Ozone Ameliorates Age-Related Oxidative Stress Changes in Rat Liver and Kidney: Effects of Pre- and Post-ageing Administration

M. H. Safwat1*, M. M. El-Sawalhi1, M. N. Mausouf2, and A. A. Shaheen1

1Biochemistry Department, Faculty of Pharmacy, Cairo University, Kasr Al-Aini Street, Cairo, 11562, Egypt; fax: +202-2363-5140; E-mail: maheerasafwat@yahoo.com; maheerahaafwat@gmail.com; mahaelsawalhi@yahoo.com
2Ozone Therapy Unit, National Cancer Institute, Cairo University, Cairo, Egypt; E-mail: nabil_mawsouf@yahoo.co.uk

Received December 28, 2013

Abstract—The ageing process is known to be accompanied by increased oxidative stress and compromised antioxidant defenses. Controlled ozone administration has been shown to be effective in various pathophysiological conditions with an underlying oxidative burden. Therefore, the present work was carried out to study the role of ozone in counteracting the state of oxidative stress associated with ageing in rat liver and kidneys using two experimental models. In the pre-ageing model, ozone was administered prior to the onset of ageing at adulthood and continued after the start of the ageing process (3-month-old rats until the age of 15 months). While in the post-ageing model, ozone was administered after ageing has begun and lasted for one month (14-month-old rats until the age of 15 months). The pre-ageing ozone administration effectively reduced lipid and protein oxidation markers, namely, malondialdehyde and protein carbonyl levels and decreased lipofuscin pigment deposition in rat liver and kidneys. Moreover, it significantly restored hepatic and renal reduced glutathione (GSH) contents and normalized cytosolic hepatic glutathione peroxidase activity. Similar but less pronounced effects were observed in the post-ageing ozone-treated group. Nevertheless, in the latter model ozone administration failed to significantly affect liver and kidney lipofuscin levels, as well as kidney GSH contents. These data provide evidences for potentially positive effects of pre-ageing ozone therapy in neutralizing chronic oxidative stress associated with ageing in rat liver and kidneys.

DOI: 10.1134/S0006297914050095

Key words: ozone, oxidative stress, liver, kidney, pre-ageing, post-ageing

Ageing is a normal complex physiological process. It is characterized by a variety of morphological and biochemical changes that occur from maturity to senescence. These changes trigger a progressive decline in multiple organ systems, thus rendering the organism more vulnerable to disease and toxicity, eventually leading to death [1, 2]. The process of ageing still remains an unresolved biological problem; it cannot be clarified by a single gene or the decline of a key body system [3, 4].

Many theories have been proposed to explain the phenomenon of ageing based on somatic mutations, accumulation of aberrant proteins, genetic programming, or changes in neural and endocrine functions. Currently, one of the most plausible and acceptable explanations for the mechanistic basis of ageing is the “free radical theory of ageing”. This theory states that free radicals elicit cumulative damage to cellular macromolecules (proteins, lipids, DNA), which in the absence of strong endogenous antioxidant defenses leads to ageing and its related diseases [5, 6]. The “oxidative stress theory” postulates that reactive oxygen species (ROS), rather than free radicals, are responsible for the functional changes that accompany ageing [7]. Oxidative stress may also provide a mechanism upon which other “damage” theories of ageing are based, such as the genomic instability as a result of DNA damage, and the accumulation of glycated crosslinks during protein damage that can result in the pathogenesis associated with cardiovascular and neurodegenerative disease [8, 9].

In the past it was proposed that the liver does not undergo significant ageing changes [10]; however, it has become clear that the liver undergoes substantial alterations in structure and function in old age. The senescent liver exhibits a number of characteristics consistent with oxidative injury, and many studies have shown that ROS tissue level impacts liver functions and is intimately linked to most age-associated diseases [11, 12].

Abbreviations: GPx, glutathione peroxidase; GSH, reduced glutathione; MDA, malondialdehyde; ROS, reactive oxygen species.
* To whom correspondence should be addressed.
One of the organs with high tendency to development of age-dependent tissue injury is the kidney [13, 14]. Excessive oxidative stress has been correlated with many age-dependent changes in kidney such as excessive fibrosis, a general lack of regenerative ability, and an increase in apoptosis [15]. A dramatic increase in the rate of H$_2$O$_2$ production in the kidneys of old rats has been reported by Gomes et al. [16], which indicates a significant increase in oxidative stress in this tissue. Moreover, the ageing kidney is highly subjected to increased lipid peroxidation, enhanced deposition of lipofuscin and advanced glycation end-products (AGEs), and increased apoptosis [17], which might contribute to the pathogenesis and progression of renal disease [18].

Improving the ageing process to achieve healthy ageing and thereby delay the onset and progression of multiple age-related diseases is one of the major challenges in the 21st century [3].

One possible intervention to achieve healthy ageing is ozone therapy, with its versatile uses and routes of administration. For several decades ozone therapy has been known to complement conventional medicine in many conditions such as resistant infections, orthopedic pathologies, as well as vascular, neurodegenerative, and inflammatory disorders. Judicious ozone doses are capable of counteracting oxidative stress by inducing cellular adaptation to it. This phenomenon is known as “oxidative preconditioning” and occurs via certain physiological messengers that are created by ozone and that act to activate numerous biological pathways [19]. The ability of ozone oxidative preconditioning to improve the redox status has been observed in a wide range of pathologies in different animal models such as cisplatin-induced nephrotoxicity [20], hepatic ischemia-reperfusion injury [21], renal ischemia reperfusion injury [22], diabetic nephropathy [23], and coronary artery disease [24].

However, very little is known about the effect of ozone on age-associated changes in various tissues. Two very recent studies conducted in our laboratory have investigated the effect of ozone on reversing certain biochemical alterations associated with ageing in rat cerebral cortex [25] and rat hippocampus and heart [26]. Nevertheless, the effect of ozone on biochemical changes related to ageing in rat liver and kidneys, to the best of our knowledge, has not been studied. Consequently, the present study was carried out to explore the possible anti-ageing effect of ozone administration in neutralizing chronic oxidative stress that accompanies ageing in rat liver and kidneys using two experimental models. In the pre-ageing model, ozone was administered prior to the onset of ageing at adulthood and continued after the start of the ageing process (3-month-old rats until the age of 15 months). In the post-ageing model, ozone was administered after ageing has begun and lasted for one month (14-month-old rats until the age of 15 months). While designing this work, we tried to avoid age-related extremes via comparing between 3- and 15-month-old rat groups. These selected ages exemplify the transition from full maturity to early ageing as it has been proposed that the differences, which emerge during this period, may create the grounds for future senescence [25, 27].

MATERIALS AND METHODS

Ozone generation and administration. Ozone was generated from medical-grade oxygen using an ozone generator system (EXT 120-T; Longevity Resources Inc., Canada). It is a high-quality oxygen-fed ozone generator for ultra-pure medical applications. The ozone concentration is precisely measured by using a built-in UV spectrophotometer set at 254 nm. The ozone obtained by this generator was administered to rats, immediately as generated, by rectal insufflation, performed with a suitable polyethylene cannula connected to a syringe. This route of ozone administration is considered as the most useful and the easiest procedure in rats [28]. The selected ozone dose in the present study was 0.6 mg/kg body weight [29]. In order to produce this concentration, the oxygen flow rate was adjusted to 125 ml/min and the voltage of the ozone generator was adjusted to 2 V.

Experimental animals. Adult male albino rats of Wistar strain (age – 3 months and weight – 180-220 g) were obtained from the animal facility of the National Institute for Vaccination, Helwan, Egypt. The animals were housed under controlled environmental conditions at constant temperature (25 ± 2°C) and a 12/12 h light/dark cycle. The rats were acclimatized to the facility for one week before any experimental procedures and were allowed standard rat chow diet and water ad libitum throughout the experimental period. Animal care was supervised and approved by the Ethical Committee for Animal Experimentation at the Faculty of Pharmacy, Cairo University.

Experimental design. Sixty rats were randomly divided into five experimental groups (n = 12, each). Group 1: aged control group was kept without any treatment until the age of 15 months. Group 2: pre-ageing ozone-treated group in which rats were treated with ozone/oxygen mixture at an ozone dose of 0.6 mg/kg body weight twice weekly for the first three months, then once per week till the age of 15 months. The volume of insufflated mixture was approximately 5 ml. Since the ozone/oxygen mixture consists of 5% ozone and 95% oxygen, it was necessary to test the effect of the oxygen vehicle on the experimental animals. Group 3: pre-ageing oxygen control group received only oxygen in the same manner as in group 2. Group 4: post-ageing ozone-treated group, animals were kept untreated until the age of 14 months, and then they were treated with ozone/oxygen mixture at an ozone dose of 0.6 mg/kg body weight three times weekly for four weeks. Group 5: post-ageing oxygen control group rats.