

# Pathophysiological Role of Peroxynitrite Induced DNA Damage in Human Diseases: A Special Focus on Poly(ADP-ribose) Polymerase (PARP)

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Received: 10 December 2014 / Accepted: 22 December 2014 / Published online: 20 January 2015  
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**Abstract** Peroxynitrite is formed in biological systems when nitric oxide and superoxide rapidly interact at near equimolar ratio. Peroxynitrite, though not a free radical by chemical nature, is a powerful oxidant which reacts with proteins, DNA and lipids. These reactions trigger a wide array of cellular responses ranging from subtle modulations of cell signaling to overwhelming oxidative injury, committing cells to necrosis or apoptosis. The present review outlines the various peroxynitrite-induced DNA modifications with special mention to the formation of 8-nitroguanine and 8-oxoguanine as well as the induction of DNA single strand breakage. Low concentrations of peroxynitrite cause apoptotic death, whereas higher concentrations cause necrosis with cellular energetics (ATP and  $\text{NAD}^+$ ) serving as control between the two modes of cell death. DNA damage induced by peroxynitrite triggers the activation of DNA repair systems. A DNA nick sensing enzyme, poly(ADP-ribose) polymerase-1 (PARP-1) becomes activated upon detecting DNA breakage and it cleaves  $\text{NAD}^+$  into nicotinamide and ADP-ribose and polymerizes the latter on nuclear acceptor proteins. Over-activation of PARP induced by peroxynitrite consumes  $\text{NAD}^+$  and consequently ATP decreases, culminating in cell dysfunction, apoptosis or necrosis. This mechanism has been implicated in the pathogenesis of various diseases like diabetes, cardiovascular diseases and neurodegenerative diseases. In this review, we have discussed the cytotoxic

effects (apoptosis and necrosis) of peroxynitrite in the etiology of the mentioned diseases, focusing on the role of PARP in DNA repair in presence of peroxynitrite.

**Keywords** Peroxynitrite · DNA · Poly(ADP-ribose) polymerase-1 · Diabetes · Cardiovascular diseases · Neurodegenerative diseases

## Introduction

The two major pathways involved in the nitric oxide (NO) induced DNA damage involve a reaction of NO with molecular oxygen, yielding  $\text{N}_2\text{O}_3$  with subsequent nitrosation of secondary amines and the formation of N-nitrosoamines or nitrosation of primary amines and nucleic acid bases [1] and the production of peroxynitrite through the reaction of NO with superoxide radical ( $\text{O}_2^{\bullet-}$ ). Peroxynitrite ( $\text{ONOO}^-$ ) is a potent oxidant and nitrating agent with a short half life ( $\sim 10$  ms) [2, 3]. The peroxynitrite formation sites are considered to be spatially associated with the sources of superoxide (such as the plasma membrane NAD(P)H oxidases and the mitochondrial respiratory complexes). Since NO is a relatively stable and highly diffusible free radical while superoxide is much short lived and has restricted diffusion rates across biological membranes. The rate of peroxynitrite production in vivo has been estimated to be as high as 50–100  $\mu\text{M}$  per min. In spite of the short half-life of peroxynitrite at physiological pH, its ability to cross cell membranes [4] influences the surrounding target cells within one to two cell diameters ( $\sim 5$ – $20$   $\mu\text{m}$ ).

The oxidant reactivity of peroxynitrite is highly pH-dependent and both peroxynitrite anion ( $\text{ONOO}^-$ ) and peroxynitrous acid ( $\text{ONOOH}$ ) can participate directly in

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one- and two-electron oxidation reactions with biological macromolecules (Fig. 1), where many of them have transition metal centres and thiols. The one of the fundamental reactions of  $\text{ONOO}^-$  in biological systems is the fast reaction with carbon dioxide (in equilibrium with physiological levels of bicarbonate anion), which leads to the formation of carbonate ( $\text{CO}_3^{\bullet-}$ ) and nitrogen dioxide ( $\bullet\text{NO}_2$ ) radicals (yield  $\sim 35\%$ ), which are one-electron oxidants [5]. Nitrogen dioxide can undergo diffusion-controlled radical–radical termination reactions with biological macromolecules, while in nitrated compounds alternatively  $\text{ONOOH}$  can undergo homolytic fission to generate one-electron oxidants, hydroxyl ( $\bullet\text{OH}$ ) and  $\bullet\text{NO}_2$  radicals (with a 30% yield). Nonetheless, this reaction is slow compared with the other reactions of  $\text{ONOO}^-$  and  $\text{ONOOH}$  in biological systems and therefore considered as a modest component of the *in vivo* reactivity of peroxynitrite. The proton-catalysed decomposition of  $\text{ONOO}^-$  to form  $\bullet\text{OH}$  and  $\bullet\text{NO}_2$  radicals becomes relevant in hydrophobic phases and results in the initiation of lipid peroxidation processes [5, 6].

Various biological macromolecules including proteins, DNA and unsaturated fatty-acid-containing phospholipids are oxidized and/or nitrated by peroxynitrite derived radicals. In fact, tyrosine nitration, dimerization and hydroxylation caused by peroxynitrite to form 3-nitrotyrosine, 3,3'-dityrosine and 3,4'-dihydrophenylalanine, respectively, are totally dependent on free-radical pathways [7]. Thiols can be oxidized through one-electron reactions by peroxynitrite-derived radicals and initiate radical-dependent chain reactions to produce higher oxidation states of

sulphur, including sulphinic and sulphonic acid derivatives [8, 9]. In DNA, purine nucleotides are susceptible to oxidation and adduct formation [10–13], with 8-oxo and 8-nitroguanine existing as two of the major products. Peroxynitrite can also cause deoxyribose oxidation and strand breaks [14].

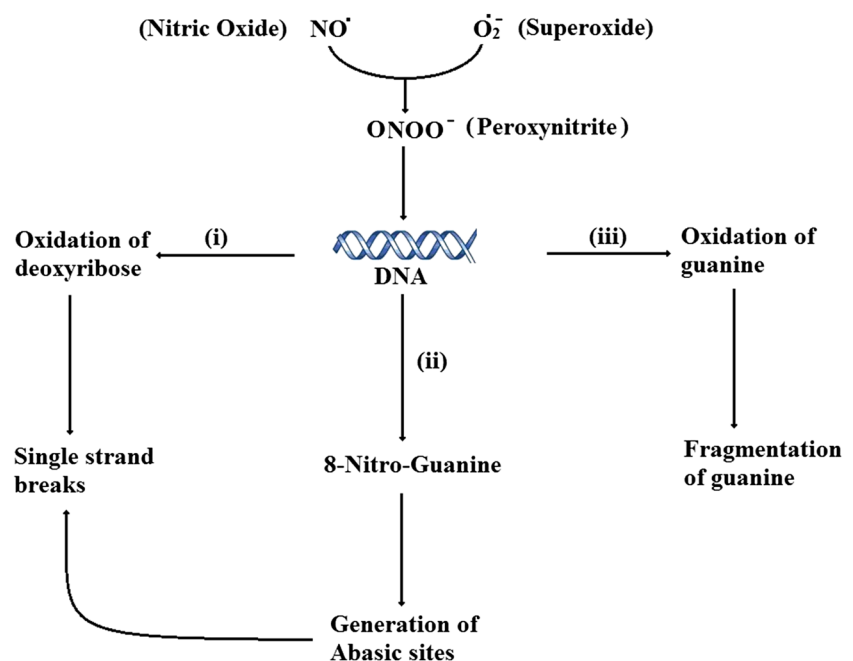
Peroxynitrite induced oxidative and nitrosative changes in lipids result in peroxidation [5] and the formation of nitrito-, nitro-, nitrosoperoxo- and/or nitrated lipid oxidation adducts (malondialdehyde, conjugated diene and lipid hydroperoxide formation) [15–17]. Peroxynitrite also causes the oxidation of arachidonic acid and leads to the formation of  $\text{F}_2$ -isoprostanes through the oxidation of low-density lipoprotein [18]. The nitration of fatty acids may lead to the secondary inhibition of protein function via thiol-based modifications [19].

Exposure of DNA to peroxynitrite has been shown to lead to the formation of 8-hydroxydeoxyguanosine [20] and 8-nitroguanine from guanine [21].

### Peroxynitrite-Induced DNA Damage

The DNA damage caused by peroxynitrite is mostly oxidative. DNA treatment with peroxynitrite usually leads to much more damage than treatment with an equivalent dose of nitric oxide. Besides the higher levels of damage present in DNA after  $\text{ONOO}^-$  treatment, the scale of damage also tends to be much more complex. This gives the assumption that peroxynitrite is intrinsically much more reactive than nitric oxide. Ischiropoulos and co-workers were among the

**Fig. 1** Effects of peroxynitrite on DNA. The superoxide ( $\text{O}_2^{\bullet-}$ ) and nitric oxide (NO) reacts to form peroxynitrite, which attacks DNA resulting in (i) oxidation of deoxyribose, (ii) formation of 8-Nitro-Guanine, ultimately result in single strand breaks, (iii) oxidation of guanine owing its fragmentation



first to observe the formation of peroxynitrite from activated macrophages [22]. They detected significant concentration of peroxynitrite (as high as  $0.11 \text{ nmol}/10^6 \text{ cells min}^{-1}$ ) using the nitration of 4-hydroxyphenylacetate in the media as a marker of peroxynitrite activity. Another group led by Lewis performed a kinetic analysis of the fate of  $\text{NO}^\bullet$  synthesized by activated macrophages using end product measurements of nitrite and nitrate ( $\text{N}_2\text{O}_3$  hydrolysis forms nitrite while  $\text{ONOO}^-$  decay leads to nitrate formation) [23]. Their results showed that approximately half of the released nitric oxide forms  $\text{N}_2\text{O}_3$  while the remainder combines with  $\text{O}_2^{\bullet-}$  to form  $\text{ONOO}^-$ . Interestingly, from this study, it comes into view that that  $\text{ONOO}^-$  formation occurs partially extracellularly and not exclusively inside the macrophage. This can be assumed from the observation that adding SOD to the media significantly reduced  $\text{ONOO}^-$  formation. If peroxynitrite would have been formed only within the cell, it would have not been influenced by the extracellular addition of superoxide dismutase.

#### Modification of Bases in DNA

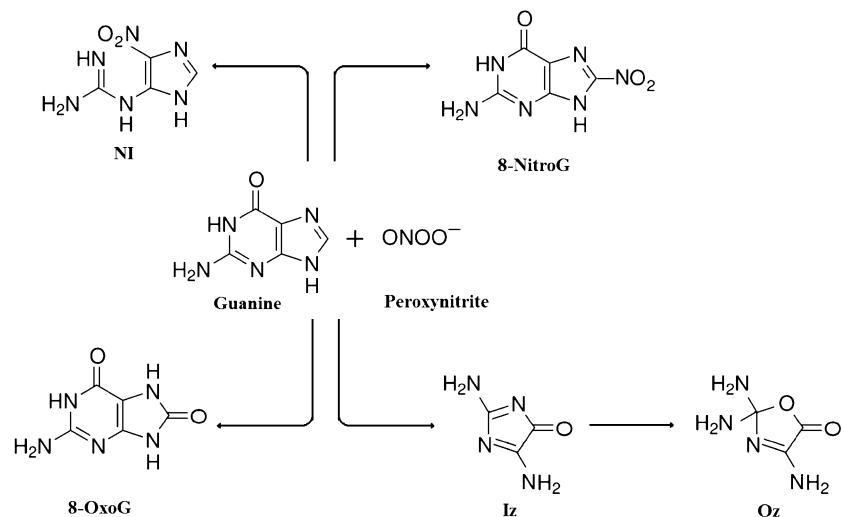
The reaction of peroxynitrite with purine bases, such as guanine and adenine, resulted in the production of a strong yellow color, whereas pyrimidine bases, such as thymine, cytosine, 5-methylcytosine, and uracil, did not [21] because guanine (G) has the lowest reduction potential among the four normal DNA bases ( $E^\circ = 1.29 \text{ V}$  [24]), and its reaction with the peroxynitrite-derived  $\text{HO}^\bullet$  ( $E^\circ = 1.9\text{--}2.1 \text{ V}$ ) [25] and  $\text{CO}_3^{\bullet-}$  ( $E^\circ = 1.5 \text{ V}$ ) [26] radicals is thermodynamically favorable. Furthermore, the bimolecular rate constants for reaction of these radicals with G ( $7.8 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$  for  $\text{HO}^\bullet$ ) [27] and ( $7 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$  for  $\text{CO}_3^{\bullet-}$ ) [28] dictate extremely fast kinetics for the initial oxidation event. The yellow coloured compound formed by the reaction between guanine and peroxynitrite has been identified by HPLC as 8-nitroguanine (first nitration product) and its formation was most favorable at pH 8 and increased dose-dependently with peroxynitrite concentration, but was not dependent on the concentration of guanine. It was proposed that either heterolytic cleavage of peroxynitrite to form a nitronium ion ( $\text{NO}_2^+$ ) or a high energy intermediate ( $\text{ONOOH}$ ) derived from *trans*-peroxynitrite (pKa 7.9) [29] could be involved in the formation of 8-nitroguanine [21]. The reaction of 2'-deoxyguanosine with peroxynitrite yields several compounds, two of which were identified as 4,5-dihydro-5-hydroxy-4-(nitrosooxy)-2'-deoxyguanosine (nox-dG) and 8-nitroguanine [30]. The 8-nitroguanine could be formed either from guanine (depurination of dG) by peroxynitrite or depurination of 8-nitro-2'-deoxyguanosine generated with peroxynitrite. The reaction of various deoxyribonucleosides with peroxynitrite was also shown to yield 2-thiobarbituric

acid (TBA)-reactive substances dose-dependently [31, 32]. Yermilov et al. have shown the formation of 8-nitroguanine dose-dependently in calf thymus DNA when incubated with low concentrations of peroxynitrite [33]. Among peroxynitrite, nitrous acid, tetra-nitromethane, and NO-releasing compounds, only peroxynitrite was responsible for the formation of 8-nitroguanine. This reaction was inhibited by antioxidants like urate, ascorbate, *N*-acetylcysteine and desferrioxamine [33]. Studies have shown that 8-Nitroguanine was depurinated rapidly from DNA incubated at physiological conditions ( $t_{1/2} = \sim 4 \text{ h}$ ), suggesting its formation in DNA is potentially mutagenic because depurination yields apurinic sites, which can induce G:C  $\rightarrow$  T:A transversions [33]. Spencer et al. have shown increased levels of both oxidized and deaminated base products, including 5-hydroxyhydantoin, 5-(hydroxymethyl)uracil, thymine glycol, 4,6-diamino-5-formamidopyrimidine (FAPy-adenine), 2,6-diamino-5-formamidopyrimidine (FAPy-guanine), 8-oxoadenine, 8-oxoguanine, hypoxanthine and xanthine in addition to 8-nitroguanine [34]. Among these, 8-nitroguanine and xanthine were formed in 100–1,000 times greater concentrations than other modified bases.

During the peroxynitrite-induced oxidation of guanine, two main oxidation products namely, 2,5-diamino-4*H*-imidazol-4-one (Iz) [30] and 8-oxoG [14] were formed. Iz has previously been identified as a product of Type I G photooxidation which undergoes hydrolysis to form 2,2,4-triamino-5(2*H*)-oxazolone (Oz) at physiologic pH [35] (Fig. 2). Oz is expected to accumulate in tissues under oxidative stress in which Iz is initially formed. Niles et al. have isolated and identified two highly oxidized products namely spiroiminodihydantoin, Sp and guanidinohydantoin, Gh [36]. 8-oxoG was detected in genomic DNA isolated from tissues not under chronically elevated levels of oxidative stress ranging from 4 to 11 in  $10^7$  bases [37]. Uppu et al. have showed that  $10^3$ -fold excess of G over 8-oxoG protected only half of the 8-oxoG from reacting with peroxynitrite implying that 8-oxoG is inherently more reactive than G with peroxynitrite [38].

There is substantial controversy existing regarding whether synthesized peroxynitrite reacts with guanine to form 8-oxoguanine in DNA. On one hand, Yermilov et al., Douki and Cadet, and Uppu et al. reported no significant increase in 8-oxoguanine in calf thymus DNA treated with synthesized peroxynitrite, compared to non-treated DNA or DNA treated with decomposed peroxynitrite [30, 33, 38]. While on the other hand, Inoue and Kawanishi, Fiala et al. and Spencer et al. showed considerable increase in 8-oxoguanine in calf thymus DNA treated with peroxynitrite [34, 39, 40]. The formation of 8-oxoguanine in peroxynitrite-treated DNA was inhibited by hydroxyl radical scavengers like ethanol and sodium formate [39]. Kennedy and co-workers also reported that low doses of peroxynitrite in pUC19 plasmid

**Fig. 2** Formation of major products from the reaction of peroxyntirite and guanine residue. The reaction between peroxyntirite ( $\text{ONOO}^-$ ) and guanine (G) residue forms 8-nitroguanine (8-nitroG), 8-oxoguanine (8-oxoG), 5-guanidino-4-nitroimidazole (NI) and 2,5-diamino-4*H*-imidazol-4-one (Iz) and it undergoes hydrolysis to form 2,2,4-triamino-5(2*H*)-oxazolone (Oz) at physiologic pH



formed 8-oxoguanine dose-dependently, where its level being decreased with a high dose of peroxyntirite [14]. A similar decrease in 8-oxoguanine formation at high concentrations of peroxyntirite was also reported [30, 33].

Some possible reasons for this discrepancy could be that (i) 8-oxoguanine formation is mediated by contaminants (e.g., hydrogen peroxide and metal ions), the concentrations of which may fluctuate in different preparations of peroxyntirite; (ii) 8-oxoguanine may be formed artificially during isolation, hydrolysis, and analyses of DNA (e.g., conversion of 8-nitroguanine to 8-oxoguanine) and (iii) under certain circumstances, especially with high concentrations of peroxyntirite, the 8-oxoguanine produced may be further oxidized into the ring cleavage product by peroxyntirite [14, 38, 39].

Human skin epidermal keratinocytes exposure to pre-formed peroxyntirite or SIN-1 led to extensive DNA base modification [34]. Large increases in xanthine, hypoxanthine and 8-nitroguanine were observed, while only small increases in some oxidized bases including 8-oxoguanine and FAPy-guanine were found in the DNA from keratinocytes [34]. A variety of DNA base modifications have also been depicted in immunostimulated macrophages which produce oxy-radicals and NO, as well as peroxyntirite. Levels of xanthine, 5-(hydroxymethyl)uracil, and 8-oxoguanine were found elevated in the DNA of macrophages activated with lipopolysaccharide and interferon- $\gamma$ , which were indicative of both oxidative and deaminative DNA damage [41, 42]. Both xanthine and 8-oxoguanine formation was inhibited by an NO synthase inhibitor, suggesting that NO plays a role in both deamination and oxidation reactions [42].

Kaneko et al. showed that 8-nitroguanosine induced oxidative damage and formation of abasic sites in DNA in AS52 cells [43]. Formation of 8-nitroguanosine has been

considered as mutagenic lesion because of its rapid removal from DNA strand by depurination to form mutagenic abasic sites, that can possibly induce G-to-T transversions [44].

#### Single Strand Breaks in DNA

Over the past few years, several studies have shown the induction of single strand breakage in DNA upon exposure to peroxyntirite or to NO and superoxide parallel in different systems. In vitro, peroxyntirite can induce DNA strand breaks in plasmid DNA such as pBR322, PM2, CMV and pUC19, converting the supercoiled form to relaxed or linear forms, which can be separated by agarose gel electrophoresis [31, 32, 41]. A significant induction of single strand breakage was observed in pBR322 plasmid treated with low peroxyntirite, whereas much higher concentrations of peroxyntirite [31] or the presence of a catalyst such as manganese porphyrin [45] were required to induce double strand breakage. DNA breakage caused by peroxyntirite or SIN-1 (donor of peroxyntirite) was observed at almost every nucleotide with a dominance at guanine residues [39]. Peroxyntirite stimulated considerably more single strand breaks at acidic pH than at neutral or alkaline pH, suggesting that hydroxy radical-like intermediate(s) ( $\text{ONOOH}^*$ ) or peroxyntirous acid ( $\text{ONOOH}$ ) are responsible for the damage [32]. Salgo et al. reported that benzoate and dimethyl sulfoxide amplified the breakage by reacting with peroxyntirite to form  $\text{NO}_2$ , which is responsible for the increased DNA damage indicating that the free hydroxyl radical is not involved in the damage [46].

Manganese porphyrins have been shown to catalyze the peroxyntirite-induced DNA single strand breakage in an in vitro system [45]. SIN-1 induced strand breakage was

inhibited by superoxide dismutase [41] as well as NO-trapping agents such as oxyhemoglobin and carboxy-PTIO [47], suggesting that simultaneous generation of NO and superoxide is necessary to cause strand breaks, supporting the assumption that these radicals react with each other to form peroxynitrite or other oxidant(s). Most of the above mentioned studies were not performed in intact cells. However, DNA single strand breakage has also been reported in intact cells exposed to peroxynitrite [10, 48, 49], indicating that extracellular peroxynitrite has the ability to enter the cells and reach the nucleus. More notably, indirect evidence supports the observation that endogenously produced peroxynitrite can also cause DNA single strand breakage during immunostimulation of various cells in addition to DNA base modifications [34, 50]. For example, the generation of DNA single strand breaks in immunostimulated macrophages parallels the production of peroxynitrite [50].

The mechanism for the formation of strand breaks by peroxynitrite includes its reaction with 2'-deoxyribose and deoxynucleosides to form malondialdehyde and base-propenals, respectively [2, 31, 32]. The initial reaction could involve hydrogen abstraction and O<sub>2</sub> attack at either deoxyribose C4' or C5' by hydroxyl radical-like intermediate(s) (ONOOH<sup>\*</sup>) or peroxynitrous acid (ONOOH). After the modification at C4', the strand breakage can be induced either by C3'–(phosphate-O) cleavage or C3'–C4' plus C1'–(ring-O) bond cleavages [51]. The strand breakage could also be induced by cleavage between C4' and C5', following the damage at C5' [52]. Studies by Habib et al. and Dixit et al. have shown the single breaks resulted from peroxynitrite upon exposure to DNA. Due to this exposure, neo-epitopes were generated in the modified DNA owing to its antigenicity [53, 54].

### Biological Effects of Peroxynitrite-Induced DNA Damage

#### Apoptosis Induced by Peroxynitrite

Once the level of cellular damage imposed by peroxynitrite supercedes any possibility of repair, the cell eventually dies through one of the two main pathways of cell demise, necrosis or apoptosis. Necrosis is associated with loss of cellular ATP, leading to membrane disruption, release of noxious cellular debris, and the development of secondary inflammation. On the contrary, apoptosis occurs in a well-strategized sequence of morphological events characterized by nuclear and cytoplasmic condensation with blebbing of the plasma membrane. The dying cell ultimately breaks up into membrane-enclosed particles termed apoptotic bodies, which are rapidly ingested and degraded by professional

phagocytes or neighboring cells, without inducing any inflammatory response. Apoptosis is organized by the proteolytic activation of cysteine proteases known as caspases, that requires preserved ATP levels to proceed properly, and which may be triggered either by the activation of death receptors (extrinsic pathway) or by the permeabilization of the outer membrane of mitochondria (intrinsic pathway) [55, 56].

Several studies have shown that NO and peroxynitrite either cause acute cell death (necrosis) or delayed cell death (apoptosis) in various cell types [2, 57–59]. Sustained low level exposure of NO or peroxynitrite cause apoptosis, whereas cell necrosis results from sudden exposure to high concentrations of peroxynitrite or NO. Therefore, necrosis induced by peroxynitrite has been suggested to play an important role in inflammation.

The cellular mechanisms of peroxynitrite-induced apoptosis have not been clearly explained which is demonstrated in CHO-Em9 cells defective in their ability to repair DNA single strand breaks and unrepaired single strand breaks lead to the formation of double strand breaks ultimately resulting in cell death [60]. There are number of regulatory factors such as p53 and CPP32 which appears to determine the fate of the cell [61, 62] in relation to NO or peroxynitrite induced DNA damage.

It is noteworthy that different types of DNA injuries can cause cell cycle arrest in different phases which consecutively can affect the fate of the cell [61, 62]. It is quite clear that the NO-induced apoptosis associated with proteolytic cleavage of PARP [63], involves mechanisms that vary from the apoptosis triggered by potent DNA single strand breaking agents, such as peroxynitrite and hydroxyl radical.

#### Poly(ADP-ribose) Polymerase PARP Activation and Acute Cell Injury

The post-translational modification of nuclear proteins that results in the poly-adenosine diphosphate (ADP) ribosylation is called as PARylation. PAR polymerase (PARP) enzymes initiate the reaction by converting the substrate nicotinamide adenine dinucleotide (NAD<sup>+</sup>) to ADP-ribose and nicotinamide, and then catalyze ADP-ribose polymerization on nuclear acceptor proteins [64, 65]. The major acceptors of poly(ADP-ribose) are PARP itself (automodification domain), topoisomerase I and II, DNA polymerases  $\alpha$  and  $\beta$ , and DNA ligase 2. The ADP-ribosylation reduced the catalytic activities of these enzymes [66]. Chromatin relaxation is also caused by the presence of ADP-ribose on histones (mainly histone H1) [66]. PAR turnover is regulated by PAR glycohydrolase (PARG), which catalyzes the degradation of poly(ADP ribose) into free ADP-ribose (ADPr) and adenosine monophosphate [67].

So far, about 17 isoforms of PARP with different structural domains and functions have been identified: PARP-1, PARP-2, PARP-3, PARP-4 (Vault-PARP), PARP-5 (Tankyrases-1 and 2), PARP-6, PARP-7 (tiPARP), PARP-8, PARP-9 (BAL1), PARP-10, PARP-11, PARP-12, PARP-13 (ZAP), PARP-14 (CoaSt6), PARP-15, and PARP-16 [68, 69]. PARP-1 is the most abundant and best characterized isoform of the PARP enzyme family. This nuclear enzyme is a highly conserved protein of 116 kDa. This protein is composed of three primary functional domains: an N-terminal DNA-binding domain (46 kDa), including a nuclear localization signal, a central auto modification domain (16 kDa), and a C-terminal catalytic domain (55 kDa). The DNA binding domain contains two zinc-finger motifs that help in binding to both single and double-stranded DNA breaks [70].

Poly ADP-ribosylation has been associated in the regulation of several physiological cellular functions such as DNA repair, gene transcription, cell cycle progression, cell death, chromatin function and genomic stability [71]. PARP-1 becomes hyperactivated in response to free radicals, reactive oxygen species and peroxynitrite resulting in the depletion of  $\text{NAD}^+$  and adenosine triphosphate (ATP) and ultimately cell death and organ dysfunction. Also, PARP-1 upregulates the expression of pro-inflammatory genes by transcription factors activation. Hence, PARP-1 plays important roles in the pathogenesis of many diseases such as stroke, myocardial infarction, circulatory shock, diabetes, neurodegenerative disorders including Parkinson's and Alzheimer's diseases, autoimmune diseases, allergy, asthma, colitis and other inflammatory disorders [72, 73].

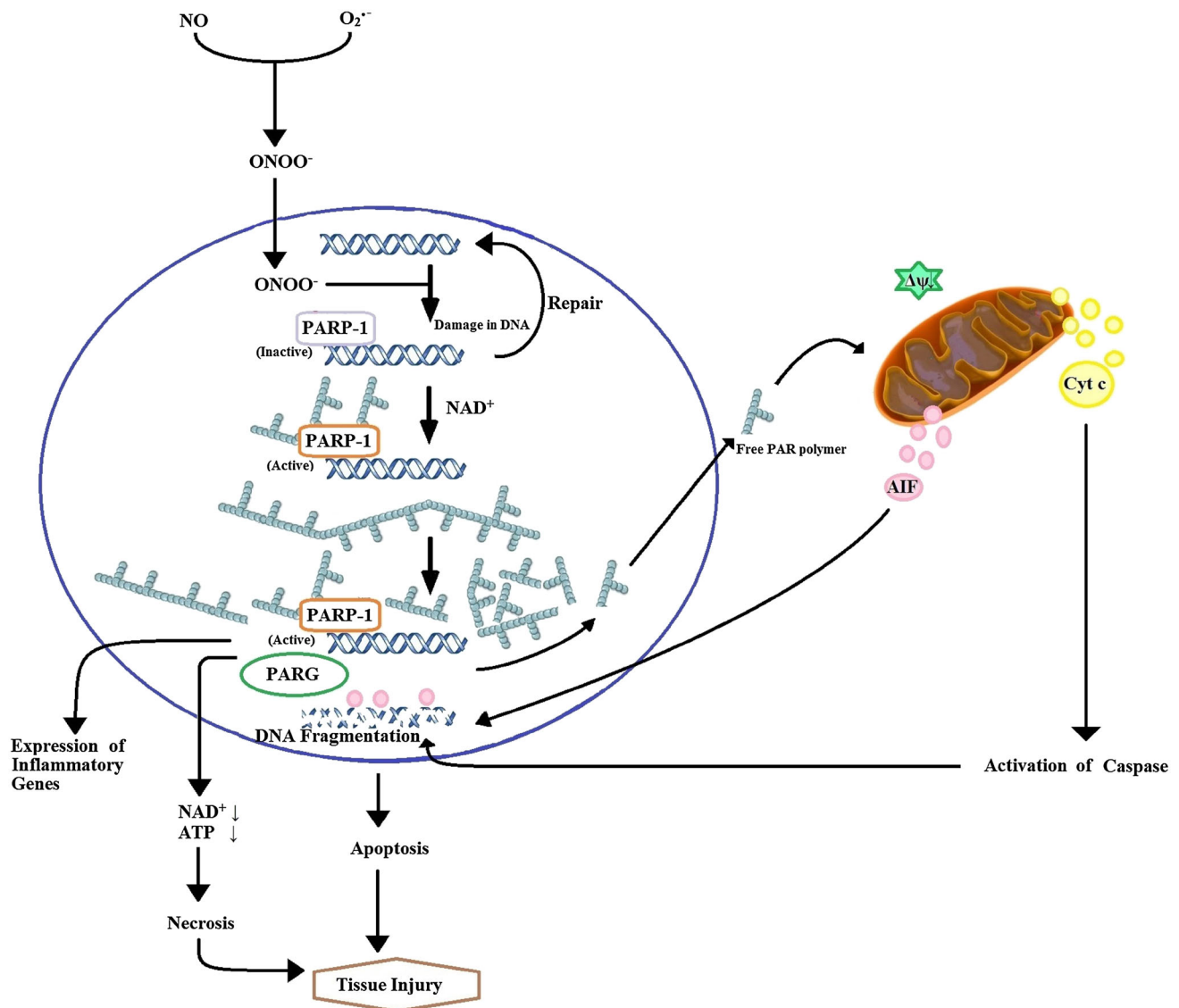
Peroxyntirite has been identified as a pathophysiological trigger of PARP activation [72, 74] and it can also induce pathophysiological alterations independently from PARP as well. These alterations are numerous and include protein modifications (of which the most studied is tyrosine nitration), DNA modifications, alterations in cellular signal transduction pathways, leading to changes in inflammatory responses and promotion of cell death via apoptotic and necrotic routes [75, 76]. The pathway of cell injury involving the pronounced activation of PARP can rapidly deplete the intracellular concentration of its substrate,  $\text{NAD}^+$ , retarding the rate of glycolysis, electron transport and, therefore, ATP formation resulting in acute cell dysfunction and cell death. It develops rapidly and is apparently distinguishable from apoptosis (Fig. 3). It has been suggested that there is an additive or synergistic relationship involving the PARP-dependent and the PARP-independent pathophysiological actions [76–78].

PARP activation can contribute to the development of disease through two main mechanisms: by driving the cell into an energetically deficit state of dysfunction and by

catalyzing the activation of proinflammatory pathways. In the first pathway, PARP-1 functions as a DNA damage sensor and signaling molecule, binding to both single and double-stranded DNA breaks. PARP-1 forms homodimers on binding to damaged DNA and catalyzes the cleavage of  $\text{NAD}^+$  into nicotinamide and ADP-ribose to form long branches of ADP-ribose polymers on target proteins such as histones and PARP-1 itself, resulting in cellular energetic depletion, mitochondrial dysfunction and ultimately necrosis [72, 74]. But in second pathway, various transcription factors, DNA replication factors and signaling molecules have also been shown to become poly(ADP-ribosylated) by PARP-1, but a PARP-mediated activation of the pluripotent transcription factor nuclear factor- $\kappa\text{B}$  (NF- $\kappa\text{B}$ ) appears to be of crucial importance [72, 79]. Notably, recent studies have suggested that PARP-1 activity can be modulated by several endogenous factors and PARP-1 can also modulate important signaling pathways [78].

The main evidence on the role of peroxynitrite and PARP in the development of disease is based on *in vitro* studies (including studies in human primary cells and cell lines), as well as on various animal models of disease [78, 80–86]. Evidences were accumulating on the pathophysiological pathways associated with the production of peroxynitrite and the activation of PARP specifically in human disease. The relationship between these two pathophysiological pathways is close and diverse, well supported by *in vitro* data and preclinical animal studies using various pharmacological inhibitors. The pathological activation of PARP requires the development of DNA single-strand breaks *in vivo* [87]. Theoretically, breaks in the DNA can be brought about by a range of oxidant and free radical species, including hydroxyl radical and peroxynitrite (but not by superoxide or nitric oxide alone). From numerous studies, however, it is quite clear that peroxynitrite is the key pathophysiological species that can trigger such DNA injury *in vivo*. Peroxynitrite can cross cell membranes, enter the nucleus and cause breaks in the strands of the DNA [74–76]. Presently, no other species is known with the capacity of travelling within and between cells and having the ability to break the DNA [76]. *In vitro* studies clearly demonstrate that exogenously applied or endogenously produced peroxynitrite induces DNA strand breakage and PARP activation, and peroxynitrite-induced cell death can be attenuated by pharmacological inhibition of PARP or specifically by genetic inactivation of PARP isoform [49, 88].

Besides, above mentioned roles, a new role of PARP has been discovered in regulating the mitochondria-to-nucleus translocation of apoptosis-inducing factor (AIF). AIF is a 67-kDa mitochondrial flavoprotein that is involved in cellular metabolism and apoptotic pathways [89]. It maintains



**Fig. 3** Peroxynitrite mediated downstream caspase dependent and independent apoptotic pathways

mitochondrial structure [90] and plays a necessary role in oxidative phosphorylation [91] under physiological conditions where it acts as a key mediator of caspase-independent cell death under pathological conditions.

The translocation of AIF from the mitochondria to the nucleus appears to be the central event, leading to induction of chromatin condensation and large-scale ( $\approx 50$  kb) DNA fragmentation and ultimately cell death [92]. AIF plays a pivotal role in mediating PAR polymerase-1 (PARP-1)-dependent cell death. Activation of PARP induces cell death by a mechanism that differs from apoptosis, necrosis and autophagy [93, 94]. This process involves translocation of AIF from mitochondria to the nucleus [95]. Harraz et al. coined the term “parthanatos” to PARP-1-mediated cell death in order to differentiate this process from other forms of cell death [96]. PARP-1 does

not cause apoptotic body formation (unlike apoptosis), cell swelling (unlike necrosis) and also does not involve autophagic vacuole formation and lysosomal degradation like autophagy. Parthanatos results in phosphatidylserine flipping onto the outer plasma membrane, dissipation of the mitochondrial membrane potential, chromatin condensation and large DNA fragmentation [97]. Reduction in AIF nuclear translocation has been shown by pharmacological inhibition of PARP activity [98].

#### Peroxynitrite-Induced DNA Damage: Aspect in Inflammation-Linked Cancer

Several risk factors that are recognized for human cancers at various sites include chronic infection by bacteria, parasites, or viruses and tissue inflammation such as gastritis,

hepatitis and colitis [99, 100]. Nitric oxide and other oxygen radicals generated in infected and inflamed tissues have been suggested as one of the contributing factors to the multistage process of carcinogenesis by damaging DNA and tissues. Since peroxynitrite is currently considered to be a major compound responsible for tissue damage caused by inflammation, it may also play an essential role in carcinogenesis [101].

Peroxynitrite causes numerous types of modifications in DNA and induces strand breakage. The transversions comprising of G:C → T:A are very common in a variety of genes from all types of human cancers [102] and these transversions account for 30 % of all p53 gene mutations in lung cancer and are also high in liver and breast cancer. It has been suggested that G:C → T:A transversions are mostly caused by polycyclic aromatic hydrocarbons such as benzo(a)pyrene present in tobacco smoke. Nonetheless, peroxynitrite also caused G:C → T:A transversions. NO (formed endogenously by NO synthase in the lung) and superoxide may react to form peroxynitrite in the lungs of smokers and play an essential role in smoking-related diseases like lung cancer. Similarly, the reaction between catechol–estrogens and nitric oxide can generate oxidants which are analogous to peroxynitrite [103]. NO is produced by constitutive and inducible types of NO synthases in human breast tissues [104, 105] which may react with superoxide generated from catechol–estrogens to produce peroxynitrite and can induce the G:C → T:A transversions observed commonly in breast tumors.

There are other numerous sites where inflammation, peroxynitrite production and DNA injury have been linked to carcinogenesis which include *Helicobacter pylori* infection, gastritis and gastric cancer [106–109], as well as ulcerative colitis and colon cancer [110–112].

### Role in Diseases

Various diseases such as diabetes, atherosclerosis, ischemic heart diseases, stroke, autoimmune and neurodegenerative diseases are resulted from the disruption of mitochondrial functions. Peroxynitrite may be either directly produced within the mitochondria or may reach mitochondria from extra mitochondrial compartments. Consecutively, peroxynitrite nitrates and inhibits Mn-SOD [113] and therefore preventing the breakdown of locally produced superoxide, which further increases the formation of peroxynitrite. The peroxynitrite toxicity in mitochondria results from both direct oxidative reactions and free radical-mediated damage [114, 115], secondary to peroxynitrite reacting with CO<sub>2</sub>, giving rise to CO<sub>3</sub><sup>•-</sup> and NO<sub>2</sub><sup>•</sup> radicals. The latter reaction is largely favored within mitochondria, which are the main organelles where CO<sub>2</sub> is produced during decarboxylation

reactions [114, 115]. Peroxynitrite further reduces energy metabolism by inhibiting the tricarboxylic acid cycle enzyme aconitase, located in the mitochondrial matrix, through oxidative disruption of the 4Fe–4S center of the enzyme [116, 117], as well as mitochondrial creatine kinase, which is present in the intermembrane space [118].

### Diabetes

Diabetes is one of the major chronic diseases with an estimated worldwide dominance of 170 million in 2002 and is expected to double by 2030 according to the World Health Organization [119]. In this disorder, system's ability to regulate blood glucose level in a physiological (5 mM) range is altered, either by a reduced production of insulin as a result of pancreatic β-cell destruction (Type 1) or by insulin resistance (Type 2). Primary Diabetes (Type 1 diabetes) is caused by the autoimmune destruction of insulin producing β-cells of the pancreatic islet resulting in prolonged periods of hyperglycemia via reduced uptake of glucose and relative increase in glucagon secretion and gluconeogenesis. However, the resulting long-term effects are far more dangerous than acute hyperglycemia itself. The endothelial cells are damaged in the vessels by chronic hyperglycemia and in fact most of the diabetic chronic complications result from pathological alterations of the micro and macrovascular system, leading to increased risk of blindness, infarction of brain and heart, kidney failure and impaired wound healing [120–122].

Atherosclerosis is the most common macrovascular complication of diabetes which increases the risk for myocardial infarction, stroke and peripheral artery disease, the latter being the principal cause of limb amputation in civilized countries whereas microvascular complications consist of retinopathy and nephropathy which are the leading causes of blindness and renal failure respectively [123]. Diabetic cardiomyopathy is a complication resulting from diabetes and is characterized by complex changes in the mechanical, structural, biochemical and electrical properties of the heart, which may underlie the development of an early diastolic and a late systolic dysfunction, or both, and increased incidence of cardiac arrhythmias in diabetic patients [124].

Considerable evidences support the pathogenetic role of endogenous peroxynitrite formation in diabetic cardiovascular complications both in experimental animals and in humans [125–127]. Peroxynitrite has been reported to attack different biological macromolecules, leading to compromised cardiovascular function in diabetes through numerous mechanisms. One of these pathways involves DNA strand breakage and consequent activation of the nuclear enzyme PARP. This PARP activation (specifically PARP-1 isoform) is a crucial process in the development of



diabetic cardiovascular dysfunction both in diabetic animals and humans [128–130] and may also contribute to the progress of other diabetic complications such as neuropathy, nephropathy and retinopathy [84]. The kidney biopsies of patients having diabetic nephropathy and of diabetic animals have shown increased peroxynitrite formation and oxidative stress [131, 132]. There is an elevation of superoxide anion and peroxynitrite formation in retinal endothelial cells maintained in high glucose [133] and in retinas of diabetic animals [133, 134] and these alterations could be attenuated with various antioxidants like NOS inhibitors or peroxynitrite scavengers [133–135]. Also, increased formation of peroxynitrite has been documented in both experimental [135] and clinical diabetic neuropathy [136]. A novel mechanism has been identified in which PARP activation induces various pathways leading to diabetic complications [137]. According to this concept, hyperglycemia induced superoxide generation from the mitochondria (directly or indirectly via generation of peroxynitrite) induces DNA strand breaks and PARP activation which, in turn, induces the poly ADP-ribosylation of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The resulting metabolic alterations activate NF $\kappa$ B, polyol pathway and aldose reductase leading to increased oxidative stress [137]. Studies have shown that inhibition of PARPs diminished the concomitant NAD<sup>+</sup> loss and prevented PAR formation [138]. Similarly, isolated  $\beta$ -cells from Parp1 knockout animals were protected from cell death induced by ROS [139]. In streptozotocin-induced diabetes, Parp1 knockout mice were protected from  $\beta$ -cell loss and subsequent type 1 diabetes. Gene dosage appeared to be essential as Parp1<sup>+/-</sup> animals were partially protected [140].

Animals were also protected after altering the application of streptozotocin from a single high dose to multiple low-dose injections, if PARP enzymes were inhibited or the Parp1 gene was disrupted [141]. Hence, inhibiting PARP activity directly suppresses islet cell loss and therefore prevents the onset of type 1 diabetes. The increased cellular damage also induces DNA strand breaks, which in turn activates PARP. PARP inhibitors significantly improved diastolic dysfunction of the heart and loss of endothelium-dependent vasodilation [129]. The administration of PARP inhibitor PJ34 even one week after onset of diabetes was effective in spite of persistent hyperglycemia [129]. Rats with streptozotocin-induced diabetes showed increased heart dysfunction and a larger infarcted area after myocardial ischemia–reperfusion injury as well as higher mortality rates during reperfusion. Blocking PARP activity with the inhibitor INO-1001 improved myocardial function in both groups. In diabetic rats, INO-1001 significantly lowered the mortality during the experiment [142]. The increase in nuclear AIF after ischemia–

reperfusion was prevented in both control and diabetic groups. These potent PARP inhibitors reduce the clinical complications usually seen in diabetes and therefore considered to be therapeutically good candidates. But PARP inhibition has been shown to promote cancer formation under genotoxic stress [143]. Likewise, because many of the cellular functions are regulated by poly ADP-ribosylation, very specific inhibitors against few isoforms of PARP have to be designed [144].

#### Cardiovascular System Diseases

The one of the major causes of death in developed countries are diseases of cardiovascular system. Therefore, improvement in treatment will have a great impact on mortality rates and average life span. After challenge with oxidants like peroxynitrite and hydrogen peroxide, PARP activation was detected in cardiomyoblasts, but not with NO-donors (*S*-nitroso-*N*-acetyl-DL-penicillamine, diethyl-triamine NONOate) [145]. All these chemicals cause reduction in mitochondrial respiration. Furthermore, hypoxia and re-oxygenation stimulated PAR formation. Unsurprisingly, PARP activity was suppressed by addition of 3AB and nicotinamide in all cases. In Parp-1 knockout mouse fibroblasts, NO-donors diminished mitochondrial respiration, whereas peroxynitrite and hydrogen peroxide did not. In brief, PARP activation depends on DNA damage induced by peroxynitrite or hydrogen peroxide. In this case, mitochondrial respiratory failure can be rescued by PARP inhibitors, but not if reduction of mitochondrial respiration is achieved by NO-donors, which do not activate PARPs.

Thiemermann and co-workers showed that PARP inhibitors (3AB, nicotinamide and 5-ISO, 1,5-isoquinolinediol) improved heart functionality and reduced infarct size after occlusion and reperfusion of the left coronary artery in ischemia–reperfusion of rabbit hearts whereas structurally similar compounds like 3-AB (3-aminobenzoic acid) and nicotinic acid, which did not inhibit PARPs, were not effective [146]. Similarly, Zingarelli and colleagues reported similar effects in rats, and additionally elevated necrosis, neutrophil infiltration and nitrotyrosine formation with loss of ATP. 3AB diminished neutrophil activation and partially preserved myocardial ATP levels [147]. The application of 3AB also proved effective in reducing infarct size and contractile dysfunction in different animal model (pigs) [148].

In the Parp1 knockout mouse model, it was shown that INO-1001 attenuated heart remodeling after ischemia–reperfusion injury (hypertrophy and formation of collagen in the hearts) in addition to preserving functionality and translocation of AIF into the nucleus [149]. In a cell culture-model of cardiomyoblasts, hypoxia followed by re-

oxygenation led to oxidative stress, PARP activation and a drop in energy-metabolite levels ( $\text{NAD}^+$  and ATP) [150]. Therefore, necrosis was the common pathway of death and AIF dependent apoptosis was increased. The administering of PJ34 attenuated the decline in levels of  $\text{NAD}^+$  and ATP, possibly due to reduced PAR formation. A shifting took place from caspase-independent to caspase-dependent apoptosis. Besides cell survival, PARP inhibition also modulates the way in which cells die, changing it from the pro-inflammatory necrosis to the less harmful apoptosis. Pacher and co-workers showed that inhibition of poly(-ADP-ribose) polymerases is also effective in a chronic heart-failure model [151]. They ligated the left anterior descending coronary artery in rats and tested functionality and stress parameters. The levels of nitrotyrosine and PAR were increased in controls and the performance of the left ventricle was reduced and relaxation was impaired (measured *ex vivo*). PJ34 facilitated the reduction of PAR amount (as expected) but had no significant impact on nitrotyrosine formation. Hence, it was not the early damage (nitrotyrosine level), but the activity of PARPs (polymer amount) that was suppressed.

In conclusion, PARP inhibition possibly does not reduce initial damage, but diminishes the ongoing formation of reactive compounds like  $\text{NO}^\bullet$  and subsequently peroxynitrite. This results in preservation of energy metabolites and blocks the translocation of AIF from the mitochondria into the nucleus. As a result, necrosis is reduced as well as overall cell death. Inflammation (neutrophil activation) is suppressed and the resulting morphological changes like hypertrophy are reduced. These all collectively lead to an improved functionality of the heart and a reduction in infarct size [86, 152, 153].

#### Neurodegenerative Diseases

Neurodegenerative diseases consist of different conditions which primarily affect the neurons in the human brain and spinal cord leading to either functional loss (ataxia) or sensory dysfunction (dementia). Peroxynitrite formation has been associated to neurodegenerative diseases like Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis (ALS) and other brain pathologies like Huntington's disease, multiple sclerosis (MS) [154–157]. The immunoreactivity of nitrotyrosine was established in early stages of all of these diseases in human autopsy samples as well as in experimental animal models. Elevated levels of nitrite, nitrate and free nitrotyrosine have been found to be present in the cerebral spinal fluid (CSF) and proposed to be useful marker of neurodegeneration [154–157]. When peroxynitrite is formed in diseased brain, it may exert its toxic effects through multiple mechanisms, including lipid peroxidation, mitochondrial damage,

protein nitration and oxidation, depletion of antioxidant reserves (especially glutathione), activation or inhibition of various signaling pathways, and DNA damage followed by the activation of the nuclear enzyme PARP [155–158].

#### *Alzheimer's Disease (AD)*

It is a progressive neurodegenerative disorder which is characterized by the formation of neuritic plaques rich in  $\beta$ -amyloid ( $\text{A}\beta$ ) peptide, neurofibrillary tangles rich in hyperphosphorylated tau protein, gliosis and a neuroinflammatory response involving astrocytes and microglia, certainly leading to progressive global cognitive decline, and accounting for the vast majority of age-related dementia [159]. This disease is known to cause loss of functional neurons from several regions of the brain, although the precise mechanisms of cell loss are still unclear, but several proposed mechanisms ultimately converge on disrupted calcium signaling [160].

It appears that increased oxidative stress is an early event in the process of neurodegeneration associated with Alzheimer's disease [161–166] and both neuronal and glial NOS may play a role in the pathogenesis of AD and peroxynitrite formation. Expression of nNOS was increased in neurons with neurofibrillary tangles in the hippocampus and entorhinal cortex of AD patients as well as in reactive astrocytes near amyloid plaques [167, 168].

Neuropathological studies have revealed fragmentation of nuclear DNA (by use of *in situ* end-labelling, ISEL) in a much higher proportion of neurons, oligodendrocytes, astrocytes and microglia in the brains of patients with Alzheimer's disease than in age-matched controls [169–172]. This led to the interpretation that the loss of neurons is due to apoptosis, probably initiated by  $\text{A}\beta$ -protein or other inducers of oxidative stress. The extent of apoptosis which is morphologically evident in Alzheimer's disease is very limited and difficult to reconcile with the high density of ISEL-positive cells. It remains unconfirmed that the fragmentation of nuclear DNA in Alzheimer's disease denotes apoptosis, particularly in the context of non-mitotic cells.

The connection between DNA damage and oxidative stress is known to activate DNA repair proteins, including poly(ADPribose) polymerase (PARP). PARP is stimulated in response to free radical-mediated injury to DNA after brain ischaemia and reperfusion [173]. Its overactivation in injured cells can cause massive consumption of  $\text{NAD}^+$  and finally resulting in cell death due to energy depletion [173–175]. Love and co-workers have shown that increased expression of PARP and intranuclear accumulation of its end-product poly(ADP-ribose) in neurons in brains from patients with Alzheimer's disease than of control brains. So, there may be the possibility that overactivation of

PARP may contribute to neuronal loss in Alzheimer's disease [176].

#### *Parkinson Disease (PD)*

It is the second most common neurodegenerative disease of adult onset, characterized by progressive loss of dopaminergic neurons within substantia nigra pars reticulata (SNr), resulting in reduced dopamine levels and a loss of dopaminergic neurotransmission in the striatum, which interferes with the function of the basal ganglia critical to motor function and coordination [177, 178]. The common symptoms are hypokinesia, rigidity, resting tremor and postural instability. The characteristics of the disease are Lewy body inclusions in the dopaminergic cells and cell loss in the substantia nigra (SN). Similar characteristics are accompanied by Lewy bodies in the cortical neurons and consequent dementia in diffuse Lewy body disease (DLBD). Both oxidative stress and excitotoxic injury play a significant role in the degeneration mechanism of the dopaminergic neurons in the SN in PD [179], resulting in intraneuronal calcium level increase and can lead to the activation of PARP to repair DNA damage. The excessive activation of PARP leads to massive consumption of  $\text{NAD}^+$  and subsequently of ATP resulting in cell death due to energy depletion [180]. PARP is needed as co-activator for the nuclear translocation and transcriptional activation of NF- $\kappa$ B in the nuclei of the dopaminergic neurons in PD [181], which has been involved in the regulation of genes involved in immune and inflammatory responses, cell survival, apoptosis, development, differentiation, cell growth and neoplastic transformation [182]. This ultimately leads to apoptosis by its influence on p53 gene expression [183].

Soós and co-workers using immunohistochemical methods confirmed that the expression of PARP is increased together with the nuclear translocation of NF- $\kappa$ B in the dopaminergic cells in the SN in PD and DLBD patients as compared with age-matched control patients with other neurodegenerative diseases and with normal controls [184]. This significant increase in PARP expression in the nuclei of a subset of dopaminergic neurons in the SN in PD and DLBD can reflect both oxidative damage and an increased intracellular calcium level leading to neuronal death by over-activation of PARP.

Besides PARP, peroxynitrite may also induce nitration of tyrosine hydroxylase, the initial and rate-limiting enzyme in the biosynthesis of dopamine, leading to inhibition of enzyme activity and consequent failure in the synthesis of dopamine [185]. The nitration of tyrosine residues in tyrosine hydroxylase paralleled the decline in dopamine levels in mouse striatum following 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) administration

[185]. Tyrosine residues are also crucial for the substrate specificity of monoamine oxidase B (MAO B) resulting in impaired dopamine catabolism once nitrated [186]. Peroxynitrite also involved in the loss of intracellular glutathione from substantia nigra (an early event in PD) by inactivating glutathione reductase, the enzyme responsible for the regeneration of glutathione from its oxidized form [187, 188], and to induce apoptosis in dopaminergic neurons in PD [189]. Some recent evidences suggested that mitochondrial complex I inhibition may be the central cause of sporadic PD and these derangements in complex I lead to  $\alpha$ -synuclein aggregation, which contributes to the demise of dopamine neurons [190]. Accumulation and aggregation of  $\alpha$ -synuclein may further assist the death of dopamine neurons through impairments in protein handling and detoxification [190].

#### *Amyotrophic Lateral Sclerosis (ALS)*

It is also known as Lou Gehrig's disease or motor neuron disease and most common adult-onset progressive fatal neurodegenerative disease, which is characterized by rapid, progressive degeneration of both upper and lower motor neurons in the motor cortex, brain stem, and spinal cord. This finally leads to progressive weakness, paralysis and premature death within 3–5 years after the onset of symptoms [191, 192]. Patients are cognitively intact and therefore completely aware of their progressive disability. About ~10 % have familial inheritance, usually with an autosomal dominant pattern, while most ALS cases are sporadic with no genetic basis. Various mechanisms like neuroinflammation, oxidative stress (in sporadic ALS, sALS), autoimmunity, defect in neuronal glutamate transport, neurofilament accumulation, exogenous factors (viruses, toxins), mitochondrial dysfunction and mutations in the SOD gene were implicated to play a role, but the pathogenesis of ALS is incompletely understood [164, 192–195].

Protein and lipid oxidation markers are elevated in postmortem tissues of sALS [196–199]. Additionally, the level of 8-hydroxy-2'-deoxyguanosine (8OH2'dG), a marker of oxidative injury of DNA, increases with disease progression [197] and correlates with disease severity [200]. These alterations were originally supposed to be mostly confined to neurons. But, recent studies using spinal cords of sALS patients or ALS animal models exhibit that markers of oxidative stress have been localized not only to motor neurons but also astrocytes, microglia, and macrophages [198–203].

DNA damage as a result of oxidation, alkylation or ionizing radiation is associated with activation of PARP [204–207]. When PARP is activated by DNA strand breaks, it subsequently transfers branched chains of ADP-

ribose to a variety of nuclear proteins, including PARP itself [208]. It can also be activated by inositol 1,4,5,-triphosphate- $\text{Ca}^{2+}$  mobilization without DNA damage [209].  $\text{Ca}^{2+}$  promotes PARP activation and  $\text{Ca}^{2+}$  acts as an activating factor in PARP-mediated cell killing [210, 211]. Even though PARP facilitates DNA repair [212], excessive PARP activation can cause massive consumption of its substrate  $\text{NAD}^+$  and results in cell death due to energy depletion [205, 208, 210, 213–215]. Recently, studies have shown that FUS (fused-in-sarcoma, member of the heterogeneous nuclear ribonucleoprotein family of proteins that bind thousands of pre-mRNAs and can regulate their splicing) is a component of the PARP-1 dependent response to oxidative chromosomal DNA damage and defect in this response might contribute to ALS [216].

Protein nitration may also play a role in ALS pathogenesis, acting directly by inhibiting the function of specific proteins and indirectly interfering with protein degradation pathways and phosphorylation cascades [217]. Peroxynitrite can participate in the pathogenesis of ALS, because it can activate spinal cord astrocytes, which normally provide excellent trophic support to motor neurons, to assume a reactive phenotype that induces the death of motor neurons [218]. Reactive astrocytes are common characteristic of neurodegeneration. The transformation of astrocytes into reactive astrocytes is the reason behind progressive nature of ALS and cause the relentless death of neighboring motor neurons.

The involvement of PARP has been emphasized as a pathogenic mechanism in MPTP-induced Parkinson's disease [219–221], Alzheimer's disease [222] and ischemic brain injury [174, 222]. Moreover, PARP inhibitors have been proposed as potential therapies in neurodegenerative diseases in which oxidative stress is suspected to play an important pathogenic role [207, 219–226].

## Conclusions

Peroxynitrite is a charged strong oxidant capable of modifying various types of biological macromolecules to produce variety of products. It primarily acts as a trigger for multiple forms of DNA damage (Base modifications, single strand breakage, and apoptotic double strand breakages). It is known to induce damage in the DNA bases predominantly at guanine and 8-oxoG nucleobases through thermodynamically and kinetically favorable oxidation reactions. Peroxynitrite induced DNA damage activates PARP and it catalyzes ADP-ribose polymerization on nuclear acceptor proteins by converting the substrate  $\text{NAD}^+$  to ADP-ribose and nicotinamide, triggering caspase-independent cell death through Apoptosis Inducing Factor. It helps in upregulation of pro-inflammatory genes expression by activating transcription factors. As a

result, activated PARP plays an important role in pathogenesis of numerous diseases like diabetes, cardiovascular diseases and neurodegenerative diseases. Several strategies were suggested for the inhibition of peroxynitrite-induced damage. One of them involves targeting of cytotoxic pathways stimulated by peroxynitrite which is now becoming a feasible approach to alleviate disease signs in numerous disorders. Controlled blocking of the activity of PARP could provide a possible direction to overcome such oxidative and nitrosative damages. But excess of DNA damage results in over stimulation of PARP, leading to an energy deficit state and ultimately necrosis. So, there is a need for exploration of more beneficial approaches in future.

**Acknowledgments** One of the authors B. U. I. is thankful to UGC-MANF for financial support as senior research fellow. Assistance from the Institution (AMU) as well as infrastructural support from DST-FIST to the department is also duly acknowledged.

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