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Group 6 - Ischaemia / reperfusion

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Mechanisms of myocardial nitroso redox imbalance following elective cardiac surgery on cardiopulmonary bypass

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Background and Aims: The mechanisms responsible for myocardial stunning after cardiac surgery are only partially understood, although ischemia - reperfusion (IR) injury associated with cardio pulmonary bypass (CPB) may play an important role. Reactive oxygen species and nitric oxide have been implicated in both myocardial injury and cardio protection suggesting that identification of their sources may refine therapeutic strategies. Here we investigated the effect of cardioplegia, CPB and reperfusion on myocardial nitroso-redox balance in patients after cardiac surgery.

Results: Paired samples of the right atrial appendages (RAA) were obtained a) prior to venous cannulation of right atrium b) after myocardial reperfusion and venous decannulation in 116 patients undergoing elective cardiac surgery on CPB. After reperfusion, 1) Atrial superoxide (O2-) release was increased (in RLU/sec/mg: from 41.3 $\pm$ 4.48 to 55.61 $\pm$ 5.92, n=46 samples from 23 patients, p = 0.01 by lucigenin enhanced chemiluminescence and in 2- hydroxyethidium (2-OH) peak area by HPLC: 267.7 $\pm$ 33.39 to 494.7 $\pm$ 29.95 n=26 samples, p=0.0002) 2) Atrial nitric oxide synthase (NOS) activity was uncoupled (L-NAME inhibitable O2- fraction in RLU/sec/mg: -18.33 $\pm$ 8.68 vs. +21.11 $\pm$ 5.66, n=40, p=0.0001) and NOX2 activity was increased (In 2-OH peak area: from 93.94 $\pm$ 15.56 to 233.6 $\pm$ 21.61, n=44, P= 0.03) along with increased O2- release from mitochondrial complex I 3) Atrial content of the NOS cofactor, tetrahydrobiopterin (BH4) (in pmol/gram/tissue: from 1.29 $\pm$ 0.13 to 0.88 $\pm$ 0.12, n=36; p = 0.004) was reduced as was the activity of the rate limiting enzyme in BH4 synthesis, GTP-cyclohydrolase 1 (GTPCH1; in μmol/mg tissue/minute: from 0.07 $\pm$ 0.01 to 0.03 $\pm$ 0.01, n=30, $p<0.0001$) without changes in oxidised biopterins. Investigation of mechanisms responsible for the reduction in atrial GTPCH1 activity revealed an increase in protein expression of GFRP (GTPCH1 feedback regulatory protein) in the absence of changes in GTPCH1 protein. Atrial NOS activity decreased after reperfusion (from 0.13 $\pm$ 0.01 to 0.05 $\pm$ 0.01, by HPLC; n=20) without changes in NOS1 and NOS3 protein content where as BH4 (10 μM) supplementation significantly increased NOS activity in both groups but did not abolish the effect of reperfusion. Evaluation of additional mechanisms of NOS uncoupling showed increased s- glutathionylation of NOS3 (n=16).

Conclusions: Cardioplegia and reperfusion are associated with suppressed myocardial NO production and increased O2- release from NOX2, mitochondrial complex I and uncoupled NOS activity secondary to S- glutathionylation.