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Role of poly(ADP-ribose) polymerases in male reproduction

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Abbreviations: 4-HNE, 4-Hydroxynonenal; ABM, Abamectin; BER, base excision repair; BRCT, (BRCA)-1 C-terminal; MMP, mitochondrial membrane potential; MNU, N-nitroso-N-methylurea; NAD, nicotinamide adenine dinucleotide; OS, oxidative stress; PAR, Poly(ADP-ribose); PARG, Poly(ADPR)glycohydrolase; PARP, Poly(ADP-ribose) polymerase; PARP-1, Poly(ADP-ribose) polymerase-1; PARPs, Poly(ADP-ribose) polymerases; PARylation, Poly(ADP-ribosyl)ation; ROS, reactive oxygen species; SDI, sperm deformity index; SSBR, single strand break repair; STS, staurosporine

Poly(ADP-ribose) polymerases (PARPs) are a family of enzymes involved in a wide variety of biological processes including DNA repair and maintenance of genomic stability following genotoxic stress, and regulates the expression of various proteins at the transcriptional level as well as replication and differentiation. However, excessive activation of PARP has been shown to contribute to the pathogenesis of several diseases associated with oxidative stress (OS), which has been known to play a fundamental role in the etiology of male infertility. Based on the degree and type of the stress stimulus, PARP directs cells to specific fates (such as, DNA repair vs. cell death). A large volume of accumulated evidence indicates the presence of PARP and its homologs in testicular germ line cells and its activity may offer a key mechanism for keeping DNA integrity in spermatogenesis. On the other hand, a possible role of PARP overactivation in OS-induced male reproductive disorders and in human sperm is gaining significance in recent years. In this review, we focus on the findings about the importance of PARP-1 and PARP-2 in male reproduction and possible involvement of PARP overactivation in various clinical conditions associated with male infertility.

Introduction

The male factor is considered a major contributory factor to infertility. Apart from the conventional causes for male infertility such as varicocoele, cryptorchidism, infections, obstructive lesions, cystic fibrosis, trauma and tumors, an important cause, oxidative stress (OS), has been determined.[1] Oxidative stress is a condition that occurs when the production of reactive oxygen species (ROS) overwhelms the antioxidant defense produced against them. Many recent studies have focused on OS and its possible role in the pathogenesis of male infertility and substantial evidence exists to suggest that small amounts of ROS are necessary for spermatozoa to acquire fertilizing capabilities.[2,3] Low levels of ROS have been shown to be essential for fertilization, the acrosome reaction, hyperactivation, motility, capacitation and ultimately fertilization.[4-6] However, excessive production and/or reduced clearance lead to OS within sperm, resulting in DNA damage,[7] reduced motility[8] and defective membrane integrity.[9] The accumulation of ROS impaires sperm and testes functions, including destruction of spermatogenesis, suppression of steroidogenesis and disruption of sperm-oocyte interaction through lipid peroxidation and DNA damages.[10] In addition, ROS-induced oxidative damage in mitochondrial proteins, leading to the collapse of mitochondrial membrane potential (MMP) and loss of sperm motility, has been documented.[11] It is a serious condition, as ROS and their metabolites attack DNA, lipids and proteins, alter enzymatic systems and cell signaling pathways, producing irreparable alterations, cell death and necrosis.[12]

DNA damage in the male germ line cells is associated with poor fertilization rates following in vitro fertilization, defective preimplantation embryonic development and high rates of miscarriage and morbidity in the offspring. Oxidative stress is also associated with high frequencies of single- and double-strand DNA breaks.[13] DNA strand break-induced catalytic activation of poly(ADP-ribose) polymerase (PARP) enzymes are involved in DNA base-excision, homologous recombination and non-homologous end-joining repair pathways.[14] Poly(ADP-ribose) polymerase-1 (PARP-1), a family of enzymes that mediate poly(ADP-ribosyl)ation (PARylation) of proteins, catalyzes synthesis of over 90% of cellular poly(ADP-ribose) (PAR) following DNA damage.[15] Poly(ADP-ribose) metabolism plays a role in a wide range of biological structures and processes, including DNA repair and maintenance of genomic stability, transcriptional regulation, centromere function and mitotic spindle formation, centrosomal function, structure and function of vault particles, telomere dynamics, trafficking of endosomal vesicles, apoptosis and necrosis.[16]

In this article, we overview the importance of PARPs in male reproduction and the possible outcomes of PARP inhibition in various clinical conditions associated with oxidative stress-related male infertility.

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Structure and Function of PARP-1 and PARP-2

Poly(ADP-ribose) polymerases constitute a family of 18 enzymes, encoded by different genes sharing a highly conserved catalytic domain that contains the "PARP signature" motif, a highly conserved sequence (100% conserved in PARP-1 among vertebrates) that forms the active site.[17,18] Two well-recognized members of the family are PARP-1, the founding member, and also PARP-2 and their catalytic activity has been shown to be stimulated by DNA strand breaks. Poly(ADP-ribose) polymerase-1 (also known as poly(ADP-ribose) synthetase and poly(ADP-ribose) transferase), is a nuclear enzyme present in eukaryotes. PARP-1 is a 116 kDa protein consisting of three main domains: the DNA binding fragment, which contains two zinc finger motifs involved in DNA strand break recognition and a nuclear location signal; the central auto-modification domain, which includes a breast cancer gene (BRCA)-1 C-terminal (BRCT) domain and the C-terminal catalytic fragment, which binds nicotinamide adenine dinucleotide (NAD).[19-21] The auto-modification domain of PARP-1 contains several glutamate residues that are likely targets for auto-modification and a BRCT motif that functions in protein-protein interactions.[18,22] This enzyme has been implicated in DNA repair and maintenance of genomic integrity and regulates the expression of various proteins at the transcriptional level as well as replication and differentiation.[20,23-28] Poly(ADP-ribosyl)ation is a post-translational modification of proteins that was first defined in the 1960s.[29] PARP enzymes use NAD as a substrate to form long branched PAR polymers on glutamic acid residues of target proteins.[30,31] Thus, the physical and biochemical properties of PARylated proteins have been altered by the covalently attached negatively charged PAR polymer molecules.[22] Altough PARP-1 activity accounts for about 90% of the total cellular PAR formation, several other PARPs including, PARP-2, PARP-3, vault-PARP and tankyrases, have been discovered that are shown to be important regulators of cellular functions. In addition to PARP-1, PARP-2 is the only other PARP isoform known to be activated by DNA strand breaks and its catalytic domain has the strongest resemblance to that of PARP-1 with 69% similarity.[32] However, in its DNA binding domain, it includes only 64 amino acids and lacks any obvious DNA-binding motif, which makes it different from that of PARP-1. PARP-2 was discovered as a result of the presence of residual DNA-dependent PARP activity in embryonic fibroblasts derived from PARP-1-deficient mice.[32,33] PARP-2 is a nuclear protein that binds to and is activated by DNase-I-treated DNA; however, its DNA-binding domain differs from that of PARP-1 and it targets DNA gaps but not nicks.[34] The crystal structure of the murine PARP-2 is very similar to that of PARP-1 but with the exception of differences in the vicinity of the acceptor site that reflect differences in terms of substrates.[35] PARP-2 acts as a chromatin modifier too but PARylates histone H2B while PARP-1 targets histone H1.[34,36]

PARP-2 interacts with PARP-1 and shares common partners involved in the single strand break repair (SSBR) and base excision repair (BER) pathways: XRCC1, DNA polymerase-β and DNA ligase III. PARP-1 and PARP-2 also interact with proteins involved in the kinetochore structure and in the mitotic spindle checkpoint. The involvement of PARP-2 in the cellular response to DNA damage has been investigated by the generation of a PARP-2-deficient mouse model.[37] Following treatment by the alkylating agent N-nitroso-N-methylurea (MNU), PARP-2-deficient cells displayed a large delay in DNA-strand breaks resealing, similar to that observed in PARP-1-deficient cells,[38] thus confirming that PARP-2 is an active player in SSBR in spite of its reduced capacity to synthesize ADP-ribose polymers. However, PARP-2-deficient mice also display specific phenotypes, indicating that PARP-1 and PARP-2 functions are complementary but do not fully overlap.

The synthesis and turnover of ADP-ribose polymers is dynamic. The counterpart enzyme of PARPs is the poly(ADPR) glycohydrolase (PARG), which plays the major role in the degradation of the polymer and it controls the level and complexity of polymerized PAR in vivo.[39]

PARPs in the Maturation and Differentiation of Germ Cells

Studies related to PARP expression in testicular tissue have been performed mainly in rodents,[40-45] and most of these studies found high PARP activity to be localized in spermatogonia and pachytene nuclei of spermatocytes. Di Meglio[46] have reported higher levels of PARP enzymes in primary spermatocytes undergoing meiotic division, which has been also supported by the findings of Tramontano et al.[44] PARP-1 mRNA and protein levels revealed high expressions in pachytene primary spermatocytes.[46] Moreover, it has been found that rat germinal cells contain both the forms of PARG (110 and 60 kDa). The large PARG fragment was present and active mainly in the nuclear fraction of primary spermatocytes, whereas round spermatids showed a higher level of the small fragment of PARG. This different expression level of PARP-1 and a different endocellular distribution of PARG suggest a role for the PAR turnover in meiotic and post-meiotic germinal cells.

The presence of PARP has been shown in rat male germ cells during specific stages of spermatogenesis.[47] Immunofluorescence staining of rat testes revealed the marked formation of PAR in elongating spermatids of differentiation steps 11–14, which may indicate that PARP-1 is highly active during spermiogenic differentiation in the seminiferous epithelium. PARP-1 was detected in spermatid steps 15–17, but was not detected in cells of later steps. Interestingly, spermatid steps 15–17 were stained positive for PARP-1 but not for ADP-ribose polymer, suggesting that the enzyme is still present but not activated. PARP-1 activation is mainly dependent on the presence of DNA strand breaks[17] and Meyer-Ficca et al. observed the occurrence of DNA strand breaks in steps 12–14 using histone H2AX phosphorylation as a DNA double-strand break marker. Their observations suggest that DNA strand breaks may be mostly responsible for the activation of PARP in elongating spermatids, although other regulatory mechanisms cannot be excluded. In the developing testis of mice, PARP-1 is expressed at high levels in the seminiferous tubules.[38] Its expression was particularly strong in the basal layers of the

seminiferous epithelium and was downregulated at the haploid stage of meiosis.

Precise localization of the PARP-1 enzyme and the PAR polymer during human spermatogenesis has also been shown recently.[48] Detection of PAR expression in human germ cells demonstrated modification of chromatin during spermatogenesis and establishes a key role for PARylation in germ cell differentiation, presumably to safeguard DNA integrity. In the study by Maymon et al.,[48] PARP-1 and PARP activity (indicated by PAR formation) were found to be present predominantly in germ cells and were not localized to somatic cells in the testis. PARP-1 was localized predominantly in primary spermatocytes, in round spermatids and in elongating spermatids in human testis. Importantly, a high level of PARP-1 was present in all types of spermatogonia, which may indicate that PARP-1 is present (even if not in an activated state) to provide a reservoir for keeping DNA integrity in the spermatogonia. Moroever, the results of Maymon et al.[48] designated a role for PAR formation in distinct steps of spermatogenesis, which is associated with high frequency of DNA strand breaks. PAR expression was present in elongating rat spermatids in differentiation steps that preceded the most pronounced phase of chromatin condensation. These results suggest that poly(ADP-ribosyl)ation may be involved in chromatin remodeling during meiosis and spermatid nuclear condensation.

Apart from PARP-1, PARP-2 is the other PARP isoform known to be expressed in the testis of mice[38] and rats,[44] and is highly expressed in human testis.[49] PARP-2 seems to be responsible for a smaller portion of PARP activity than PARP-1 in rat testis.[50] Moreover, PARP-2 is found in the ejaculated spermatozoa in both mice and in humans.[51,52] Interestingly, it has been suggested that PARP-2 gene might be associated with azoospermia by meiotic arrest in humans.[52]

PARP-1-/- mice are fertile,[53] whereas PARP-2-/- mice suffer severe hypofertility.[51] A study by Dantzer et al.[51] has shown that the absence of PARP-2 in mice leads to male hypofertility associated with important abnormalities at various steps throughout spermatogenesis that are in agreement with the presence of PARP-2 in meiotic and post-meiotic germinal cells at high levels. PARP-2-deficient mice display defective meiosis I characterized by segregation failure in the first meiotic division and delayed spermiogenesis. Moreover, they found that regulation of histone methylation and acetylation in PARP-2-/- spermatocytes was disrupted, which indicates an interaction between PARP-2-dependent PARylation and histone modifications that could account for various meiotic defects. A study by Dantzer et al. provided in vivo evidence for the role of PARP-2 in both meiosis and spermiogenesis and highlighted multifunctional roles of this protein throughout spermatogenesis in mice, which raise the possibility that an impairment of this protein could be a novel cause of infertility in humans. During testis development in mice, in contrast to PARP-1, PARP-2 signal was weak and rather homogeneous, throughout the seminiferous tubules and the interstitial tissue.[38]

Evidence about the presence and role of both PARP-1 and PARP-2 in testis suggests a possible important role for these molecules in male fertility.

Overactivation of PARP in Male Reproduction

Oxidative stress is a state related to increased cellular damage caused by oxygen and oxygen-derived free radicals known as ROS, which are by products of aerobic metabolism. Elevated levels of ROS are seen in up to 30–80% of men with male infertility.[54] Oxidative stress plays a fundamental role in the etiology of male infertility by negatively affecting sperm quality and function.[55] Many conditions or events associated with male infertility are inducers of OS. X-irradiation, for example, or exposure to environmental toxicants and the physical conditions of varicocele and cryptorchidism have been demonstrated to increase testicular OS, which leads to an increase in germ cell apoptosis and subsequent hypospermatogenesis.[12] Although an association between ROS and male infertility was proposed, the downstream cellular mechanisms involved after ROS generation in testes have not yet been clearly recognized.

The PARP pathway has been recently established as a major downstream intracellular pathway of OS.[56] PARP-1's basal enzymatic activity is very low, but is stimulated dramatically in the presence of damaged DNA.[22,57] Oxidant species are endogenous inducers of DNA strand breakage and are required for PARP activation. Once activated, the enzyme rapidly catalyzes the transfer of ADP-ribose moieties from NAD to the acceptor protein and to itself.[58] Under normal conditions, catalytically inactive PARP-1 resides in the nucleoplasm.[21] PARP-1 and other PARPs are also localized within mitochondria.[59] Under physiological conditions or limited DNA damage, the primary function of PARP-1 is to detect and repair DNA damage, thus PARP plays a protective role.[60,61] On the other hand, after massive DNA damage, PARP plays a crucial role in cell death program by its overactivation. The excessive activation of PARP-1 in pathological conditions results in a rapid depletion of intracellular NAD.[62,63] In an attempt to restore the NAD pools, NAD is resynthesized with a consumption of two to four molecules of ATP per molecule of NAD. This slows the rate of glycolysis and mitochondrial respiration, eventually leading to cellular dysfunction and death via apoptosis or necrosis.

As mentioned above, overactivation of PARP represents an important mechanism of tissue damage in various pathological conditions related to OS.[23,64,65] Therefore, the activation of the PARP pathway as a consequence of increased OS and subsequent DNA strand breaks can be hypothesized to explain male infertility related with OS. The possible role of PARP activation in testis has been summarized in **Figure 1**.

Role of the PARP pathway in the pathogenesis of male infertility in animal models. Although an association between increased OS and varicocele-induced testicular damage was reported, the downstream cellular mechanisms involved after ROS generation in testes with varicocele have not yet been known. We investigated the possible role of PARP pathway activation in the pathogenesis of infertility in a rat model of varicocele.[66] Our immunohistochemistry results showed that both PARP-1 and PAR expression in germ cells increased as a consequence of elevated OS in varicocele-induced rat testes. The expression patterns of the enzyme PARP-1 and PAR were somewhat similar to each other.

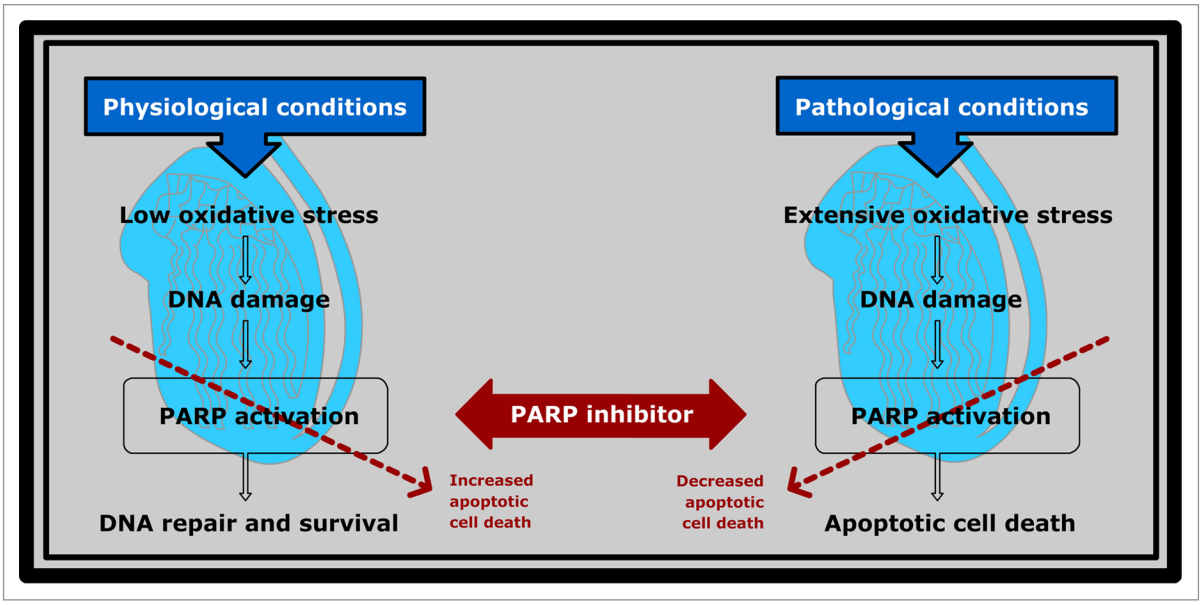

Figure 1. Possible role of PARP activation in testis. Under physiological conditions, mild DNA damage is repaired by PARP activation. Pathological conditions, associated with more severe oxidative stress, may cause excessive PARP activation and result in apoptotic cell death. Under physiological conditions, blockage of PARP activity with PARP inhibitors may inhibit DNA repair and lead to apoptotic cell death. On the other hand, the inhibition of extensive activation of PARP in pathological conditions may prevent apoptotic cell death.

In control and sham groups; PARP-1 was present in spermatogonia and spermatocytes, indicating its possible role in stem cells of the testis and meiotic processes. In contrast, PAR was localized to spermatogonia and elongating spermatids only, indicating the contribution of PARylation during stem cell activity and chromatin modeling of elongating rat sperm. In the varicocele group, expressions of PARP-1 and PAR increased, and cellular localizations of these proteins changed. PARP-1 was expressed in spermatogonia, spermatocytes and round spermatids. Similarly, PAR was immunolocalized to spermatogonia, spermatocytes, round spermatocytes and elongating spermatocytes, which could indicate that the shutdown of protein expression for PARP-1 and PAR was delayed in varicocele, leading to overactivation of PARP-1 and PAR. In accordance with these results, the quantitative analysis of PARP-1 and PAR by western blot indicated an increase in the varicocele group when compared with control and sham groups.

Moreover, our results indicated that the cleavage of a 113 kDa PARP to a 29 kDa fragment occurs in germ cells in varicocele-induced rats.[66] It is known that PARP-1 is cleaved in fragments of 89 and 24 kDa during apoptosis, which has become a useful hallmark of this type of cell death.[67] Apoptotic cell death allows cells with irreparable DNA damage to become eliminated in a safe way. The cleavage of PARP-1 by various caspases results in the formation of two specific fragments: an 89 kDa catalytic fragment and a 24 kDa DNA-binding domain.[68,69] The 89 kDa fragment containing automodification domain and the catalytic domain of the enzyme has reduced DNA binding capacity and is translocated from the nucleus into the cytosol.[70] The 24 kDa cleaved fragment with two zinc-finger motifs is retained in the nucleus, irreversibly binding to nicked DNA where it acts as a trans-dominant inhibitor of active PARP-1. Cleavage of PARP might prevent its overactivation, energy depletion and necrosis by preserving cellular energy required for the apoptotic mode of cell death. The cleaved form of PARP is catalytically inactive; PARP cleavage has been considered as an endogenous mechanism that serves to prevent PARP-dependent metabolic suppression and necrosis.[71] In our study, it is most likely that increased OS in varicocele-induced testicular tissue might be responsible for the cleavage of full-length PARP into its apoptotic fragment.[66] Accordingly, the number of apoptotic cells increased in germ cells of varicocele-induced rats. These results suggested PARP overactivation in testis might be important with regard to impaired testicular function associated with increased OS in experimental varicocele.

In recent years, a large volume of accumulated evidence suggests that the trend of decreasing male fertility (in terms of sperm count and quality) might be due to exposure to environmental toxicants.[72] Male infertility caused by exposure to environmental toxicants such as cadmium, mercury, bisphenol A and dioxin is a global problem, particularly in industrialized countries.[73] Most environmental toxicants have been shown to induce ROS, thereby causing a state of OS in various compartments of the testes. Studies in the testis and other organs have illustrated the importance of environmental toxicant-induced OS in mediating disruption to cell junctions.[73] These environmental contaminants can target testicular spermatogenesis, steroidogenesis and the function of both Sertoli and Leydig cells.[72] This leads to reproductive dysfunction such as reduced sperm count and reduced quality of semen. Previous reports have indicated a strong link between male infertility and exposure to more than 50 pesticides.[74-77] One of these pesticides, abamectin (ABM), is used extensively all over the world. Recently, we have shown that ABM administration was associated with decreased sperm count and

motility and increased seminiferous tubule damage.[78] In addition, significant elevations in the 4-HNE (4-Hydroxynonenal, a marker of oxidative stress)-modified proteins were observed in testes of rats exposed to ABM. These results showed that ABM exposure induces testicular damage and affects sperm dynamics via increasing OS. As a consequence of increased OS due to ABM exposure, the overactivation of the PARP pathway in testis tissue may cause harmful effects on testes. The quantitative analysis of PAR showed an increase in PAR expression of ABM-exposed rat testes. Moreover, marked inhibition of sperm motility after ABM exposure may be related with low levels of ATP content as a consequence of increased enzymatic activity of PARP. These results showed that ABM exposure induces testicular damage and affects sperm dynamics. Thus, OS-mediated PARP activation might be one of the possible mechanism(s) underlying testicular damage induced by ABM. It is most likely that exposure to ABM may lead to ATP failure and testicular damage as a result of increased PARP enzyme activity. The activation of PARP results in a rapid depletion of intracellular ATP,[79] a source of energy for the forward movement of spermatozoa. Full ATP pool is also crucial for normal spermatozoal movement and a slight deprivation of ATP leads to reduction in motility, which may cause infertility.[80]

Studies regarding role of PARP in human testis and sperm. The role of PARP has recently been shown in male germ cells during spermatogenesis.[81] High PARP expression has been reported in mature spermatozoa and in proven fertile men. The presence of low PARP levels in mature sperm of infertile patients compared with the mature sperm of donors suggests a role of PARP in male infertility.[82] Whenever there are strand breaks in sperm DNA due to OS, chromatin remodeling or cell death, PARP is activated.[81] However, the cleavage of PARP by caspase-3 inactivates it and inhibits PARP's DNA-repairing abilities. Therefore, cleaved PARP may be considered a marker of apoptosis. The presence of higher levels of cleaved PARP in sperm of infertile men adds a new proof for the correlation between apoptosis and male infertility. Maymon et al.[48] investigated the role of PAR and PARP-1 during human spermatogenesis in azoospermic men who underwent testicular biopsies for sperm recovery. Expression of both markers was localized in germ cells' nuclei in seminiferous tubules showing full spermatogenesis; PAR expression was exhibited in round spermatids and sperm and in a subpopulation of primary spermatocytes. In seminiferous tubules showing maturation arrest at the spermatocytes level, strong PAR expression was identified in spermatocytes.

There are limited studies in the literature regarding the role of PARP activity in male fertility disorders in humans. In one of these studies, possible relationships between PARP and varicocele have been investigated. It has been reported that cleaved PARP-1 expression was significantly higher in spermatogonia of varicocele patients compared with fertile subjects.[83] Meanwhile, in spermatocytes, significantly more cells expressed PARP-1, active caspase 3 and cleaved PARP-1 in varicocele patients compared with young fertile subjects. This indicated that, overall, both DNA repair and apoptosis are elevated in the early stages of spermatogenesis in varicocele patients. Moreover, Chang et al.[84] assessed the expression of somatic apoptosis-related proteins in ejaculated sperm from both patients with varicocele and normal donors. This study indicated that PARP was expressed substantially more in the sperm cells of the varicocele group when compared with the normal group. An increased expression of PARP for varicocele-afflicted individuals seems to be related with increased apoptosis in the ejaculated semen from patients with varicocele. It has been reported that in varicocele patients, the long-term persistence of high DNA repair and apoptosis activity in the testes may be detrimental and trigger metabolic stress that over time will lead to increased apoptosis.[85]

Advancing male age has multiple genomic defects such as, DNA fragmentation index, chromatin integrity, gene mutations and numeric chromosomal abnormalities, in human sperm.[86] In aged testes, significant differences were observed in DNA repair and apoptotic marker expression in both spermatogonia and spermatocytes.[87] PARP-1 protein expression was more frequently expressed in spermatocytes in the old age group testes specimens compared with the young, which may indicate that aging is associated with increased DNA repair due to increased DNA breaks.[87] When repair mechanisms such as PARylation are overactivated, it may lead to apoptosis.[88] This could explain the increased expression of active caspase 3 and cleaved PARP-1 in spermatocytes after PARP-1 overactivation and the initiation of apoptosis in them. Additionally, apoptotic markers active caspase 3 and cleaved PARP-1 expression were significantly higher in the spermatogonia and spermatocytes in the old testes compared with the young.

There are also some studies investigating the effect of PARP inhibition in human ejaculate. In the absence of PARP inhibition, exposure to staurosporine (STS), one of the chemical inducers of sperm damage, only resulted in a large increase in the percentage of early apoptotic sperm.[89] This increase in early apoptotic sperm with STS declined after PARP inhibition. Furthermore, a more than 2-fold increase in the percentage of late apoptotic sperm has been observed after PARP inhibition in STS-induced sperm injury. In this study, early apoptotic sperm were defined as sperm that were positive for only annexin V, and late apoptotic sperm were those that were positive for both annexin V and propium iodide.[90,91] The increase in late apoptotic sperm with chemical exposure and PARP inhibition are more apparent in immature than in mature sperm fractions, suggesting mature spermatozoa are more protected from chemical-induced damage. On exposure to OS, PARP inhibition caused a significant increase only in the percentage of early apoptotic sperm. Immature spermatozoa show more resistance to this damage compared with mature sperm, suggesting that mature sperm are more susceptible to OS. The OS-induced increase in early apoptotic sperm after PARP inhibition also suggests that overactivation of PARP can increase late apoptosis and necrosis. Collectively, mature and immature spermatozoa showed a significant decline in the percentage of late apoptotic sperm after PARP inhibition in chemical and OS-induced sperm damage.

Aziz et al.[92] have reported that normal sperm morphology correlated negatively with the late apoptotic marker after OS both in the presence or absence of PARP inhibitor. The positive correlation of cleaved PARP with percentages of sperm with acrosome defects, midpiece damage and tail abnormalities, may be attributed to the localization of this enzyme in the head and midpiece area. When the data were considered collectively (immature and mature fraction), a significant positive interdependence was seen

between sperm deformity index (SDI) scores and cleaved PARP in the presence of PARP inhibitor. In conclusion, SDI scores are associated with PARP cleavage as an early marker of apoptosis. Normal sperm morphology correlates negatively with the late apoptotic marker after OS both in the presence and absence of PARP inhibitor. The relationship between acrosomal and midpiece defects appears to be influenced by the localization of PARP in these regions in the sperm cell.

Future Prospects

To date, few reports have investigated the role of PARP during mammalian spermatogenesis and male infertility, and most of these have been limited to the rodent species. Based on the evidence reviewed herein, we conclude that OS-PARP pathway might play important roles in the pathogenesis of male infertility in pathophysiological conditions associated with OS. Thus, PARP inhibitors may be effective intervention to prevent OS-associated male infertility in humans. A multitude of novel PARP inhibitors are in various stages of preclinical or clinical development, many with potency that greatly exceeds the prototypic agents successfully used in earlier animal studies. It remains to be studied whether the remarkable efficiency of new PARP inhibitors would also be benefical for various clinical conditions regarding male infertility that are associated with increased OS. Importantly, it should be kept in mind that PARP activity and PARylation seems to be necessary for maintaining normal spermatogenesis. Therefore, the idea of its inhibition during increased OS conditions except for testis should be considered carefully since it could be unnecessarily detrimental to male fertility.

Since the evidence about the possible effect of PARP inhibition in testicular tissue is at its initial stages, some commentaries may be borrowed from the findings of other cell or tissue types where PARP inhibitors seem as good candidates in conditions related with its overactivity. Importantly, a concern with the clinical use of PARP inhibitors relates to the general role of PARP in DNA repair: when PARP is blocked, is it possible that DNA repair is compromised and cells may be sensitized to malignant transformation? Because, failure of DNA repair and fixation of DNA damage as mutation eventually leads to cellular transformation and carcinogenesis. Could this scenario be the case also for normal spermatogenic cells? Would their DNA repair systems get damaged? When there is a pathological condition related with OS, would the inhibition of PARP make the spermatogenic cells more vulnerable to OS or alkylating agents in terms of DNA repair or cell death? Recently, our laboratory has found that when PARP-1-knockout adult mice are treated with doxorubicin, a drug used in cancer chemotherapy, spermatogenic cells are more rapidly lost when compared with their wild-type counterparts (in preparation). Thus, as far as risks of PARP inhibition stand there, the most frequently raised issue relates to the preservation of cells with broken DNA.[93] Although this is a difficult question to answer, one must make clear that PARP inhibitors do not actually induce these DNA damage conditions: the DNA damage is actually induced by the disease itself. In this respect, by inhibiting PARP, one only would expect to do more harm to the tissues than what the disease is already inducing, if PARP inhibition would interfere with the repair of the damaged DNA. While some studies demonstrated delays in DNA strand rejoining in PARP inhibitor-treated cells, or in PARP-1-deficient cells, others found no remarkable differences. Another issue is related to the interaction of PARP-1 with other PARP isoforms. It is unlikely that therapeutic use of PARP inhibitors would inhibit all PARP isoforms, and it is also unlikely that it would lead to a complete inhibition of the enzymatic activity. Interestingly, when doxorubicin treated wild-type mice (in this model DNA strand breaks are formed due to increased OS) were treated with a potent PARP inhibitor PJ34, germ cells were protected from this condition to some extent (in preparation). Based on the evidence established in the literature so far, one can tentatively conclude that short-term inhibition of PARP, in an incomplete fashion, may be acceptable, in terms of risk/benefit ratio, for the experimental therapy of severe, life-threatening diseases. Since the current information in the literature commonly indicate that the treatment of various clinical conditions with PARP inhibitors seems to be useful, the role of PARPs in male fertility and the outcomes of PARP inhibition in situations related with OS-associated male infertility urgently need further investigations.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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