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BAKER SCIENTIFIC APPLICATION BULLETIN

Physiological oxygen and its importance in redox biology

Reactive oxygen species (ROS); oxidative stress; oxygen; physoxia; metabolism, inflammation, Good Scientific Practice

Krista Rantanen, Ph.D. Director of Scientific Applications

CONTENT

When cellular oxygen levels change, the first response is metabolic: a response that rapidly translates into physiological changes. The central event for metabolic activity in most cells is mitochondrial oxidative phosphorylation, both in ATP production and oxygen consumption. Treating cells in aberrant levels of oxygen during experiments has direct effects on the function of oxidative phosphorylation thus perturbating all cellular events. In attempt to detect and understand subtle cellular reactions, attention needs to be paid to the basic conditions of the cells in order to establish solid grounds for translational results.

Oxidative environment has forced aerobic life forms to adapt, and in order to distribute oxygen to various tissues and organs, complex respiratory systems have evolved. The ambient atmospheric contains about 21% of O2 but when inhaled the O2 levels fall gradually when reaching organs and tissues. O2 utilization through metabolism as well as the rate of capillary blood flow dictates its distribution among tissues (Brahimi-Horn et al).

For the sake of convenience, cell culture is most often done in ambient O2 and so the in vitro cells grow in 2 – 10 -fold higher O2 concentrations than what cells in vivo ever experience (Atkuri et al). Strong bodies of evidence shows that cell culture done in such high O2 leads to unbalanced production of reactive oxygen species (ROS), hydrogen peroxide, hydroxyl radicals and singlet oxygen (Halliwell et al), all of which are highly damaging to cells.

Even though cells are capable processing some ROS and maintaining an optimal redox environment, in ambient oxygen cell culture systems ROS production greatly exceeds cellular capacity to process them. In in vivo conditions where O2 levels are at their peak (between 10-13%), the highest ROS production (approximately 1-4% of O2 consumed) occurs in the mitochondrial respiratory chain and is controlled by moderating the flow rate of electrons through respiratory chain complexes. This production level equals to roughly 1 billion ROS molecules produced per cell per day. When cells are grown in vitro in 21% O2 this production increases several fold, leading to irreversible damage to biomolecules (Sesti et al). Conformational changes of proteins caused by peroxyl radical and cysteine residue oxidation under oxidative stress have been widely reported. As an example, entire signaling cascades can be dysregulated due to protein kinase and phosphatase damage leading to untraceable amount of changes in cellular functions (Fedorova et al).

Mouse embryonic fibroblasts (MEFs) have been shown to increase their growth rate and saturation densities in 3% O2 versus that in 20% of O2. Moreover, MEFs lacked any signs of

normal replicative senescence even after 60 population doublings in the lower oxygen condition (Parrinello et al). Studies done in human peripheral blood mononuclear cells (PBMC) show that at 21% O2, cells were continuously sending out inflammatory signals whereas the same cells grown under physiological oxygen levels of 5% and 10% displayed very little inflammatory signaling.

This phenomenon is due to the fact that prolonged ROS generation, as is the case in ambient O2 (21%), contributes to chronic inflammation.

Chronic inflammation in turn induces oxidative stress and reduces cellular anti-oxidant function (Sesti et al; Atkuri et al).

Taken together, cell culture done in physiological O2 levels lessens variation in results and in general will be in accordance with Good Scientific Practice. However, practical issues have so far existed. In organs and tissues, O2 distribution is varied. Many organs exhibit O2 gradients that is the result of differences in capillary blood supply (Brooks et al; Wang et al; Wild et al). O2 consumption rate, culture dish shape, cell density, all give great experimental variation and make physoxic cell culture seem too impractical. However, care taken in the initial steps of an experiment and experimental design is the only way to ensure biologically relevant, reproducible, economically justified and translatable results.

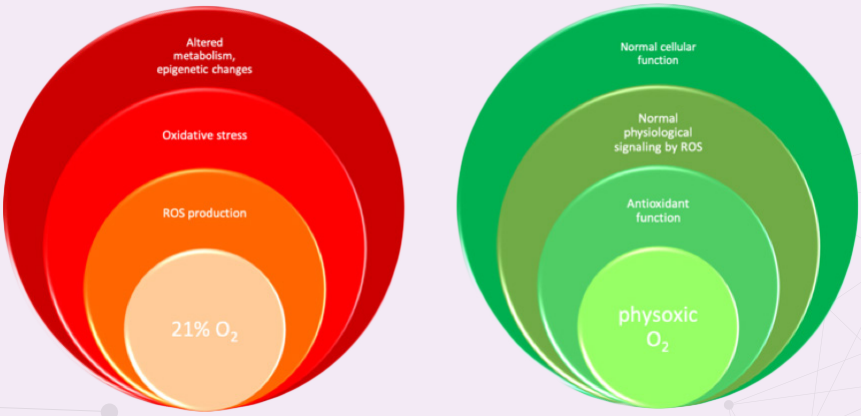

Figure 1. Summary of ROS – mediated consequences of excess vs physiological oxygen levels

WHAT TO LOOK FOR?

- angiogenesis
- immune response
- apoptosis
- synaptic communication
- protein methylation
- cancer metastasis
- inflammation
- tumour metabolism
- tumour metastasis
- cellular immortalization
- anaerobic glycolysis
- oxidative damage

HOW TO CONTROL OXYGEN IN AN EXPERIMENT?

Using instruments designed to produce and maintain optimal, controlled biomimicking experimental conditions, one ensures that all cellular responses occur as they do in vivo.

REFERENCES

Brahimi-Horn MC, Pouysségur J. Oxygen, a source of life and stress. FEBS Lett. 2007 Jul 31; 581(19):3582-91.

Atkuri KR, Herzenberg LA, Herzenberg LA. Culturing at atmospheric oxygen levels impacts lymphocyte function. Proc Natl Acad Sci U S A. 2005 Mar 8; 102(10):3756-9.

Halliwell B. Cell culture, oxidative stress, and antioxidants: avoiding pitfalls. Biomed J. 2014 May-Jun; 37(3):99-105.

Parrinello S, Samper E, Krtolica A, Goldstein J, Melov S, Campisi J. Oxygen sensitivity severely limits the replicative lifespan of murine fibroblasts. Nat Cell Biol. 2003 Aug;5(8):741-7.

Sesti F, Tsitsilonis OE, Kotsinas A, Trougakos IP. Oxidative stress-mediated biomolecular damage and inflammation in tumorigenesis. In Vivo. 2012 May-Jun; 26(3):395-402.

Fedorova M, Bollineni RC, Hoffmann R. Protein carbonylation as a major hallmark of oxidative damage: update of analytical strategies. Mass Spectrom Rev. 2014 Mar-Apr; 33(2):79-97.

Brooks AJ, Eastwood J, Beckingham IJ, Girling KJ. Liver tissue partial pressure of oxygen and carbon dioxide during partial hepatectomy. Br J Anaesth. 2004 May; 92(5):735-7.

Wang W, Winlove CP, Michel CC. Oxygen partial pressure in outer layers of skin of human finger nail folds. J Physiol. 2003 Jun 15; 549(Pt 3):855-63.

Wild JM, Fichele S, Woodhouse N, Paley MN, Kasuboski L, van Beek EJ. 3D volume-localized pO2 measurement in the human lung with 3He MRI. Magn Reson Med. 2005 May; 53(5):1055-64.

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