that the administration of lexaptepid did not induce hepcidin production by itself. In addition, hepcidin production may have been influenced by the major changes in serum iron as observed throughout the experiment.

There was a marked induction of serum iron in both the lexaptepid-treated and placebo group at 3 and 6 hours after endotoxin administration (rise to 203% ± 13% and 221% ± 22% from baseline respectively in the lexaptepid treated group, versus 181% ± 14% and 146% ± 14% in the placebo (Figure 1C,D and supplemental Figure 1). This immediate increase was previously observed during experimental endotoxemia and may be due to an acute inflammation-induced abrogation of erythropoiesis and iron uptake by the bone marrow, whereas the export of iron from iron storing cells persists as long as ferroportin is expressed. In concordance,

**Results and discussion**

As hepcidin is the principal regulator of iron homeostasis, it has become a target for the development of novel therapeutics for iron disorders. Lexaptepid has proven effective in counteracting hepcidin’s effects in animal models, including an IL-6-induced anemia model in cynomolgus monkeys. In the present study, we extend the concept of hepcidin inhibition to humans for the first time.

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serum iron levels were comparable in both groups at T = 3 hours and started to decline in the placebo-treated subjects concurrent with the increase of hepcidin concentrations. In contrast, lexaptepid administration resulted in a prolonged increase in serum iron, peaking at T = 6 hours. The transient LPS-induced hypoferremia was effectively prevented by lexaptepid. Accordingly, the predefined primary end point, change in serum iron concentration 9 hours after LPS administration, was met (P < .0001). At this time point, no differences between groups were found in changes in hematologic parameters and cytokine levels (supplemental Table 2). A total of 24 to 48 hours after LPS administration, serum iron was actually lower in the lexaptepid-treated group compared with controls. We suspect that the prolonged and strongly elevated serum iron concentrations may have stimulated hepcidin production to overcome the effect of lexaptepid, eventually resulting in hepcidin-induced hypoferremia. We do not anticipate that treatment of patients with anemia of chronic disease and functional iron deficiency, who typically have serum iron concentrations below 10 μmol/L, would react with similarly high increases in serum iron concentrations when treated with lexaptepid, and we therefore do not consider this observation as predictive for actual patients. Currently, a phase 2 trial (clinicaltrials.gov #NCT01691040) investigates whether lexaptepid is able to elevate hemoglobin in cancer patients, and initial results from a pilot phase are encouraging.19

Although an increase over time was noted for both total iron-binding capacity and plasma ferritin concentration after endotoxin administration, no differences between groups were observed (supplemental Figure 2A,B). No effect of lexaptepid was observed on innate immunity. Laboratory parameters of inflammation (Figure 2A-F) and inflammatory signs and symptoms (supplemental Figure 3A,B) caused by LPS were not different between the groups.

In conclusion, the antihepcidin treatment with lexaptepid was well tolerated and effective in blocking the inflammation-induced reduction in serum iron during systemic inflammation in humans. It therefore represents an interesting compound for the future treatment of anemia of inflammation.
Acknowledgments

This study was supported by research funding from NOXXON Pharma AG, Berlin, Germany and the German Federal Ministry of Education and Research (BMBF) through a grant for small and midsize companies (KMU-innovativ-7).

Authorship

Contribution: Data were analyzed by L.T.v.E., F.S., S.V., S.Z., M.K., and K.R., and all other authors had access to the primary data; L.T.v.E. codesigned the study, conducted the endotoxemia experiments, analyzed the data, interpreted findings, and wrote the manuscript; A.S.E.J. participated in the execution of the experiments and gathering and corrected the manuscript; F.S. contributed to designing the study, interpreting findings, and reviewing the manuscript; L.S. took part in designing the study and gathering and cleaning of the data; S.V. and S.Z. planned, performed, and interpreted the pharmacokinetics, monitored hepcidin measurements, and corrected the manuscript; C.M.L. developed and validated an assay for total plasma hepcidin-25, performed the hepcidin measurements, and corrected the manuscript; M.K. analyzed the study data results and corrected the manuscript; J.G.v.d.H. interpreted findings and edited the manuscript; D.W.S. took part in the design of the study, supervised measurements of hepcidin and development of an assay for total hepcidin, interpreted findings, and edited the manuscript; K.R. took part in designing the study, analyzing the results, and correcting the manuscript; and P.P. was primary investigator, supervised the endotoxemia experiments and the data analyses, and corrected the manuscript.

Conflict-of-interest disclosure: The study was sponsored by NOXXON Pharma AG, Berlin, Germany. F.S., L.S., S.V., S.Z., and K.R. are employees of NOXXON Pharma AG. D.W.S. and C.M.L. are involved in the hepcidinanalysis.com initiative, by which the Radboud University Medical Center offers high-quality hepcidin measurements to the scientific and medical community. The remaining authors declare no competing financial interests.

Correspondence: Peter Pickkers, Department of Intensive Care Medicine (710), Radboud University Medical Center, PO Box 9101, 6500 HB, Nijmegen, The Netherlands; e-mail: peter.pickkers@radboudumc.nl.

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