Mitochondrial DNA and Ischemia Reperfusion Injury: Development of an Experimental Extra Corporeal Lung Perfusion (EVLP) Rat Model

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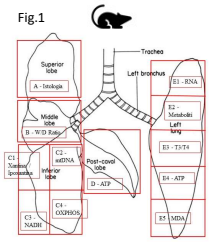
BACKGROUND: Ex-vivo lung perfusion (EVLP) is a tool to evaluate and recover marginal grafts in lung transplant (LuTx). Nevertheless, EVLP is still not a reconditioning so that the ischemic damage worsens over time during perfusion. Steen solution is the gold standard in this setting, and its composition is not known precisely due to patent's protection, but high hormones concentrations in this solution may afflict negatively lung function. The lung metabolism is influenced by triiodothyronine (T3), that acts on mitochondrial function and energy production, as documented in studies on cardiac-ischemia animal models. The aim of this study was to identify, in a DCD lung rat model, the effects of Steen solution and T3 titration on IRI and mitochondrial DNA (mtDNA), release during 180 minutes of EVLP as a marker of mitochondrial damage.

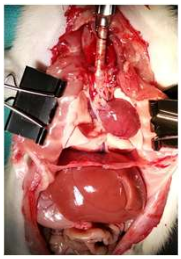
METHODS: 8 groups of five rats were scheduled (Tab 1), the harvesting and EVLP were conducted as previously described by our group. DNA was extracted and mtDNA levels were measured in frozen parenchima, plasma and in perfusate at 30, 60, 120 e 180 min. Functional parameters (EGA, vascular resistance, compliance), biomolecular markers (ATP, ROS, freeT3) and gene expression were evaluated at the same time points (Fig.1, Tab 2).

Fig.1



Tab.1

EXPERIMENTAL GROUPS OF RATS (n=40).	
Native	Lungs retrieved after anesthesia
Ischemia	60' hot ischemia+ flushing with Perfadex + lungs retrival + 60' cold ischemia
EVLP (Sham)	Flushing with Perfadex + lungs retrival+ 180' EVLP
EVLP-1 T3 (Sham-1T3)	Flushing with Perfadex + lungs retrival + 180' EVLP with Steen plus T3 650 mcg/l (concentration of 1 µM)
EVLP-1,5 T3 (Sham-1,5T3)	Flushing with Perfadex + lungs retrival + 180' EVLP with Steen plus T3 975 mcg/l (concentration of 1,5 µM)
EVLP ischemia	60' hot ischemia+ flushing with Perfadex + lungs retrival + 60' cold ischemia + 180' EVLP
EVLP ischemia-1T3	60' hot ischemia+ flushing with Perfadex + lungs retrival + 60' cold ischemia +180' EVLP with Steen plus T3 650 mcg/l (concentration of 1 µM)
EVLP ischemia-1,5T3	60' hot ischemia+ flushing with Perfadex + lungs retrival + 60' cold ischemia +180' EVLP with Steen plus T3 975 mcg/l (concentration of 1,5 µM)



Tab.2

A. FUNCTIONAL PARAMETERS: emo-gas, compliance, mPAP, PEEP, pPeak, lung vascular resistance
B. BIOMOLECOLAR MARKERS : inflammatory mediators, free mitocondrial DNA, freeT3 e freeT4, ATP, NADH, genes expression on perfusate at 45', 60', 120', 180' and on lung tissue
C. STEEN ANALYSIS: dosage in two different laboratories anb batches of free fatty acids, vitamins, hormones not already clearly described before («phisiological concentration» in the patent)

RESULTS: As expected, mtDNA tissue levels were similar in all the groups analyzed. The free mtDNA detected in perfusate increased in a time dependent manner in ischemic group and EVLP T3 groups (Fig.2, 3), as NADH and ATP (in tissue), confirming the mitochondrial dysfunction and cell damage induced by too much ischemic stress. However, this increase is more pronounced in T3 groups (Fig.3). This is confirmed also by functional results, as vascular resistances increase in T3 groups (Fig.4, 5).

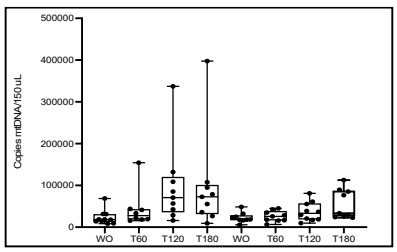


Fig.2

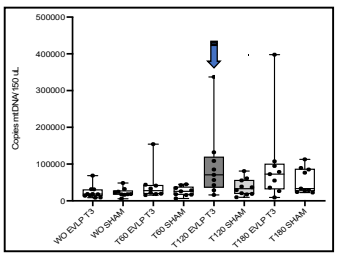


Fig.3

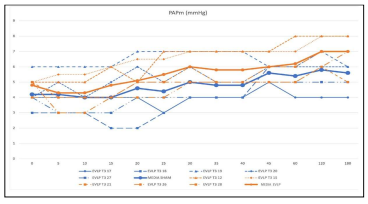


Fig.4

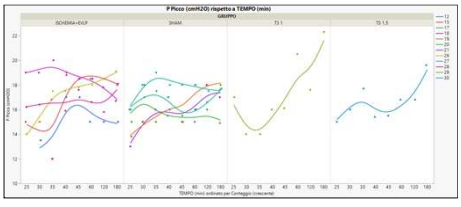


Fig.5

CONCLUSIONS: Our results showed the metabolic effect of Steen solution and T3 on IRI during EVLP. Time-dependent increase of mtDNA suggests that EVLP does not improve IRI and too high T3 concentration increases mitochondrial dysfunction and likely promotes inflammation, as mtDNA can act as a proinflammatory molecule. Finding the best T3 concentration should allow a better metabolic effect on mitochondria and the best reconditioning during EVLP.