Toll-like receptor 4 inhibition restores cytochrome C oxidase mitigating hyperammonemia-induced hepatocyte mitochondrial dysfunction

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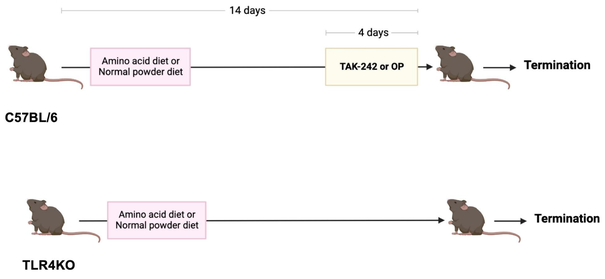
Introduction & Aim

- Hyperammonemia has been demonstrated to induce mitochondrial dysfunction in hepatocytes, with a particular impact on oxidative phosphorylation (OXPHOS).[1,2]
- While inhibition of Toll-like receptor 4 (TLR4) has shown potential in preserving mitochondrial stability, supporting urea cycle enzyme (UCE) function, and reducing oxidative stress, the underlying mechanisms remain unclear.
- This study investigates how TLR4 inhibition can restore mitochondrial function under hyperammonemic conditions.

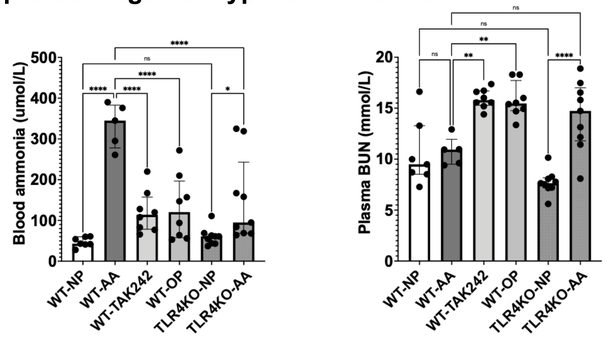
Methods



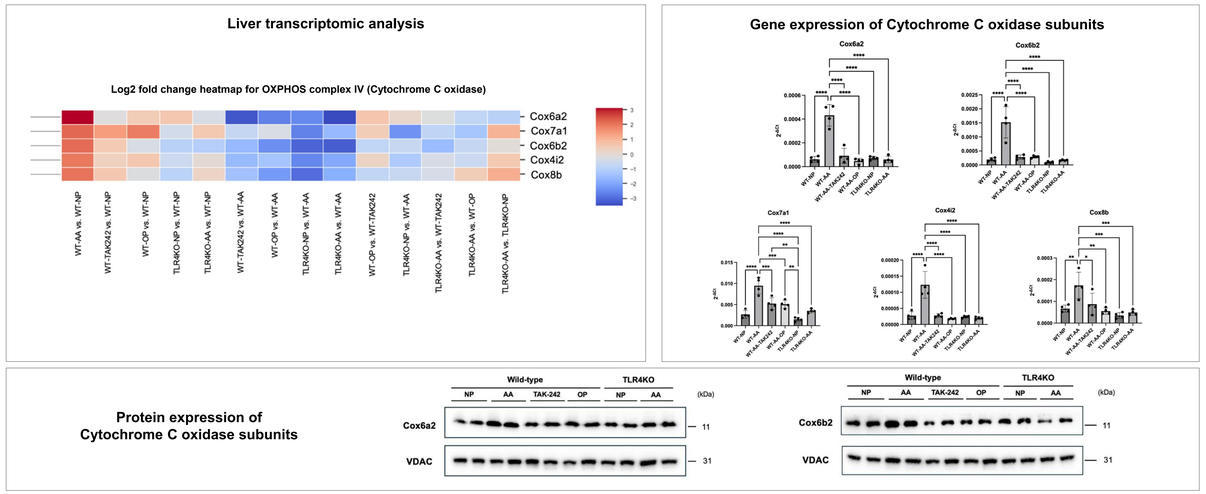
- Hyperammonemia was induced in wild-type (WT) and TLR4 knock-out (TLR4KO) mice via a 14-day amino acid (AA) diet.
- WT-AA mice were treated with either the TLR4 antagonist, TAK-242 (10 mg/kg i.p.) or the ammonia scavenger Ornithine Phenylacetate (OP, 300 mg/kg i.p.) from days 10 to 14.
- Liver transcriptomic analysis was performed using whole RNA sequencing, with specific focus on **OXPHOS genes**. Data were analysed with KEGG and Gene Ontology databases.
- Quantitative activity of cytochrome C oxidase in isolated hepatocyte mitochondria was assessed using spectrophotometric enzyme assay.

Main findings

1 TLR4 inhibition protects against hyperammonemia.

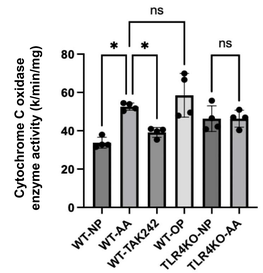


2 Among all OXPHOS complexes, only complex IV (Cytochrome C oxidase) was significantly affected by hyperammonemia, which was normalised following TAK-242 treatment.



3 Mitochondrial cytochrome C oxidase activity assays further confirmed significantly elevated enzyme activity in WT-AA mice compared to controls, which was significantly reversed by TAK-242.

4 TLR4 knock-out mice showed no changes in cytochrome C oxidase gene or protein expression, nor in enzyme activity, regardless of the AA diet.



Conclusions

- Hyperammonemia drives mitochondrial dysfunction by selectively upregulating cytochrome C oxidase subunits, which are critical for mitochondrial OXPHOS function.
- TLR4 inhibition effectively mitigates this dysregulation, stabilising mitochondrial bioenergetics and preserving UCE activity.
- These results suggest that TLR4 antagonist may offer a promising therapeutic approach for hyperammonemia by restoring cytochrome C oxidase regulation.

Abbreviations: AA, amino acid; COX, Cytochrome C oxidase; KO, knock-out; NP, normal protein; OP, Ornithine Phenylacetate; TAK242, toll-like receptor 4 inhibitor; TLR4KO, TLR4 knock-out; WT, wild-type

References

1. Kerbert A et al. *Sci. Adv. 2025;*11(10):eado1648
2. Sriphoosanaphan S et al, EASL Congress 2024

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