Cutaneous mitochondrial respirometry in rats

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Introduction

Adequate supply of oxygen to tissues and the subsequent use of oxygen by the mitochondria are paramount for sustaining cellular integrity. Monitoring of these parameters on the cellular level in vivo has not been possible till to date. The PpIX triplet state lifetime measurement (PpIX-TSLM) technique for measurement of mitochondrial oxygen tension (mitoPO2) in vivo provides a potential means to do so [1–2]. In this pilot study we describe the use of this technique for measuring mitoPO2 and oxygen consumption in skin. Methods

Animals

A total of 4 male anesthetized and mechanically ventilated male Wistar rats (Charles River, Wilmington, MA, USA,) body weight 281 ± 6.3 g) were used in this pilot study.

Measurements

The background of the PpIX-TSLM is described in more detail by Mik et al. [1–2] Briefly, PpIX is induced in the mitochondria by administration of 5-aminolevulinic acid (ALA) (Fig. 1a). PpIX pos- sesses a triplet state (T1) of which the lifetime is oxygen-dependent (Fig. 1b). Population of T1 occurs upon photo-excitation with a pulse of light, and bidirectional intersystem crossing causes the emission of oxygen-dependent red delayed fluorescence. This lifetime is related to mitoPO2 according to the Stern- Volmer equation, in which kq is the quenching constant and s0 is the lifetime at zero oxygen (Fig. 1c).

To induce PpIX, 2.5 % ALA cream was applied to the abdominal skin of rats. Oxygen consumption was determined by repeated mitoPO2 measurements (one flash every 3 s) during blockage of the oxygen supply by applying local pressure with the measurement probe. This pressure temporally occludes the microvessels and gradually prolongs the delayed fluorescence lifetime due to oxygen consumption. The oxygen consumption was calculated from the slope of the decrease in mitoPO2 (Fig. 2).

Results

Oxygen disappearance curves were acquired at de abdominal skin of 4 rats. One of the PO2 time courses recorded before, during, and after a 1.5-min compression period is show in Fig. 3. The results of the all measurements are shown in Table 1.

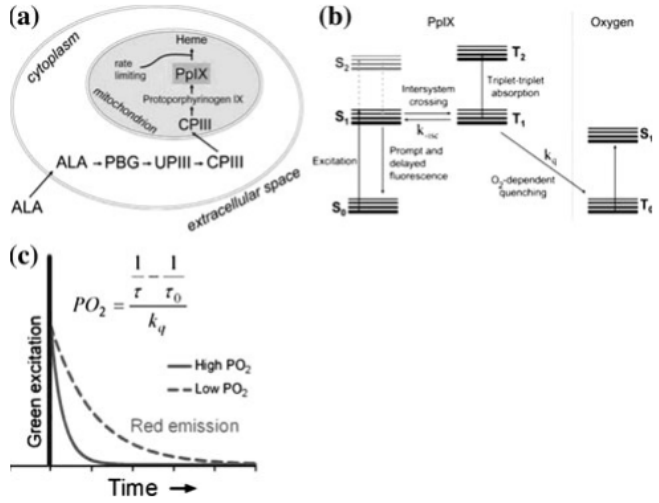

Fig. 1 Principle of mitoPO$_2$ measurement by oxygen-dependent quenching of ALA enhanced PpIX. **a** Principle by which ALA administration enhances mitochondrial PpIX levels. **b** Jablonski diagram of states and state transitions of PpIX and its interaction with oxygen. **c** PpIX emits delayed fluorescence after excitation by a pulse of *green* (510 nm) light. The delayed fluorescence lifetime is oxygen-dependent according to the Stern–Volmer equation, in which kq is the quenching constant and τ0 is the lifetime at zero oxygen

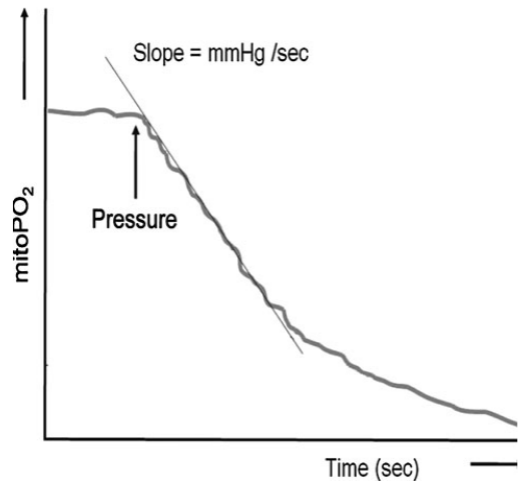

Fig. 2 Principle of mitochondrial respiration by mitoPO$_2$ kinetics

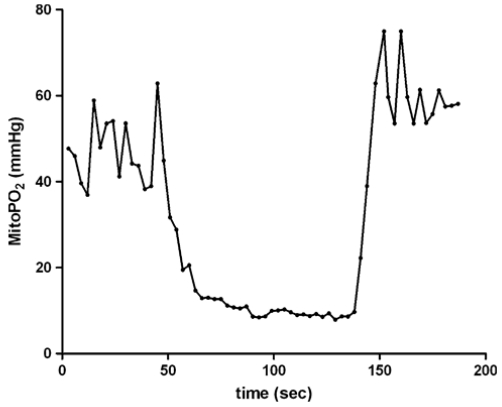

Fig. 3 A typical time course of mitochondrial PO$_2$ during the microcirculation compression experiment in de skin of a rat. The pressure was introduced at 50 s after the beginning of the PO$_2$ record and released at 140 s. The baseline mitochondrial PO$_2$ (PO$_2$ during the first 50 s) in this example was 46 ± 6.9 mmHg, the slope −2.15 mmHg/s

Table 1 Parameters of mitochondrial cutaneous oxygen consumption

Rat number	Baseline MitoPO$_2$ (mmHg)	Slope (mmHg/s)
Rat 1	46 ± 6.9	−2.15
Rat 2	58 ± 10	−3.25
Rat 3	47 ± 2.1	−2.32
Rat 4	30 ± 8.2	−2.38

Conclusion

This pilot study shows that PpIX-TSLM is an optical spectroscopic method that allows assessment of mitochondrial oxygenation and oxygen consumption, based on measurement of the oxygen-dependent triplet state lifetime of endogenous protoporphyrin IX (PpIX). We aim at further developments of this technique to ultimately enable measurements in humans. This will provide clinicians with novel technology that allows for the first time assessment of oxygen tension and oxygen consumption at the subcellular level, and permits a look at oxygen supply and demand at the place where it matters most, the mitochondria.

References

[1] E.G. Mik, T. Johannes, C.J. Zuurbier, A. Heinen, J.H. Houben- Weerts, G.M. Balestra, J. Stap, J.F. Beek and C. Ince. In vivo mitochondrial oxygen tension measured by a delayed fluores- cence lifetime technique. Biophys J 95, 3977–3990 (2008).

[2] E.G. Mik, J. Stap, M. Sinaasappel, J.F. Beek, J.A. Aten, T.G. van Leeuwen and C. Ince. Mitochondrial PO2 measured by delayed fluorescence of endogenous protoporphyrin IX Nat Methods 3, 939–945 (2006)