Delayed fluorescence lifetime technique; exploring the use of PpIX for monitoring cellular metabolism in different types of tissue for clinical applications

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Introduction

Measurement of mitochondrial oxygen tension (MitoPO2) could be a powerful tool for monitoring metabolism; oxygen supply and con- sumption provide insight into cellular function. In clinical syndromes such as sepsis but also in isolated organ dysfunction, transplantation procedures and hypoxic environments, such as tumorous tissue, oxygen supply is limited (1, 2). Insight can reveal much about the clinical consequences and mechanisms; such as tumours' resistance to hypoxia (2). In this study we explore oxygen dependent phospho- rescent lifetime measurements through protoporphyrine IX for determining MitoPO2 in several tissues to further develop this tech- nique for clinical use.

Methods

Animal experiments conducted in accordance to institutional guidelines were performed on 18 anesthetized (ketamine 90 mg/kg, medetomidine 0.5 mg/kg, atropine 0.05 mg/kg) male Wistar rats (284gr ± 17grams, Harlan, Horst, The Netherlands). A tracheostomy was placed for mechanical ventilation and arterial Carotid and venous Jugular line were installed for monitoring of arterial blood pressure and heart rate, blood samples (metabolic and respiratory monitoring) and continuous NaCl infusion (15 ml/kg/h). MitoPO2 measurements by delayed fluorescence lifetime technique utilizing endogenous mitochondrial protoporphyrine IX (PpIX) which was ALA enhanced (5-aminoleulinic acid, Sigma-Aldrich) (1, 3, 4). Oxygen dependent quenching of fluorescence emission was recorded (3, 5). Surgical midline laparotomy provided peritoneal access.

Results

MitoPO2 measurements during normoxia, hyper- and hypoxia are shown for kidney, liver and intestinal tissue (Fig. 1). Control sample times (t1–t3) are corresponding to those applied in the FiO2 steps. Arterial blood gases are taken to compare and confirm proper FiO2 ventilation (normoxia 182 mmHg ± 17, hyperoxia 486 mmHg ± 49, and hypoxia 87 mmHg ± 4). Stabile normoxic PO2 values are shown

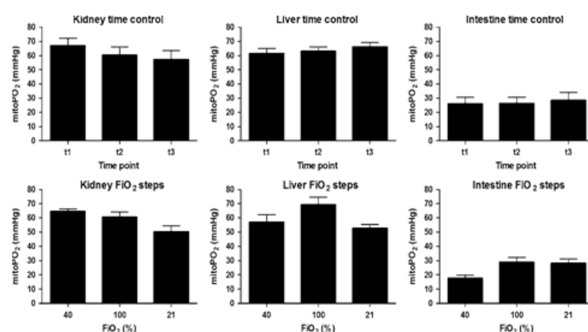

Fig. 1 MitoPO2 during normoxia versus varied FiO_2 (40, 100 and 21 oxygen %) in kidney, liver and intestine (ileum). Results show FiO2 dependent MitoPO2 values during hyper- and hypoxia. Normoxia sample times (t1–t3) are conform FiO_2 steps

in during normoxia. Hyper- and hypoxic FiO2 ventilation shows clear deviation from controls.

Discussion

Hyper- and hypoxic FiO2 PO2 clearly deviate from control and FiO2 readings meaning oxygen dependency of the delayed fluorescence signal. Our findings of tissue MitoPO2 match those found in other literature (1, 4, 6). Delayed fluorescence lifetime measurements for determining MitoPO2 seems to be a promising technique to gain insight into cellular metabolism. Further research and adjustments to enhance the technique

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