INTRODUCTION:

Ischaemia-reperfusion (IR) injury is a common surgical event, with tourniquet use in orthopaedic surgery a recognised cause. The pre-emptive induction of intra-cell stress proteins by sub-critical injury, or preconditioning, is known to protect against subsequent critical injury. Finding a clinically safe and acceptable method of induction has proven difficult. Glutamine, a known inducer of heat shock proteins (HSP) suggests itself as a safe clinical pre-conditioning agent. The effect of parenteral glutamine treatment has not been assessed in the setting of limb IR. This study tests the hypothesis that glutamine protects against tourniquet-induced regional and remote organ IR injury.

MATERIAL AND METHODS:

Forty adult male Sprague-Dawley rats were randomised into four groups: control, IR injury, normal saline pre-treated IR injury, and glutamine pre-treated IR injury. Pre-treated groups received either normal saline or glutamine (0.5g/kg) in saline by intravenous bolus 24 hours before injury. Bilateral hindlimb tourniquets were placed proximal to the greater trochanter for two hours. Blood samples were taken before and after tourniquet release, and following 2 hours of reperfusion. The animals were then sacrificed, bronchioalveolar lavage (BAL) performed and skeletal muscle and lung harvested for evaluation.

All animals were humanely killed at the conclusion of two hours reperfusion. Experimentation was performed under licence from the Animal Ethics Committee.

RESULTS

No effect of pre-treatment of renal or hepatic biochemical markers. Although pre-treatment with normal saline and glutamine lead to lower Urea and Creatinine levels than the untreated groups, renal and hepatic biochemical markers in blood did not demonstrate any statistically significant benefit for glutamine pre-treatment.

Muscle damage attenuated in Glutamine pretreated group.

Creatine Kinase levels in the glutamine pre-treated group were significantly lower following ischaemia than in the group pre-treated with saline (p = 0.024), and following reperfusion, were significantly lower than either the normal saline pre-treated group (p = 0.001) or the injury alone group (p = 0.02). Myeloperoxidase levels in the glutamine pre-treated group were significantly reduced in muscle samples when compared to the injury group (p = 0.042).

Glutamine pre-treatment attenuates lung parenchymal damage.

In lung tissue, levels of myeloperoxidase were reduced, but not significantly so (p = 0.195). However, the measurement of the number of neutrophils in the bronchoalveolar lavage fluid revealed that pre-treatment with glutamine significantly reduced neutrophil infiltration into parenchymal tissue compared to the normal saline pre-treated group (p = 0.001), the injury alone group (p = 0.001) and even the uninjured control group (p = 0.016).

HSP72 detected only in glutamine pretreated tissue.

Samples of lung and muscle tissue from all groups were tested for Heat Shock Protein 72. Only those samples from the glutamine treated group demonstrated an upregulation in the protein, and in the glutamine pre-treated group, both were strongly expressed, with muscle tissue demonstrated a higher levels of upregulation than lung.

CONCLUSION

This study demonstrates that preconditioning, with a single intravenous bolus prior to a planned ischaemia-reperfusion injury. To our knowledge, no effective prophylactic intervention strategies currently exist for to modulate clinically relevant reperfusion injury and its sequelae.

Only the group of rats pre-treated with glutamine caused an upregulation in HSP72, doing so for both lung and muscle tissue. This implicates enhanced HSP72 expression as a potential mechanism for attenuating the expression of MPO into muscle and lung tissue and for reducing the levels of creatine kinase released following both the ischaemia and the reperfusion phases of the experiment. Similarly the reduction in capillary dysfunction and vascular permeability and consequent reduction in neutrophils found in the bronchoalveolar lavage of the glutamine pre-treated group is suggestive of a HSP-induced lung protection.

It is interesting that glutamine pre-treatment did not seem to afford any attenuation of the reperfusion ‘hit’ as measured by renal and hepatic biochemistry. Although HSP72 has been induced in both kidney and liver, pre-induction of these proteins has proven highly difficult to replicate in a clinically acceptable manner. It is possible that the design of this study was not conducive to upregulation of HSP72 given the moderate dose of glutamine given (0.5g/Kg), and the relatively short, if intense, insult provided. Alternatively, the excretive functions of both kidney and liver may confer these organs with an endogenous protection of their own.