ROS

REVIEW ARTICLES

Oxygen and Oxygen Toxicity: The Birth of Concepts

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ABSTRACT | Molecular dioxygen (O_2) is an essential element of aerobic life, yet incomplete reduction or excitation of O_2 during aerobic metabolisms generates diverse oxygen-containing reactive species, commonly known as reactive oxygen species (ROS). On the one hand, ROS pose a serious threat to aerobic organisms via inducing oxidative damage to cellular constituents. On the other hand, these reactive species, when their generation is under homeostatic control, also play important physiological roles (e.g., constituting an important component of immunity and participating in redox signaling). This article defines oxygen and the key facts about oxygen, and discusses the relationship between oxygen and the emergence of early animals on Earth. The article then describes the discovery of oxygen by three historical figures and examines the birth of the concepts of oxygen toxicity and the underlying free radical mechanisms. The article ends with a brief introduction to the emerging field of ROS-mediated redox signaling and physiological responses.

KEYWORDS | Oxygen; Oxygen toxicity; Reactive oxygen species; Redox signaling

ABBREVIATIONS | CNS, central nervous system; EPR, electron paramagnetic resonance; GOE, Great Oxidation Event; ROS, reactive oxygen species; SOD, superoxide dismutase

CONTENTS

1. OXYGEN: DEFINITIONS AND KEY FACTS

1.1. Definitions

Oxygen, if not specified, may refer to: (i) element oxygen (O), which is also known as oxygen atom with an atomic number of 8 and is a member of the chalcogen group on the periodic table; (ii) ground (triplet) state molecular dioxygen (O_2), which is commonly called molecular oxygen or simply, oxygen or oxygen gas; or (iii) ozone (O_3), which is a pale blue gas with a distinctively pungent smell and is formed from O_2 by the action of ultraviolet light as well as atmospheric electrical discharges.

Ozone is also known as trioxygen and is an allotrope of oxygen (see Section 1.3) that is present in low concentrations throughout the Earth's atmosphere. It has been suggested that ozone may be generated by antibodies via a water oxidation pathway and may play a role in phagocyte-mediated innate immunity [1–3]. Ozone is also present in atherosclerotic lesions and possibly formed by the antibodies and immune cells located there. The locally generated ozone might contribute to plaque formation by oxidizing cholesterol [4].

1.2. Abundance

Oxygen is a highly reactive nonmetallic element that readily forms compounds with most other elements. By mass, oxygen is the third most abundant element in the cosmos after hydrogen and helium. It is the most abundant chemical element by mass in the Earth's biosphere, air, sea, and land, constituting ~46−47% of the Earth's crust and ~86−87% of the world's oceans.

Molecular dioxygen is the second most common component of the Earth's atmosphere, taking up about 21% of its volume and 23% of its mass. Oxygen is also the most abundant element in the human body, accounting for approximately 65% of total body mass.

1.3. Isotopes and Allotropes

Naturally occurring oxygen exists as three stable isotopes, namely, ^{16}O, ^{17}O, and ^{18}O, with ^{16}O being the most abundant form (~99.8% of natural abundance). Radioactive, short-lived isotopes of oxygen with mass numbers ranging from ^{12}O to ^{26}O have also been identified [5].

Allotropes are different forms of the same chemical element that exhibit different physical and chemical properties. There are several known allotropes of oxygen, with O_2 being the most familiar one. The second most familiar allotrope is the highly reactive ozone (also see Section 1.1). Other allotropes of oxygen include tetraoxygen (O_4) and the dark-red solid oxygen O_8, both of which are formed under high pressures [6, 7].

1.4. Chemical and Physical Properties of O_2

1.4.1. General Properties

The molecular weight of O_2 is 31.9988 g/mol. O_2 gas is colorless, odorless, and tasteless with a density of 1.429 g/L at 0°C. O_2 changes from a gas to a liquid at a temperature of −182.96°C when it takes on a slightly bluish color. Liquid oxygen can then be solidified or frozen at a temperature of −218.4°C.

1.4.2. Triplet State

An electron configuration with two unpaired electrons, as found in O_2, occupying orbitals of equal en-

ergy is termed a triplet spin state or ground state. Hence, the oxygen in the air we breathe is ground (triplet) state molecular dioxygen.

1.4.3. Paramagnetism

O_2 contains two unpaired electrons and is thus paramagnetic. Paramagnetism refers to the magnetic state of a chemical species with one or more unpaired electrons. The unpaired electrons are attracted by a magnetic field due to the electrons' magnetic dipole moments. The paramagnetic feature makes it possible to detect and measure O_2 in biological systems using electron paramagnetic resonance (EPR) spectrometry, a specific technique for detecting radicals.

2. OXYGEN AND THE EMERGENCE OF ANIMALS ON EARTH

Most of us take our richly oxygenated world for granted and expect to find oxygen everywhere–after all it makes up ~21% of the modern atmosphere (also see Section 1.2). But free oxygen, at levels mostly less than 0.001% of those present in the atmosphere today, was anything but plentiful during the first half of Earth's 4.5-billion-year history [8].

The atmosphere of Earth was anaerobic until the advent of oxygenic photosynthesis. The rise of oxygen in Earth's early atmosphere and ocean led to the emergence and diversification of animals [9, 10]. Accumulating evidence suggests a permanent rise to appreciable concentrations of oxygen in the atmosphere sometime between 2.4 and 2.1 billion years ago. This increase, now popularly known as the Great Oxidation Event (GOE), left clear fingerprints in the rock record [8], and is believed to lead to the emergence of multicellular organisms and earliest animals on Earth [11].

3. DISCOVERY OF OXYGEN

Three historical figures are generally credited with the discovery of oxygen. They are Carl Wilhelm Scheele, Joseph Priestley, and Antoine-Laurent Lavoisier (**Figure 1**).

3.1. Carl Wilhelm Scheele

Between 1771 and 1772, Scheele (1742−1786), a Swedish chemist, did a series of experiments with

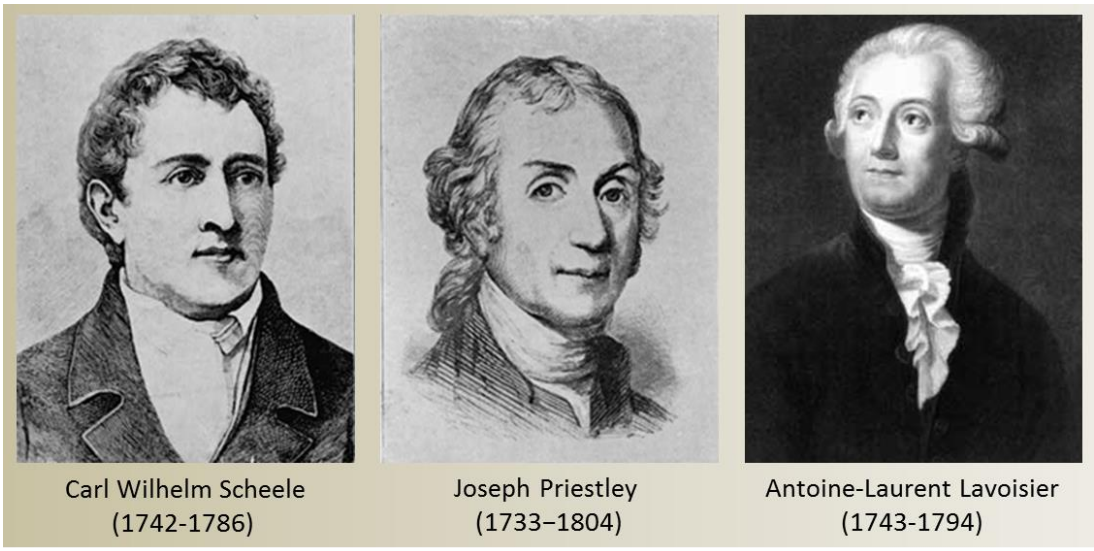

FIGURE 1. Historical figures who discovered oxygen. Carl Wilhelm Scheele, Joseph Priestley, and Antoine-Laurent Lavoisier are generally credited with the discovery of oxygen (source: Library of Congress; www.loc.gov).

mercuric oxide and potassium nitrate. On heating the two he obtained a gas that caused candles to burn more brightly. He did not, however, rush to publish his observation until 1777.

3.2. Joseph Priestley

Working independently in 1774, Joseph Priestley (1733−1804), an English radical Unitarian minister and chemist, observed that a gas was liberated when he heated the mineral mercuric oxide. In the atmosphere of this gas, a candle burned more brightly and a mouse could live longer than when sealed in a comparable volume of ordinary air. He reported this remarkable experiment in 1775, 2 years earlier than did Scheele (also see Section 3.1). Priestley did not have much training in chemistry, and was unable to abandon the phlogiston theory of his time (the theory stated that all combustible material contains something known as a phlogiston, that on heating, is transformed into fire). By heating a burnt substance, such as mercury oxide, Priestley believed that he was removing phlogiston from the atmosphere into the mercury, thereby purifying the air. As such, he referred to the gas as "dephlogisticated air".

3.3. Antoine-Laurent Lavoisier

Inspired by Priestley's experiment conducted in 1775, Lavoisier (1743−1794), a French chemist, began his own experiment with the gas during the same year, and came to agree that air contained an element that supported life and combustion. Lavoisier noted the element's tendency to form acids by combining with many different substances and thereby incorrectly believed the element to be a component of all acids, and hence named the gas oxygen ("*oxygène*" in French), meaning acid former. In fact, the word oxygen comes from Greek words: oxus (acid) and gennan (generate).

4. DISCOVERY OF OXYGEN TOXICITY

4.1. An Evolutionary View

The accumulation of O_2 in the Earth's atmosphere as well as the ocean changed the environment for, and therefore changed the selection pressures on, all living organisms. It also increased the mutation rate and therefore hastened subsequent evolution. Advantages could be gained by using O_2 to increase the useful energy derivable from foodstuffs, to carry out novel metabolic transformations, to solubilize and detoxify numerous compounds, and even to generate heat and light [12]. But there was a price to pay for these benefits and that was to provide defenses against the considerable toxicity of oxygen, a paramagnetic gas (also see section 1.4.3). Those organisms that succeeded in developing the requisite defenses could reap the benefits, and they gave rise to the enormous variety of aerobic life forms that are now so evident on Earth. Those that could not accommodate to the challenge of oxygen toxicity evolved into anaerobic organisms [12] (**Figure 2**). Interestingly, a recent study reports that multicellular animals (metazoans) live in deep hypersaline anoxic basins of the Mediterranean Sea, suggesting that certain forms of multicellular animals may live in permanently anoxic conditions [13].

4.2. Joseph Priestley's Conjecture on Oxygen's Harm

Priestley, who discovered oxygen, an element essential to life, was himself among the first to suggest that there might be adverse effects of the gas. In 1775, he conjectured that "though pure dephlogisticated air [oxygen] might be very useful as a medicine [for sick persons], it might not be so proper for us in the usual healthy state of the body; for, as a candle burns out much faster in dephlogisticated than in common air, so we might, as may be said, live out too fast, and the animal powers be too soon exhausted in this pure kind of air". Priestley's speculation on oxygen toxicity was subsequently proved correct, and it is now well-established that oxygen therapy is like a two-edged sword–at one edge, oxygen is essential for human survival, whereas at the other edge, the same gas may cause injury at an elevated partial pressure [14].

4.3. Historical Overview of Oxygen Toxicity in Experimental Animals

The first important contribution to oxygen toxicity was made in 1878 by Paul Bert (1833−1886), a French physiologist. His pioneering work has withstood the test of time in a most impressive manner. He showed that oxygen at increased pressures was

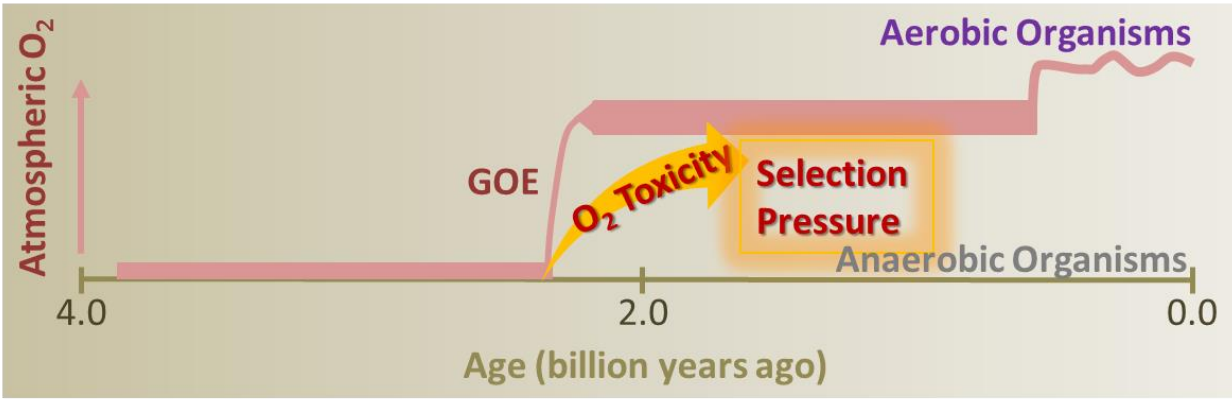

FIGURE 2. Oxygen and emergency of animals on Earth. The increase of oxygen in the atmosphere 2.4−2.1 billion years ago, commonly known as the Great Oxidation Event (GOE), is believed to lead to the emergence of earliest animals on Earth. On the other hand, oxygen toxicity is thought to exert evolutional pressure contributing to the diversification of animals. Organisms that could not accommodate to the challenge of oxygen toxicity evolved into anaerobes. This scheme is based on Ref. 8.

highly poisonous and that no living matter was exempt. Larks exposed to 15–20 atmospheres of air convulsed and finally died. In a large series of experiments, Bert showed that the oxygen tension was the decisive factor in the immediate effect of air or of any mixture of nitrogen and oxygen. This is why the central nervous system (CNS) effect of hyperbaric oxygen is sometimes referred to as the "Paul Bert effect". Subsequently in 1899, James Lorrain Smith (1862−1931), a Scottish pathologist demonstrated that animals breathing oxygen at moderately high tensions over prolonged periods suffered from severe and finally fatal pulmonary damage, and as such, the pulmonary oxygen toxicity is also known as the "Lorrain Smith effect" [15]. In fact, the CNS and pulmonary effects remain as the major features of oxygen toxicity in both experimental animals and human subjects.

4.4. Historical Overview of Oxygen Toxicity in Humans

Although oxygen therapy has been used in medical practice for over two centuries, the recognition of oxygen toxicity as an important clinical problem is relatively recent. Reports in the early 1950's linked oxygen therapy to retrolental fibroplasia (also known as retinopathy of prematurity) in premature infants [16]. It was shown in the early 1970s that breathing 50–100% oxygen at one atmosphere was potentially toxic to the lungs [17]. Since then, the toxic effects of oxygen on other organs and systems of the body have also been recognized. These include the eyes, liver, heart, kidneys, blood, and endocrine system [18]. Meanwhile, progress has been made to understand the molecular basis of oxygen toxicity.

4.5. Free Radical Mechanisms of Oxygen Toxicity

Although the harmful effects of oxygen were noticed in 1878, insights into the molecular mechanisms of oxygen toxicity were provided in several articles published during the 1950's and 1960's. In 1954, Rebeca Gerschman and associates published an article in the prominent journal Science, hypothesizing that oxygen poisoning and radiation injury have at least one common basis of action, possibly through the formation of oxidizing free radicals [19]. Another important event in the investigation of oxygen toxicity was the finding by Joe M. McCord and Irwin Fridovich in 1969 of an enzymatic function of a protein containing both copper and zinc, which then was known alternatively as erythrocuprein, hepatocuprein, or cerebrocuprein [20]. The function of this enzyme is the catalysis of dismutation of superoxide anion radical ($O_2^{\bullet-}$) (superoxide for simplicity) to produce hydrogen peroxide and molecular oxygen, and the enzyme is known as superoxide dismutase (SOD).

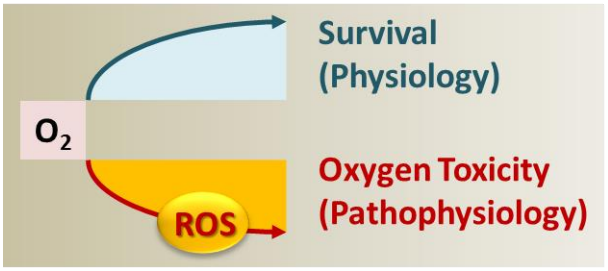

FIGURE 3. Free radical mechanisms of oxygen toxicity. On the one hand, oxygen is essential for aerobic life. On the other hand, utilization of oxygen also inevitably results in the formation of reactive oxygen species (ROS). The ROS, when their formation is abnormally increased, may cause oxidative damage to cellular constituents, which constitutes the molecular basis of oxygen toxicity.

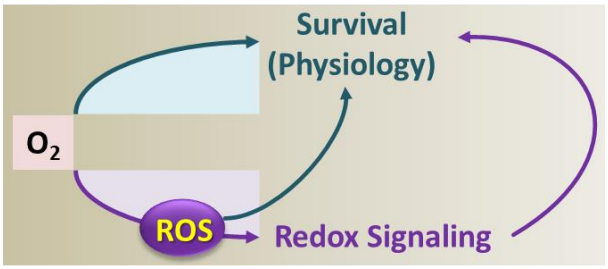

FIGURE 4. Reactive oxygen species (ROS) in physiology. The involvement of ROS in physiology is two-fold: On the one hand, ROS derived from O_2 contribute to killing of pathogenic microorganisms by phagocytic cells, thereby promoting the survival of the host. On the other hand, ROS act as second messengers to activate cell signal transduction, leading to desired physiological responses.

This discovery has triggered extensive investigations into the free radical mechanisms of oxygen toxicity (**Figure 3**). Substantial evidence accumulated over the past several decades suggests a critical involvement of free radicals and related reactive species, especially ROS, in the pathophysiology of a wide variety of human diseases as well as drug action and toxicity [21–23].

4.6. Discovery of ROS Physiology

Four years after the discovery of SOD by McCord and Fridovich, Bernard Babior and colleagues reported in 1973 that one of the principal bactericidal actions of leukocytes was the enzymatic generation of superoxide [24]. Now we know that superoxide formed from the NAD(P)H oxidase of phagocytic cells during respiratory burst and the resulting hydrogen peroxide and hypochlorous acid constitute an important mechanism of innate immunity against pathogenic microorganisms [25–27]. More recently, mitochondria-derived ROS are shown to also be required for antigen-specific T cell activation [28]. In addition to the antimicrobial activity, ROS have been demonstrated to play an important role in redox regulation of cell growth, differentiation, apoptosis, autophagy, and senescence [29–31]. Notably, self-renewal of certain stem cells may actually require ROS [32, 33]. Elucidation of the physiological roles of ROS along with their harmful effects over the past decades has greatly broadened the concepts of oxidative stress and redox signaling (**Figure 4**).

5. SUMMARY OF KEY POINTS

- Oxygen, if not specified, may refer to element oxygen (O), ground state (triplet) molecular dioxygen (O_2), or ozone (O_3). O_2 was discovered by Carl Wilhelm Scheele, Joseph Priestley, and Antoine-Laurent Lavoisier over 240 years ago.
- By mass, oxygen is the third most abundant element in the cosmos after hydrogen and helium. It is the most abundant chemical element by mass in the Earth's biosphere, air, sea, and land, as well as the human body.
- Naturally occurring oxygen exists as 3 stable isotopes: ^{16}O, ^{17}O, and ^{18}O, with ^{16}O being the most abundant form. Radioactive, short-lived isotopes of oxygen with mass numbers from ^{12}O to ^{26}O have also been identified. Allotropes of oxygen include O_2 and O_3, as well as the recently identified dark-red solid oxygen O_8.
- O_2 has two unpaired electrons and is paramagnetic. As such, this gas can be measured by using electron paramagnetic resonance (EPR) spectrometry.
- A permanent rise of oxygen in the atmosphere sometime between 2.4 and 2.1 billion years ago, known as the Great Oxidation Event (GOE), is

believed to lead to the emergence of multicellular organisms and earliest animals on Earth.

- The accumulation of O_2 in the Earth's atmosphere as well as the ocean changed the environment for, and therefore changed the selection pressures on, all living organisms. Metabolic advantages, such as efficient production of energy from foodstuffs could be gained by using O_2, but the organisms would need to pay a price– that was the evolvement of defenses against oxygen toxicity.
- Organisms that succeeded in developing the requisite defenses could reap the benefits, and they gave rise to the enormous variety of aerobic life forms that are now so evident on Earth. Those that could not accommodate to the challenge of oxygen toxicity evolved into anaerobic organisms.
- Joseph Priestley, who discovered oxygen, an element essential to life, was himself among the first to conjecture in 1775 that there might be adverse effects of this gas.
- The first important contribution to oxygen toxicity was made in 1878 by Paul Bert, who demonstrated the CNS toxicity of high oxygen tensions in animals, and as such, the CNS effect of hyperbaric oxygen is also referred to as the "Paul Bert effect".
- In 1899, James Lorrain Smith demonstrated the pulmonary injury caused by high oxygen tensions in experimental animals, and, hence, pulmonary oxygen toxicity is also known as the "Lorrain Smith effect".
- Recognition of oxygen toxicity as a clinical problem began in the early 1950's, and now oxygen toxicity is a well-recognized clinical problem of oxygen therapy. Also in the 1950's, scientists began looking into the molecular mechanisms of oxygen toxicity. The subsequent discovery of SOD in 1969 triggered an explosion of investigations on free radical mechanisms of oxygen toxicity.
- Over the past 4−5 decade since the discovery of SOD, substantial evidence supports ROS as important contributors to the pathogenesis of a wide variety of human diseases. On the other hand, the past 2−3 decades have also witnessed the emergence and evolvement of the concept that ROS also play important physiological roles (e.g., constituting innate immunity and participating in cellular redox signaling). While our knowledge of oxygen chemistry dates back to the 18th century there is still much to learn about the various roles of molecular oxygen-derived species in both normal cellular processes and pathophysiology.

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