O45. HYDROXYCHLOROQUINE IS CARDIOPROTECTIVE IN NEONATAL RAT CARDIOMYOCYTES EXPOSED TO SIMULATED MYOCARDIAL ISCHAEMIC/REPERFUSION INJURY: AN EFFECT MEDIATED THROUGH ERK PHOSPHORYLATION

Lauren Bourke¹, James McCormick², Jack Franklin¹, Anastasis Stephanou³ and Yiannis Ioannou³
¹Department of Rheumatology, University College London, London, ²MMBU, University College London, London, ³Arthritis Research UK Centre for Adolescent Rheumatology, University College London, London, UK

Background: A significant amount of myocardial damage during a myocardial infarction (MI) occurs during the reperfusion stage which is known as ischaemic reperfusion (I/R) injury and can account for up to 50% of cell death. SLE is a condition associated with a high burden of cardiovascular morbidity and mortality, when compared with the matched healthy population. A large proportion of these patients are treated with the anti-malarial drug HCQ and whilst retrospective studies have suggested that SLE patients prescribed HCQ have a reduced risk of suffering an MI, inevitably there are patients who still do. Therefore the purpose of this study is to determine whether HCQ has an effect on I/R injury.

Methods: We utilized an established in vitro model simulated ischaemic reperfusion (I/R) injury. Cardiomyocytes were isolated from 1–2 day old rat pups and when beating synchronously were treated with 1–2 μg/ml HCQ and the following day exposed to simulated I/R injury in a hypoxic chamber (argon, 9% CO₂) followed by reoxygenation. Apoptosis was assessed using a TUNEL kit and caspase-3 cleavage detected by immunoblot.
Results: Neonatal rat cardiomyocytes treated with HCQ prior to and during simulated I/R injury showed reduced cell death, specifically apoptosis when compared with control cells. This was observed using TUNEL which showed that cells exposed to hypoxia alone saw a 20.7% (s.d. 7.4) increase in TUNEL positivity when compared with cells in optimal conditions. This was further increased to 30.1% (s.d. 7.0) (P < 0.001) after reoxygenation and in the presence of HCQ was abrogated back down to 16.93% (s.d. 3.0) (P < 0.0001). This was confirmed by an increase in cleaved caspase-3 (0.24 relative to GAPDH (s.d. 0.10) (P < 0.0001)) in cells exposed to simulated I/R injury when compared with cells in optimal conditions (0.03 relative to GAPDH (s.d. 0.03)). In the presence of HCQ this increase in caspase-3 cleavage was reduced by 54% (0.11 relative to GAPDH (s.d. 0.06) (P < 0.01)). Correlating with decreased cell death, enhanced ERK phosphorylation in HCQ treated cells was observed in a dose-dependent manner. Cells treated with HCQ and exposed to simulated I/R injury were incubated with the ERK inhibitor U1026 and protective effects of HCQ was completely reversed. Additional experiments indicate pre-treatment of cells with HCQ prior to simulated I/R injury leads to the most significant protection and treatment only during the simulated reperfusion stage offers no protection.

Conclusion: HCQ is cardioprotective in this in vitro I/R injury model and results suggest this protection is dependent upon up-regulation of ERK phosphorylation. Experiments are now being performed to confirm this observation using an in vivo I/R injury animal model.

Disclosure statement: The authors have declared no conflicts of interest.