329. PURIFIED IgG FROM PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS ENHANCES APOPTOSIS IN NEONATAL RAT CARDIOMYOCYTES EXPOSED TO SIMULATED MYOCARDIAL ISCHAEMIC REPERFUSION INJURY

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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. Circulating autoantibodies such as antiphospholipid antibodies (aPL), which are found in 30–40% of SLE patients increase the risk of suffering an arterial thrombotic event. Studies performed by other groups have shown accelerated I/R injury in other systems such as mesenteric I/R injury in a lupus mouse model, however to date there has been no research focusing on the heart. The purpose of this study is to assess I/R injury in the presence of polyclonal IgG from patients with SLE aPL in an established in vitro model of simulated myocardial I/R injury utilizing neonatal rat cardiomyocytes.
Methods: Polyclonal IgG was isolated by protein G purification from serum of patients with SLE (n = 23) and healthy controls (n = 11) which were age and gender matched. Endotoxin was removed using Detoxi-Gel columns to a level below 0.225 endotoxin U/ml. We utilized an established in vitro model of anoxia/reoxygenation to I/R injury. Cardiomyocytes were isolated from 1–2 day old rat pups and when beating synchronously were treated with 500 μg/ml polyclonal IgG from each group and the following day exposed to simulated I/R injury using a hypoxic chamber (argon, 5% CO₂) followed by reoxygenation. Apoptosis was measured by assessment of caspase-3 cleavage using immunoblot and TUNEL.

Results: In cells exposed to simulated I/R injury caspase-3 cleavage was not significantly increased in the presence of IgG from healthy volunteers (mean increase in caspase-3 cleavage of cells treated with healthy control IgG above untreated cells exposed to simulated I/R injury is 12.28% ± s.d. 26.01, n = 10). However, in the presence of IgG from patients with SLE/aPL +ve and SLE/aPL –ve, caspase-3 cleavage was increased above untreated cells exposed to simulated I/R injury by 58.74% (± s.d. 17.8, n = 6) and 63.98 (± s.d. 17.9, n = 8) and therefore significantly increased in comparison with healthy controls (P = 0.0001). The effect observed with IgG from SLE patients was not altered when serum used in the buffers was heat inactivated suggesting a non-complement mediated mechanism.

Conclusion: In this in vitro simulated I/R injury model IgG purified from patients with SLE significantly enhanced I/R injury as assessed by caspase-3 cleavage and TUNEL. This novel pathogenic role of these antibodies will now be tested in vivo to validate this finding and explore potential mechanisms of action.

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