Porto Biomed. J. 2016;xxx(xx):xxx–xxx

PORTO BIOMEDICAL JOURNAL
WHERE SCIENCE MEETS KNOWLEDGE

Porto Biomedical Journal

http://www.portobiomedicaljournal.com/

Rostrum

Relevance of peroxynitrite formation and 3-nitrotyrosine on spermatozoa physiology

Daniel Filipe Cruz, Margarida Fardilha*

Signal Transduction Laboratory, Institute for Research in Biomedicine – iBiMED, Health Sciences Program, Department of Medical Sciences, University of Aveiro, Aveiro, Portugal

ARTICLE INFO

Article history:
Received 9 May 2016
Accepted 18 July 2016
Available online xxx

Keywords:
3-Nitrotyrosine
Nitric oxide
Peroxynitrite
Spermatozoa
Superoxide anion

ABSTRACT

Infertility is a clinical condition that affects around 15% of reproductive-aged couples worldwide. Around half of these cases are due to male factors, the most owing to idiopathic causes. The increase of reactive oxygen species (ROS), which leads to oxidative stress (OS), has been discussed in the last years as a possible cause of male idiopathic infertility. Superoxide anion ($O_2^{\bullet -}$) and nitric oxide (NO) can react with each other contributing to the formation of peroxynitrite ($ONOO^-$). This molecule can then act on spermatozoa proteins, leading to nitration of protein tyrosines – addition of a nitro (NO_2) group – that is then manifested by the formation of 3-nitrotyrosine (3-NT). In turn, 3-NT may be responsible for the alteration or inactivation of the protein function.

This review will focus on the description of spermatozoa ROS, namely $O_2^{\bullet -}$, NO and $ONOO^-$ and in their contribution to protein tyrosine nitration, namely by 3-NT formation. Previous results about the effect of $ONOO^-$ and 3-NT in spermatozoa will be presented, as well as, the methods that can be performed to detect the protein oxidation by these species. The impact of measuring, at the clinical level, 3-NT, considered a marker of OS, in spermatozoa will be discussed.

Introduction

According to World Health Organization, infertility is defined as a "disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse".[1] Currently, this clinical situation affects around 15% of the reproductive-aged couples worldwide, of which around half are directly or indirectly related with male infertility.[2,3]

In the last years, the increase of oxidative stress, which corresponds to an imbalance between the levels of reactive oxygen species (ROS) and the levels of antioxidant systems, where the ROS are increased, has been indicated as a factor that leads to male infertility.[3–7]

Reactive oxygen species are molecules and free radicals with one unpaired electron derived from molecular oxygen, which, in the ground state, is a bi-radical with two unpaired electrons with the same spin in the outer shell. As the electrons have the same spin, oxygen can only react with one electron at a time. However, if there is a change in any spin of the two electrons, which occurs when one of the two unpaired electrons is excited, the two electrons with opposing spins can quickly react with other pairs of electrons, especially through double bonds, resulting in powerful oxidant species.[8]

Human spermatozoa are characterized by a paucity of cytoplasm, which leads to a lack of an adequate reserve of defensive enzymes. Furthermore, human spermatozoa membranes are mainly constituted by polyunsaturated fatty acids. The combination of these two characteristics makes these cells very sensitive to oxidative attack by ROS that are generated by neutrophils or by defective or immature spermatozoa.[9–11]

High concentration of ROS and reactive nitrogen species (RNS) induce pathologic effects in the spermatozoa biomolecules, namely in proteins, lipids and mitochondrial and nuclear DNA. In fact, the peroxidation of phospholipids of the plasma membrane promotes alterations in its fluidity, causing a loss of motility, as well as, a decrease of membrane enzymes and ionic channels activity, contributing to fertility impairment.[4,12,13] ROS are also responsible for damaging mitochondrial and nuclear DNA in human spermatozoa due to their action on phosphodiester backbones and DNA bases, leading to its fragmentation.[14,15] These species can also damage proteins, interfering with enzymatic activity or with structural protein function. At last, ROS promote a decrease in the number of spermatozoa by activating apoptosis [14]. Indeed, it has been shown

* Corresponding author.
E-mail address: mfardilha@ua.pt (M. Fardilha).

http://dx.doi.org/10.1016/j.pbj.2016.07.004

Please cite this article in press as: Cruz DF, Fardilha M. Relevance of peroxynitrite formation and 3-nitrotyrosine on spermatozoa physiology. Porto Biomed. J. 2016. http://dx.doi.org/10.1016/j.pbj.2016.07.004

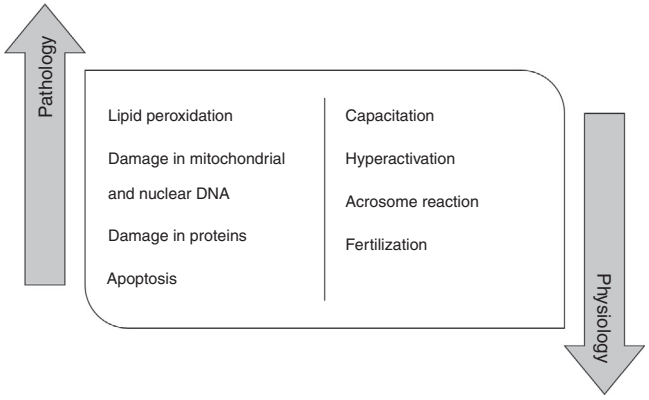

Fig. 1. Effects of ROS in the pathophysiology of sperm cells. High levels of ROS contribute to pathologic effects while low levels have an important role in the physiology of sperm cells.

that antioxidants improve rat sperm viability during storage and in several disease condition, including diabetes.[16–18]

However, ROS do not play just a pathological role in the male germ cells (Fig. 1). Currently, it is believed that these molecular species have a physiological role when they are in an ideal concentration. Low ROS levels regulate fertilization, acrosome reaction, hyperactivation, motility and capacitation.[19]

This review will focus in the description of the ROS contributing to the protein oxidation, namely by tyrosine nitration. First, we will make a short introduction on superoxide anion ($O_2^{\bullet-}$), nitric oxide (NO) and peroxynitrite ($ONOO^-$), followed by a discussion on the contribution of these ROS to protein tyrosine nitration and the effects in the spermatozoa. Lastly, we will describe the methods that can be applied to evaluate the presence of peroxynitrite and 3-nitrotyrosine in the sperm cells and discuss their utility at the clinical level since they are considered markers of oxidative stress.

Reactive oxygen species involved in 3-nitrotyrosine formation

Superoxide anion

Superoxide anion is the product of the one-electron reduction of oxygen, which can be produced both enzymatically and non-enzymatically.[8] Enzymatically, the sources of $O_2^{\bullet-}$ include NADPH oxidases, which are located on the cell membrane of several cells, such as polymorphonuclear cells, macrophages and endothelial cells[20,21] and cytochrome P450-dependent oxygenases.[8,22] Another enzymatic source of $O_2^{\bullet-}$, but also of hydrogen peroxide (H_2O_2), corresponds to the proteolytic conversion of xanthine dehydrogenase to xanthine oxidase.[23] In turn, the transfer of a single electron to oxygen by reduced coenzymes or prosthetic groups or by reduced xenobiotics corresponds to the non-enzymatic production of $O_2^{\bullet-}$. In the majority of human tissues, the mitochondrial electron transport chain is the primary source of $O_2^{\bullet-}$.[8] Furthermore, $O_2^{\bullet-}$ can also result from oxidation of peroxide (O_2^{2-}).[24]

The standard reduction potential for the conversion of molecular oxygen to $O_2^{\bullet-}$ is highly dependent on the nature of the medium. Mitochondria is the principal source of $O_2^{\bullet-}$, since 0.2–2% of the oxygen consumed is transformed to $O_2^{\bullet-}$.[25,26] The electron transfer chain, namely complexes I and III, is responsible for the majority of the $O_2^{\bullet-}$ produced during cellular respiration. Superoxide anion is then released in the matrix and in the intermembrane space.[8,26] Due to its anionic character ($pK_a = 4.7$), $O_2^{\bullet-}$ has a limited capacity to diffuse through membranes.[26–28]

Nitric oxide

Nitric oxide (NO) is a short-lived and highly reactive free radical that is produced in all mammalian cells during the oxidation of L-arginine to L-citrulline, by a family of NO synthase (NOS) isoforms.[11,29,30]

Three different NOS isoforms have been reported. Neuronal or brain NOS (nNOS), first described in neuronal tissues, and endothelial or constitutive NOS (eNOS or cNOS), first described in endothelial cells, which are Ca^{2+}-calmodulin-dependent isoforms. Inducible NOS (iNOS), originally found in macrophages, is expressed only in response to inflammatory cytokines and lipopolysaccharides and is an isoform Ca^{2+}-calmodulin-independent.[11,30–33]

The described isoforms can be found in human spermatozoa, namely in its head and/or flagellum regions, and their presence and activity depends on the maturity of male germ cells.[34–36] However, as the iNOS produces higher NO levels and remains active for a longer time period, when compared to nNOS and eNOS, it is responsible for a more negative effect in the sperm function.[31,32]

The activation of cNOS is dependent on calcium levels, so when the intracellular levels of calcium increase, a cascade that leads to cNOS activation and to NO synthesis is initiated. In this process, the intracellular calcium binds to calmodulin, forming a calcium-calmodulin complex. This linkage also regulates the binding of calmodulin to the "latch domain", which permits electron transfer from NADPH via flavin groups within the reductase domain to a haem-containing active site, thereby facilitating the conversion of O_2 and L-arginine to NO and L-citrulline.[37–39] In turn, activation of iNOS does not require alterations in intracellular calcium levels, because this isoform contains calmodulin tightly bound to each subunit of the enzyme, resulting in the permanent activation of the enzyme.[39,40] Furthermore, calcium chelators have been shown to reduce cNOS activity significantly. Other factors can also regulate NOS activity, such as pre- and post-translational and transcriptional mechanisms.[33,34] In sperm, which is virtually devoid of transcription, the post-translational modifications may be preponderant. Constitutive NOS can be linked to cell membranes due to the possession of a site for myristylation.[43] Phosphorylation of cNOS by protein kinase C (PKC) or protein kinase A (PKA) is associated with the detachment of the enzyme from the cell membrane and loss of activity.[44]

Nitric oxide has the ability to diffuse through cell membranes, reaching the intracellular targets, where it can act through two different signaling pathways: cGMP-dependent signaling and cGMP-independent signaling.[30,33,45] The first pathway involves the activation of soluble guanylyl cyclase (sGC), generation of 3′,5′-cyclic guanosine monophosphate (cGMP) and finally the activation of specific cGMP-dependent enzymes, such as protein kinases, channels and phosphodiesterases.[45] The other pathway occurs through covalent post-translational modification of target proteins, such as S-nitrosylation, S-glutathionylation and tyrosine nitration.[46–48] This last pathway needs a higher NO concentration to occur when compared with the activation of sGC.[30]

Peroxynitrite

Nitric oxide and $O_2^{\bullet-}$, when at physiological conditions, are present at very low concentrations.[49] These two molecules may combine to form peroxynitrite ($ONOO^-$), by a reaction that occurs at 6.7×10^9 mol^{-1}/l/s and that is considered irreversible due to its highly exothermic nature.[49–51]

In contrast to NO and $O_2^{\bullet-}$, $ONOO^-$ is not a free radical because the unpaired electrons of these radicals have combined to form a new N–O bond; however, it is a strong oxidant and nitrating agent.[49,52]

Please cite this article in press as: Cruz DF, Fardilha M. Relevance of peroxynitrite formation and 3-nitrotyrosine on spermatozoa physiology. Porto Biomed. J. 2016. http://dx.doi.org/10.1016/j.pbj.2016.07.004