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A Selective IκB Kinase Inhibitor (IKK16) Attenuates The Organ Injury / Dysfunction Associated With Haemorrhagic Shock In The Rat

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Introduction: Haemorrhagic shock (HS) is one of the most common types of shock and occurs due to uncontrollable bleeding, often resulting in organ ischaemia, tissue hypoperfusion and organ injury (1). Restoration of blood flow and reoxygenation is associated with an exacerbation of tissue injury and an excessive inflammatory response (2). Many drugs (or interventions) that have reduced inflammation and organ injury in HS attenuate the activation of nuclear factor kappa B (NF-κB), which controls the transcription of several proinflammatory proteins (3). Thus, the aim of the present work is to investigate the effects of a specific and potent inhibitor of IκB kinase, which plays a pivotal role in the activation of NF-κB complex, in the organ dysfunction associated with HS.

Methods: Male Wistar rats were subjected to HS under sodium thiopentone anesthesia (120 mg/kg; i.p.). Specifically, blood was removed from the carotid artery in order to reduce mean arterial pressure to 30 ± 2 mmHg for 90 min. This was followed by resuscitation with the shed blood over 5 min. Rats were treated with the inhibitor of IκB kinase (IKK16; 1 mg/kg; i.v.) or vehicle (10% DMSO) on resuscitation. At 4 h after resuscitation, blood was removed to measure the following indicators of organ injury: Creatinine (renal dysfunction), lactate (tissue ischaemia), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (liver injury). Creatinine was also measured in urine to calculate creatinine clearance. Myeloperoxidase (MPO) activity was measured in the lung as an indicator of neutrophil recruitment. In addition, organs were removed for Western blot analysis of signaling events that lead to the activation of NF-κB. Data was analyzed by one or two-way ANOVA followed by Bonferroni's post hoc test for multiple comparisons. $P < 0.05$ was considered significant.

Results: When compared to sham-operated rats (n=8), HS resulted in a significant decrease in creatinine clearance (from 1.4 ± 0.09 to 0.12 ± 0.03 mL/min) as well as rises in serum creatinine (from 33.5 ± 1.7 to 108.8 ± 5.6 umol/L), AST (from 146.4 ± 23.7 to 1138.0 ± 114.1 U/L), ALT (from 50.0 ± 4.1 to 347.6 ± 44.6 U/L) and lactate (from 1.0 ± 0.15 to 3.4 ± 0.6 mmol/L) (n=10; $p<0.05$ for all parameters measured), indicating the development of renal dysfunction, liver injury and organ ischaemia. HS also resulted in a significant increase in lung MPO activity (from 30.6 ± 9.3 to 93.3 ± 4.7 U/tissue g; n=4; $p<0.05$), indicating the development of lung inflammation. Western blot analysis of kidney and liver tissue from HS rats revealed increases in phosphorylation of IκB, nuclear translocation of NF-κB subunit p65, increases in phosphorylation of ERK 1/2 and enhanced expression of inducible nitric oxide synthase (iNOS). Treatment of HS-rats with IKK16 protected animals against the organ injury and dysfunction induced by HS. In addition, IKK16 attenuated phosphorylation of IκB and nuclear translocation of NF-κB subunit p65, indicating

that this molecule does indeed prevent the activation of NF-κB caused by HS. Surprisingly, IKK16 also caused an increase in the phosphorylation of Akt; and activation of the Akt-survival pathway may well contribute to the observed beneficial effects of IKK16.

Conclusion: In conclusion, administration of IKK16 on resuscitation attenuated the organ dysfunction associated with HS. This effect may be (at least in part) due to the observed inhibition of NF-κB and/or the activation of the Akt-survival pathway.

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