Protective effects of adiponectin receptor agonists in an *in vitro* model of endoplasmic reticulum stress in equine laminitis

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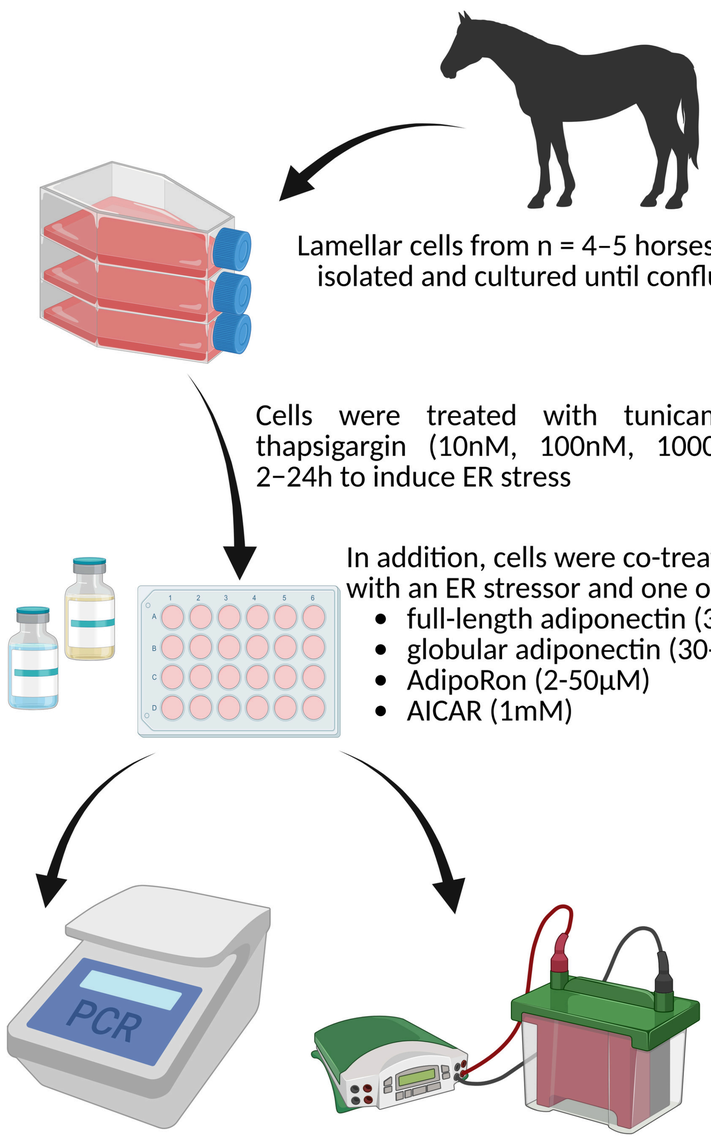
Introduction

- Lamellar endoplasmic reticulum (ER) stress is reported in naturally occurring endocrinopathic laminitis[1]
- Adiponectin is associated with a reduced risk of laminitis[2]
- In other species, adiponectin inhibits ER stress[3] and is associated with better outcomes in patients with sepsis[4]

Aims

1. Develop a model of ER stress in equine lamellar cells
2. Investigate the potential of adiponectin receptor agonists to reduce ER stress

Methods



The expression of the following was determined using PCR and western blotting:

- binding immunological protein (BiP), as a marker of ER stress
- interleukin (IL)-6 and IL-8

Conclusions and implications

- Equine lamellar ER stress was induced using two pharmacological agents
- ER stress was associated with upregulation of IL-6 and IL-8, which are implicated in laminitis pathogenesis
- Co-treatment with adiponectin receptor agonists reduced the expression of BiP
- Modulating adiponectin *in vivo* may be useful in the treatment and/or prevention of laminitis
- Further work is necessary to determine whether low adiponectin predisposes to poor outcome, including laminitis, in SIRS cases

References

[1]Cassimeris, L., Engiles, J. B. & Galantino-Homer, H. Detection of endoplasmic reticulum stress and the unfolded protein response in naturally-occurring endocrinopathic equine laminitis. BMC Vet Res 15:24, 1-16 (2019).

[2]Menzies-Gow, N. J., Harris, P. A. & Elliott, J. Prospective cohort study evaluating risk factors for the development of pasture-associated laminitis in the United Kingdom. Equine Vet J 49, 300–306 (2017).

[3]Hou, Y. et al. Adiponectin is protective against endoplasmic reticulum stress-induced apoptosis of endothelial cells in sepsis. Brazilian Journal of Medical and Biological Research 51 (12), 1-10 (2018).

[4]Hillenbrand, A. et al. Circulating adipokine levels and prognostic value in septic patients. Journal of Inflammation (United Kingdom), 13(1), 1–7 (2016).

Results

1. ER stress model

- Both tunicamycin and thapsigargin upregulated BiP expression in a time- and concentration-dependent manner (**Figure 1**)

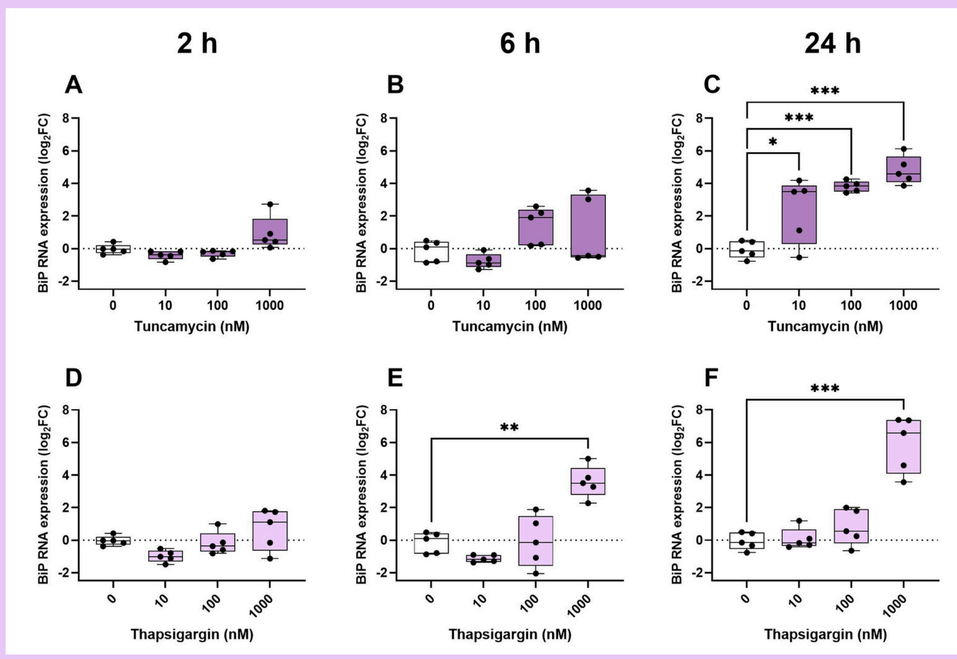


Figure 1. BiP RNA expression in equine lamellar cells exposed to tunicamycin or thapsigargin for 2, 6, or 24 h

RNA expression was determined via qPCR and normalised to β-actin and GAPDH. (A-C) tunicamycin treatment; (D-F) thapsigargin. Data are presented as median (line), interquartile range (box), and range (error bar) of n=5 of log_2 fold expression relative to the control. *P<0.05, **P<0.01, ***P<0.001.

- Treatment with ER stressors also upregulated expression of IL-6 (2 h) and IL-8 (6 h and 24 h; **Figure 2**)

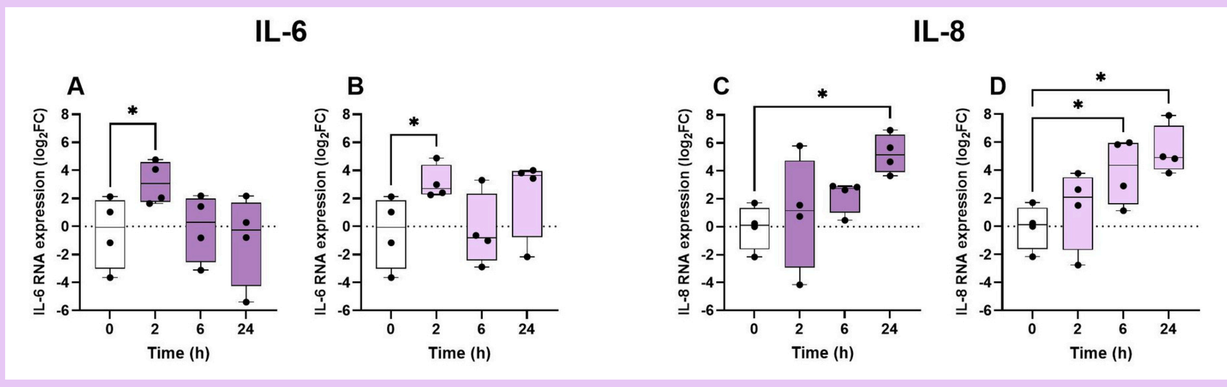


Figure 2. RNA expression of IL-6 and IL-8 in equine lamellar cells exposed to tunicamycin or thapsigargin for 2, 6, or 24 h

RNA expression was determined via qPCR and normalised to β-actin and GAPDH. (A, C) tunicamycin treatment; (B, D) thapsigargin; 1000 nM each. Data are presented as median (line), interquartile range (box), and range (error bar) of n=4 of log_2 fold expression relative to the control. *P<0.05.

2. Protective effects of adiponectin receptor agonists

- ER stress-induced BiP expression was reduced by full-length adiponectin and AdipoRon (protein level), and globular adiponectin (RNA level; **Figure 3**)

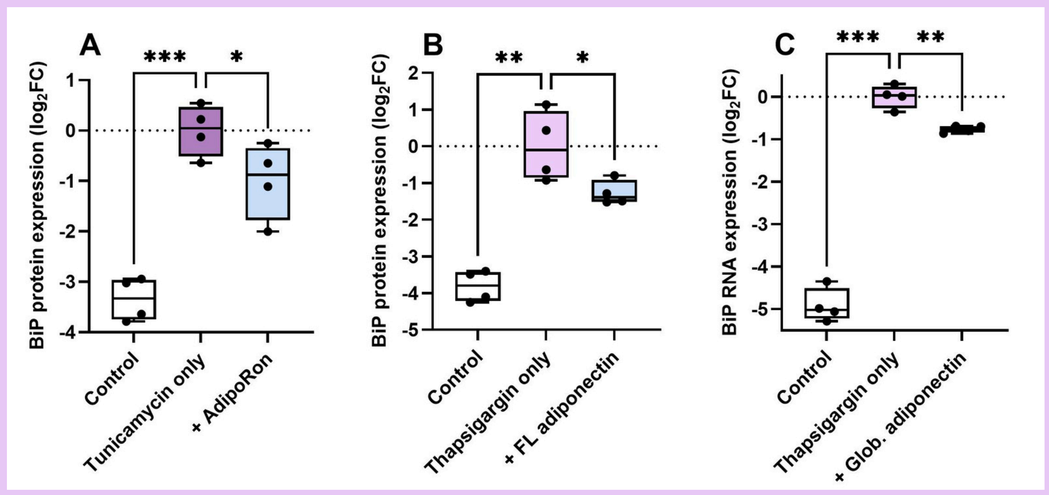


Figure 3. BiP expression in equine lamellar cells exposed to tunicamycin or thapsigargin and adiponectin receptor agonists for 24 h

(A,B) BiP protein expression in cells treated with (A) tunicamycin (1000 nM) and AdipoRon (50 µM), and (B) thapsigargin (1000 nM) and full-length adiponectin (3000 ng/mL). (C) BiP RNA expression in cells treated with thapsigargin (1000 nM) and globular adiponectin (3000 ng/mL). Protein expression was determined via western blotting and normalised to vinculin. RNA expression was determined via qPCR and normalised to β-actin and GAPDH. Data are presented as median (line), interquartile range (box), and range (error bar) of n=4 of log_2 fold expression relative to the tunicamycin or thapsigargin only treatment. *P<0.05, **P<0.01, ***P<0.001.

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