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The William Harvey Research Institute

Inhibition of Bruton's tyrosine kinase activity reduces organ injury and dysfunction in a rat model of severe haemorrhage

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Abstract

Trauma and/or haemorrhagic shock (HS) drives an excessive systemic inflammatory response, which contributes to multiorgan dysfunction syndrome (MODS), and is the main cause of death in the late post-injury phase[1]. There is no specific therapy for MODS. Bruton's tyrosine kinase (BTK) is known to play a role in the activation of the NLRP3 inflammasome which is a key component of the innate inflammatory response[2]. However, its role in trauma-haemorrhage is unknown. BTK activity can be blocked by acalabrutinib (irreversible) and fenebrutinib (reversible). We hypothesised that **inhibition of BTK activity would reduce MODS** in two rat models of HS.

Introduction

Trauma is one of the **leading causes of death**, affecting 6 million people annually worldwide. Approximately 40% of deaths associated with trauma are due to HS, which causes **hypoperfusion of organs** and subsequently **ischaemia**. This leads to MODS, which occurs in approximately 30% of injured patients.

Although guidelines for the early management of HS (including resuscitation and organ support strategies) have decreased the rates of immediate (on scene/within 60min) and early (emergency department and operating room/within 1-4h) deaths, post-injury MODS is still associated with **significant morbidity and mortality**.

The mechanisms underlying MODS are not fully understood, although it is thought to be associated with **excessive systemic inflammation**, secondary to the release of **damage-associated molecular patterns (DAMPs)** from extensive tissue damage and **ischaemia-reperfusion (I/R) injury**.

To date, there are **no specific pharmacological interventions** for the MODS associated with HS. Therefore, a therapeutic agent that reduces the **incidence and severity of MODS** is urgently needed and could have a major global impact on both patient outcomes and resource utilisation.

Aims

- To investigate the potential of the BTK inhibitors (BTKi) acalabrutinib and fenebrutinib to reduce **MODS** in an acute and chronic HS rat model
- To examine whether treatment with either BTKi attenuates **BTK, NF-κB and NLRP3 activation** in HS

Methods

Rats: The animal protocols used in this study were approved by the AWERB of QMUL in accordance with Home Office Guidance (acute model) and Universidade Federal de Santa Catarina Institutional Committee for Animal Use in Research (chronic model). Male Wistar rats were subjected to HS by withdrawal of blood from the carotid (acute model) or femoral (chronic model) artery to maintain MAP at 35±5mmHg for 90min. Resuscitation was initiated by rapid infusion of shed blood plus Ringer's lactate. Animals received acalabrutinib (3mg/kg), fenebrutinib (3mg/kg) or the vehicle (5% DMSO, 95% Ringer's lactate).
At 4h (acute model) or 24h (chronic model) after resuscitation, organ injury and dysfunction were evaluated by measuring creatinine (renal dysfunction), ALT (liver injury) and LDH (general tissue damage). The activation of BTK, NF-κB and NLRP3 pathways were analysed by western blot. Pulmonary and hepatic myeloperoxidase (MPO) activity were determined as an indicator of neutrophil infiltration.

Statistical analysis: One-way ANOVA followed by Bonferroni's *post-hoc* test; level of significance $p<0.05$.

Conclusions

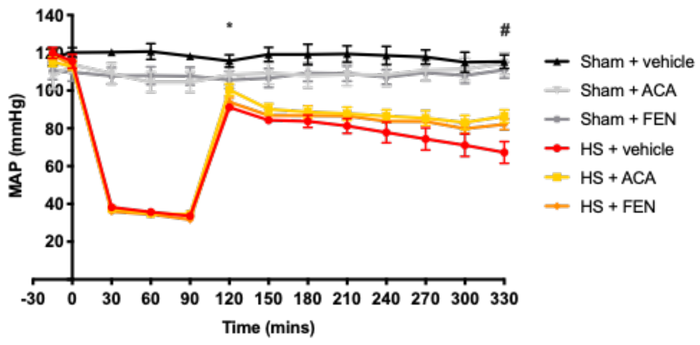
The results point to a role of BTK in the **pathophysiology of organ injury and dysfunction** caused by trauma-haemorrhage and indicate that **BTK inhibitors may be a potential therapeutic approach for MODS** after trauma and/or severe haemorrhage. Notably, no significant differences were found between the two structurally and mechanistically different inhibitors, suggesting that the observed beneficial effects in experimental trauma-haemorrhage are most likely due to a drug class related effect.

References

1. Dewar *et al.* (2009) ***Injury***
2. Weber *et al.* (2017) ***Frontiers in Immunology***

Results

Treatment with BTKi improves HS-induced circulatory failure in an acute HS model



Data are expressed as mean ± SEM of 8-10 animals per group. *p<0.05 Sham + vehicle vs. HS + vehicle; #p<0.05 HS + vehicle vs. HS + BTKi (ACA or FEN).

Figure 1. Treatment with BTKi improves HS-induced circulatory failure. MAP was measured from the completion of surgery to the termination of the experiment. Baseline MAP values were similar amongst all six groups. Rats subjected to HS demonstrated a decline in MAP which was ameliorated by resuscitation, but MAP still remained lower than that of sham rats during resuscitation (at the equivalent time points). The MAP of HS rats treated with either BTKi was significantly higher than that of vehicle treated HS rats at the end of the resuscitation period ($p<0.05$).

Treatment with BTKi attenuates HS-induced organ damage in acute and chronic HS models

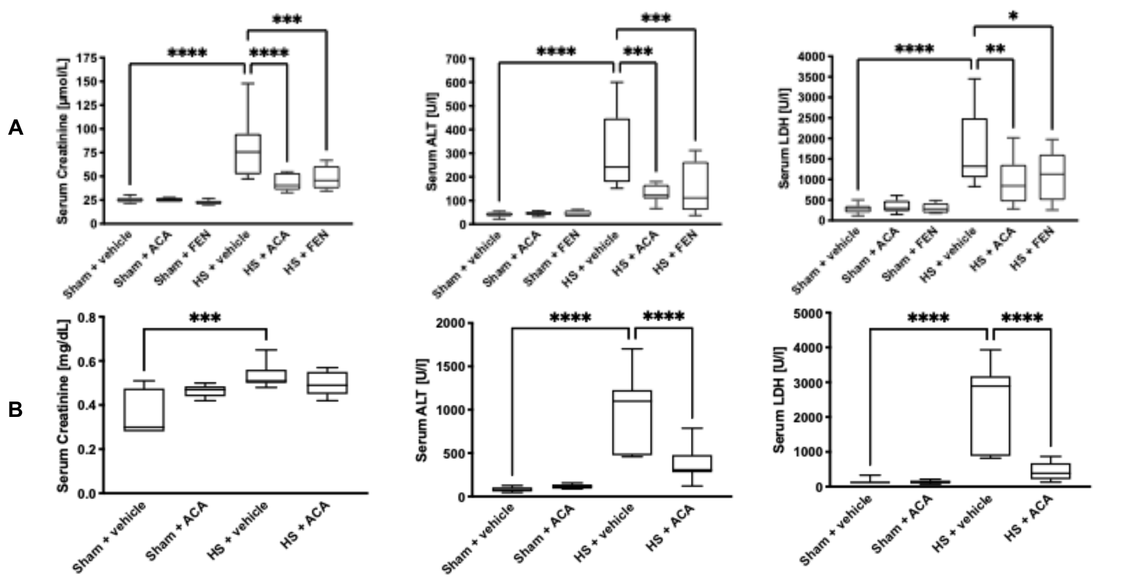


Figure 2. Treatment with BTKi attenuates HS-induced organ damage. Serum levels of creatinine, alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) were determined in vehicle and BTKi treated (acalabrutinib, ACA; fenebrutinib, FEN) rats from the (**A**) acute and (**B**) chronic HS models. When compared to sham rats, vehicle treated HS rats displayed significant increases in creatinine, ALT and LDH in both models. The rises in ALT and LDH were significantly attenuated following BTKi treatment in both models ($p<0.05$). The increase in creatinine was only significantly reduced in the acute model ($p<0.05$). Data are expressed as box and whiskers plotted from min to max of 8-10 animals per group.

Treatment with BTKi attenuates BTK, NF-κB and NLRP3 activation in an acute HS model

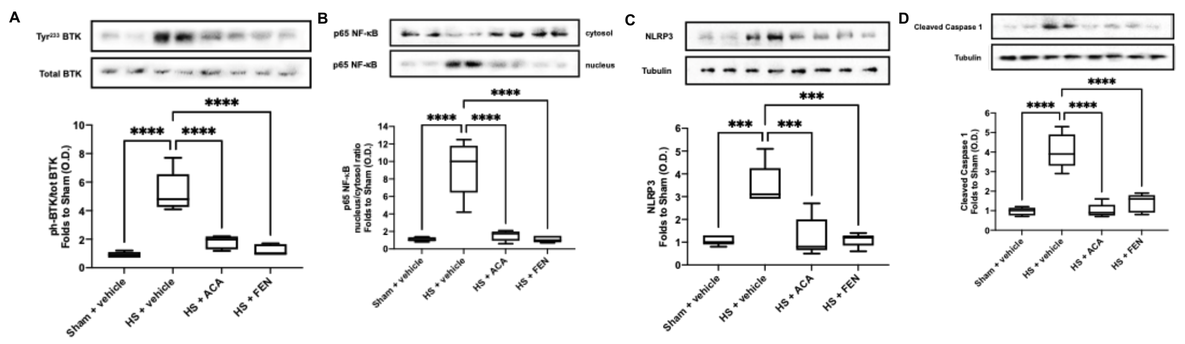
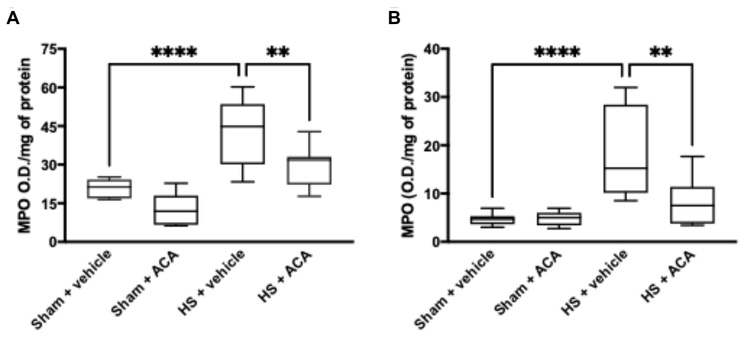


Figure 3. Treatment with BTKi attenuates BTK, NF-κB and NLRP3 activation. (**A**) Phosphorylation of BTK at Tyr[223], (**B**) nuclear translocation of p65, (**C**) activation of NLRP3 and (**D**) cleaved form of caspase 1 of vehicle and BTKi treated (acalabrutinib, ACA; fenebrutinib, FEN) rats were determined by western blotting in the kidney. Protein expression was measured as relative optical density (O.D.) and normalised to the sham band. When compared to sham rats, vehicle treated HS rats displayed significant increases in the phosphorylation of BTK at Tyr223 ($p<0.05$; Figure 3A), translocation of p65 to the nucleus ($p<0.05$; Figure 3B), expression of the NLRP3 inflammasome ($p<0.05$; Figure 3C) and cleaved (activated) form of caspase 1 ($p<0.05$; Figure 3D) in the kidney. These rises were significantly attenuated following BTKi treatment. Data are expressed as box and whiskers plotted from min to max of five animals per group.

Treatment with acalabrutinib reduces MPO activity in a chronic HS model



Data are expressed as box and whiskers plotted from min to max of 9-10 animals per group.

Figure 4. Treatment with acalabrutinib reduces MPO activity. Myeloperoxidase (MPO) activity in the (**A**) lung and (**B**) liver were determined for vehicle and acalabrutinib (ACA) treated rats as an indicator of neutrophil infiltration. When compared to sham rats, vehicle treated HS rats showed a significant increase in MPO activity in the lung ($p<0.05$; Figure 4A) and liver ($p<0.05$; Figure 4B) which was attenuated following treatment with acalabrutinib ($p<0.05$; Figure 4).