Molecular mechanisms of myocardial nitroso redox imbalance during cardiac surgery on cardiopulmonary bypass and therapeutic implications

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BACKGROUND

The mechanism responsible for LV dysfunction after cardiac surgery is only partially understood. In isolated rat hearts subjected to a prolonged ischemia-reperfusion protocol, LV dysfunction was associated with uncoupling of endothelial nitric oxide synthase (eNOS) activity secondary to oxidation of the NOS cofactor, tetrahydrobiopterin (BH4) (Dumitrescu et al. PNAS 2007). Here we investigated the effect of cardiopulmonary bypass (CPB) and reperfusion on myocardial NOS activity and superoxide production in 116 patients who underwent elective cardiac surgery.

METHODS

Samples of the right atrial appendages (RAA) were obtained before (PRE) and following CPB. For the analysis of RAA homogenates methods used include: Measurement of superoxide release by Lucigenin enhanced Tiron- inhibitable chemiluminescence and measurement of 2-hydroxyethidium by HPLC; Contribution of individual oxidases to superoxide release by specific pharmacologic inhibitors - Rotenone (100µmOL/L) for mitochondrial complex I, gp91 ds tat peptide (10µmOL/L) for NOX2-NADPH Oxidase, L-NAME (1mMOL/L) for coupled and and measurement of 2-hydroxyethidium by HPLC; Contribution of individual oxidases to superoxide production.

RESULTS

1: Right atrial superoxide production is increased after CPB and reperfusion due to uncoupling of NOS and increased activity of Complex-I of Mitochondrial electron transport chain and NOX2-NADPH Oxidase.

2: Right atrial content of the NOS cofactor, tetrahydrobiopterin (BH4) is reduced after CPB and reperfusion without changes in oxidised bioperins (BH2 and B) by HPLC; chromatographic analysis of Tetrahydrobiopterin (BH4) and its oxidised products BH4 and Bioprin by HPLC electrochemical detection; GTPCH I activity by HPLC fluorescent detection; Measurement of NOS activity by HPLC detection of conversion of 14C-L-arginine to L-citrulline.

3: Activity of the rate limiting enzyme in BH4 synthesis, GTP - cyclohydrolase 1 (GTPCH 1) is decreased after CPB and reperfusion without concomitant changes in GTPCH1 protein.

4: Protein expression of GFRP (GTPCH1 feedback regulatory protein) is increased after CPB and reperfusion.

5: Reduction in NOS activity after CPB and reperfusion not restored by pre treatment of atrial myocardium with BH4.

6: Reduction in NOS activity after CPB and reperfusion is not associated with changes in protein expression of NOS isofoms.

7: Atrial levels of S-glutathionylated eNOS is increased after CPB and reperfusion.

8: Reversal of S-Glutathionylation abolishes the difference in NOS activity associated with CPB and reperfusion.

CONCLUSIONS

- NOS S-Glutathionylation, rather than BH4 depletion, accounts for NOS dysfunction in patients undergoing cardiac surgery on cardiopulmonary bypass and imply that in this patient cohort, BH4 supplementation is not effective in restoring atrial bioavailability of nitric oxides.
- Whether redressing atrial oxidative stress by deglutathionylation of eNOS will preserve NO-redox balance and prevent perioperative myocardial dysfunction in patients undergoing on-pump cardiac surgery remains to be established.