

**Consequences of Cold and Warm Ischemia
on Pulmonary Mitochondrial Respiratory Function**

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In recent years, pulmonary transplantation has become the treatment of choice of several end-stage lung diseases, but is still limited by the scarcity of suitable donors and the lack of reliable prolonged method of lung preservation.¹ Transplantation of lung harvested since more than 6 hours should lead to an increased incidence of primary lung graft dysfunction, due in part to ischemic damage of pulmonary cell structure and metabolism, and to acute reperfusion injury. However, very little is known about the real mechanisms of pulmonary cell injuries before, during, and after lung transplantation. Ischemia promotes intracellular depletion of high-energy compounds, and is associated with loss of normal mitochondrial function and characteristics.² Mitochondrial ischemic dysfunction may contribute to cell death by one of two processes: (1) failure of oxidative phosphorylation with loss of mitochondrial adenosine triphosphate (ATP) synthesis leading to cellular ATP depletion; and (2) a permeability transition of the inner mitochondrial membrane, leading to cell death independently of cellular ATP concentrations. The aim of this present study was to investigate the consequences of warm and cold ischemia on the oxidative phosphorylation function in mitochondria isolated from lungs.

MATERIAL AND METHODS

Surgical procedure

Twenty-three Pure Pietrain pigs weighing 25 to 30 kg were anesthetized (pentobarbital; sufentanyl), paralysed (pancuronium) and ventilated with room air. After systemic heparinization (300 U/kg), 500 µg of prostaglandin E1 (PGE1) were injected intravenously.

Control and cold ischemia groups: The lungs were then flushed with 2,000 ml of cold (4°C) standard Euro-Collins solution (ECS). The ventilation was continued during the flush. After completion of the flush, the ventilation was discontinued, and the lungs were isolated and stored in inflation in cold (4°C) ECS. We analysed the respiratory function of the mitochondria isolated from these lungs immediately after harvesting (control group, n=5), or submitted to 24 hr or 48 hr of cold ischemia (CI) (24 hr CI group, n=5, and 48 hr CI group, n=5, respectively).

Warm ischemia groups: After heparinization and PGE1 injection, the pigs were euthanased by intravenous injection of a lethal sodium pentobarbital bolus, and the ventilation was discontinued. After 30 min or 45 min of warm ischemia (WI) (30 min WI group, n=5, and 45 min WI group, n=5, respectively), the ventilation was restarted, and the lungs were flushed by 2,000 ml of ECS and harvested. In these WI groups, mitochondria isolation was started immediately after the WI period.

Combined warm and cold ischemia groups: After 30 min of WI, the lungs of these groups were harvested and stored in inflation in cold (4°C) ECS. The mitochondria were then isolated after 24 hr or 48 hr of CI (30 min WI + 24 hr CI group, n=5, and 30 min WI + 48 hr CI group, n=5, respectively).

Mitochondrial Isolation and Respiratory Function Assessment

The respiratory parameters were determined on isolated mitochondria by in vitro measurement of oxygen consumption rates in an hypotonic medium at pH 7.4 (15 mM KCl, 2 mM ()EDTA, 5 mM MgCl₂, 50 mM Tris.Cl), in the presence of 1% fatty acid free bovine serum albumine (BSA), with 5 mM ketoglutarate and pyruvate as oxidizable substrates, and 2.5 mM phosphate. The added ADP concentration is 2.5 mM, 16 μg/ml of oligomycin and 5 mM of uncoupler () FCCP. For each adenosine diphosphate (ADP) pulse, 330 nanomoles of ADP were added. Functional parameters of mitochondria were defined as: (1) respiration rate in the presence (V_3) or in the absence (V_4) of externally added ADP; (2) respiratory control (RC) given by the ratio V_3/V_4 ; (3) respiration rate when the ATP synthase is blocked by oligomycin (V_{Olig}); (4) respiration rate in the presence of uncoupler FCCP (V_{FCCP}); (5) uncoupled respiratory control (URC) given by the ratio V_{FCCP}/V_{Olig} ; (6) the yield of the oxidative phosphorylation i.e. the number of moles of ADP phosphorylated by Atg of oxygen consumed (ADP/O).

Statistics

All results were expressed as the mean \pm SEM. Statistical significance was assumed for a *P*-value less than 0.05.

RESULTS

Related to control group, 24 hr CI group developed a significant decrease in V_3 (27%), V_3S (29%), V_{FCCP} (32%), V_4 (16%), RC (10%), RCs (15%), and URC (14%). There was no differences in V_{Olig} and ADP/O. Related to 24 hr CI group, 48 hr CI group underwent a

significant decrease in RC, RCs and ADP/O, and no differences in VFCCP, VOlig, and URC. Related to control group, 30 min WI group underwent a significant decrease of V3s (13%), and the other parameters were not significantly modified. Related to control group, 45 min WI group developed a highly significant ($p < 0.01$) decrease in V3 (30%), V3S (20%), RC (27%), RCs (21%), and ADP/O (30%). VFCCP, VOlig and URC were not modified. Related to the 30 min WI group, 24 hours of CI added to 30 min of WI promoted a decrease in V3 (10%) and V4 (9%), and no significant changes in the other parameters, and 30 min WI + 48 hr CI group developed a decrease in V3 (30%), V3S (31%), VFCCP (29%), V4 (15%), RC (18%), RCS (20%), URC (20%) and ADP/O (7%).

DISCUSSION

We developed an original model of respiratory function analysis of mitochondria isolated from swine lungs. In this model, both cold and warm ischemia induced significant alterations of the respiratory functions of pulmonary mitochondria. After 24 hr of CI, the changes in the respiratory parameters indicated a decrease of the oxidoreductase activity, and 48 hr of CI promoted a decrease in the oxidative phosphorylation functions. The lesions induced by 30 min of WI seem to be moderate. However, 45 min of WI induced significant changes in the function of the oxidoreductases, as deleterious for the pulmonary mitochondria than 24 hr of CI. The combination of 30 min WI and 24 hr or 48 hr of CI does not seem to add significant changes in the mitochondrial respiratory functions, related to 24 hr or 48 hr of CI alone, respectively. These results should be a major concern in the issue of the procurement of lung grafts from non-heart beating donors (NHBD).³ Our model of WI corresponds to the Category 3 defined by Kootstra et al.,⁴ as organs retrieved from NHBD dying in the intensive care unit from "withdrawal of support", so called "ventilator switch-off" or "controlled NHBD". In this category, the WI

duration may be kept very short, below 30 min, and this short WI might allow the use of the lungs for transplantation.

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