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Translational Medicine & Therapeutics
The William Harvey Research Institute

The inhibition of MIF by ISO-1 attenuates trauma-induced multiple organ failure in rats

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Abstract

Trauma and/or hemorrhagic shock (HS) drives an excessive systemic inflammatory response, which contributes to multiple organ failure (MOF), and is the main cause of death in the late post-injury phase[1]. There is no specific therapy for MOF. The cytokine macrophage migration inhibitory factor (MIF) is an important modulator of the inflammatory response and the effects of MIF can be inhibited by ISO-1[2].

We hypothesized that:
(i) MIF levels are increased in the serum of trauma patients;
(ii) Inhibition of the effects of MIF with ISO-1 reduces MOF in a rat model of HS.

We found patients with trauma and rats with severe hemorrhage had elevated serum levels of MIF. The MIF inhibitor ISO-1 attenuated the trauma-induced MOF in rats with HS, indicating that MIF drives MOF in trauma/hemorrhage.

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 **ischemia**. This leads to MOF, 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 MOF is still associated with **significant morbidity and mortality**.

The mechanisms underlying MOF 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 **ischemia-reperfusion (I/R) injury**.

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

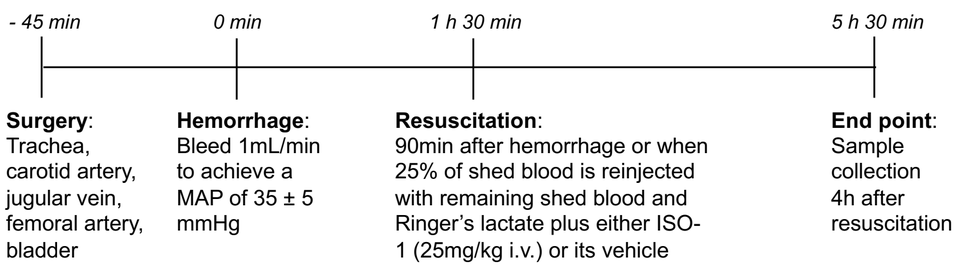
Aims

- To examine the role of MIF in trauma patients
- To evaluate the extent of organ damage following treatment with ISO-1 in a rat model of HS

Methods

Patients: MIF levels were measured in the blood of trauma patients with ISS ≥ 16 (n=208) at time of admission, after 2 days, 5 days and 7 days by ELISA. Healthy donors served as control.

Rats: The animal protocols used in this study were approved by the AWERB of QMUL in accordance with Home Office Guidance. Male Wistar rats (250-350g) anesthetized with sodium thiopentone (120mg/kg i.p.) were subjected to HS by withdrawal of blood from the carotid artery to maintain MAP at 35±5mmHg for 90min. Resuscitation was initiated by rapid infusion of shed blood plus the same volume of Ringer's Lactate (RL). Animals received either ISO-1 (25 mg/kg i.v., n=8) or its vehicle (DMSO 5%, RL 95%; n=8). 4h after resuscitation, organ injury and dysfunction were evaluated by measuring creatinine, urea, creatinine clearance (renal dysfunction), ALT, AST (hepatic injury) and lactate. The activation of NF-κB and NLRP3 pathways were analyzed by western blot in the liver and kidney.



Statistical analysis: One-way ANOVA followed by Bonferroni *post hoc* test; $p<0.05$ was considered statistically significant.

Conclusions

- Trauma resulted in a rapid, but transient rise in MIF in patients and a similar increase in rats
- MIF levels on admission correlated with longer ICU and hospital stay, hence could be a disease biomarker used to predict patient outcomes
- Inhibition of the effects of MIF with ISO-1 significantly reduced the renal dysfunction and hepatic injury caused by HS in rats
- Western blot analysis highlighted the significant reduction in NF-κB and NLRP3 activation upon ISO-1 treatment in the liver and kidney of rats
- Thus, prevention of the synthesis or effects of MIF could be a potential therapeutic target in patients with trauma and/or severe hemorrhage

References

1. Dewar *et al.* (2009) ***Injury***
2. Al-Abed *et al.* (2005) ***J Biol Chem***

Results

MIF levels are elevated in the serum of polytrauma patients and are associated with longer stay on ICU and in hospital

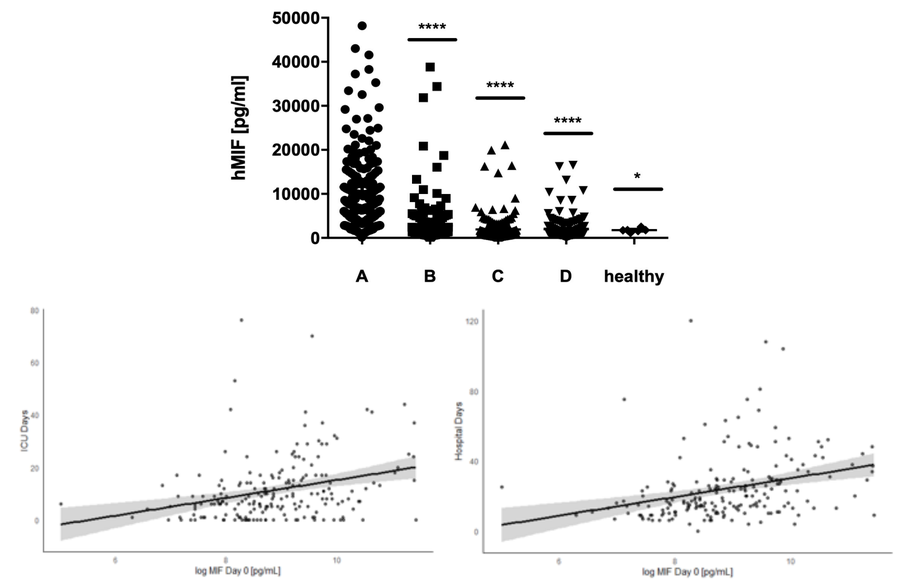


Figure 1. MIF serum levels are elevated in polytrauma patients. Trauma patients admitted to hospital were entered into a pilot study examining the role of MIF. MIF levels were significantly increased at timepoint A in comparison to all other timepoints and healthy controls as measured by ELISA. MIF levels on admission also showed a significant, positive correlation with days spent on ICU ($p<0.01$) and in hospital ($p<0.01$). Trauma patients (n=208); healthy donors (n=6). Timepoints: A = emergency room, B = 2 days; C = 5 days; D = 7 days. Data are expressed as mean ± SEM. Statistical analysis was performed using one-way ANOVA followed by Bonferroni *post hoc* test; with statistical significance considered as $p<0.05$ vs A.

MIF levels are elevated in the serum of rats after induction of HS

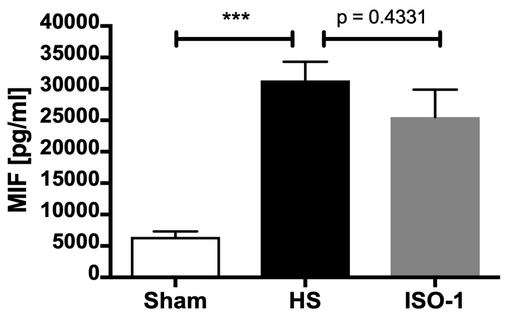


Figure 2. MIF serum levels are increased in HS rats. Treatment with ISO-1 did not result in a significant decrease in MIF levels in rats subjected to HS. Serum MIF levels were measured by ELISA. The following groups were studied: sham + vehicle (n=8); HS + vehicle (n=8); HS + ISO-1 (25mg/kg i.v., n=8). Data are expressed as mean ± SEM. Statistical analysis was performed using one-way ANOVA followed by Bonferroni *post hoc test*; with statistical significance considered as $p<0.05$ vs HS.

Treatment with ISO-1 attenuates HS-induced organ damage in rats

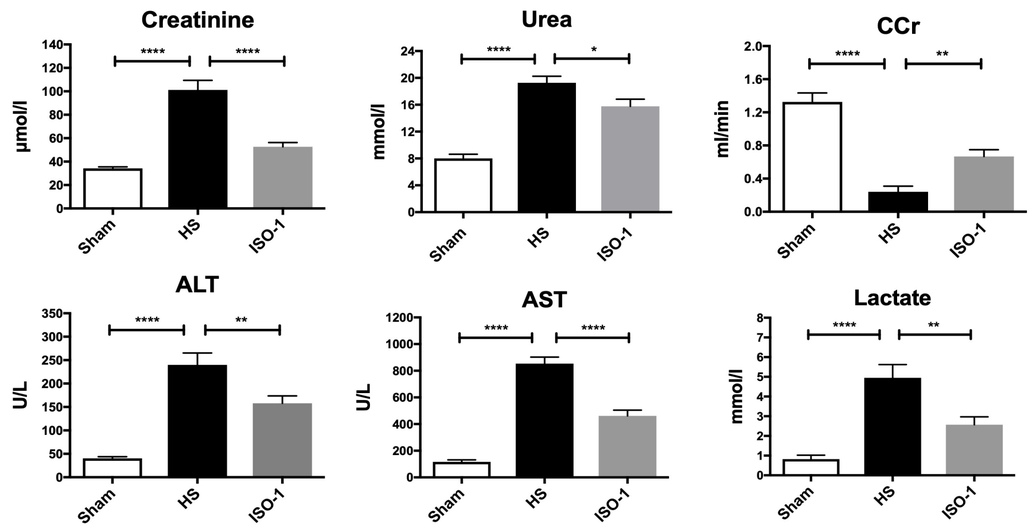


Figure 3. ISO-1 attenuates organ failure after induction of HS in rats. Treatment with ISO-1 resulted in significant decreases in parameters evaluating renal dysfunction (creatinine, urea, creatinine clearance), hepatic injury (ALT, AST) and overall tissue damage (lactate) when compared to non-treated rats. The following groups were studied: sham + vehicle (n=8); HS + vehicle (n=8); HS + ISO-1 (25mg/kg i.v., n=8). Data are expressed as mean ± SEM. Statistical analysis was performed using one-way ANOVA followed by Bonferroni *post hoc* test; with statistical significance considered as $p<0.05$ vs HS.

Treatment with ISO-1 attenuates NF-κB and NLRP3 activation in rats

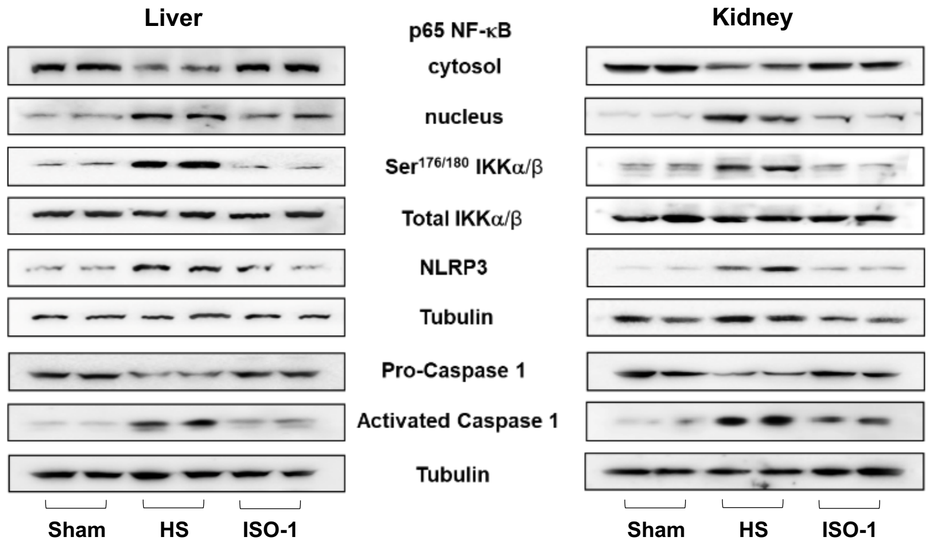


Figure 4. ISO-1 attenuates NF-κB and NLRP3 activation after induction of HS in rats. Treatment with ISO-1 resulted in significant decreases in the translocation of p65 to the nucleus ($p<0.05$), phosphorylation of IKKα/β at $Ser^{176/180}$ ($p<0.05$), expression of the NLRP3 inflammasome ($p<0.05$) and cleavage of pro-caspase 1 to caspase 1 ($p<0.05$) in both the liver and kidney. The following groups were studied: sham + vehicle (n=8); HS + vehicle (n=8); HS + ISO-1 (25mg/kg i.v., n=8). Data are expressed as mean ± SEM. Statistical analysis was performed using one-way ANOVA followed by Bonferroni *post hoc* test; with statistical significance considered as $p<0.05$ vs HS.