Poly(ADP-ribose) Polymerase 1 (PARP1) in Atherosclerosis: From Molecular Mechanisms to Therapeutic Implications

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Abstract: Poly(ADP-ribosyl)ation reactions, carried out by poly(ADP-ribose) polymerases (PARPs/ARTDs), are reversible posttranslational modifications impacting on numerous cellular processes (e.g., DNA repair, transcription, metabolism, or immune functions). PARP1 (EC 2.4.2.30), the founding member of PARPs, is particularly important for drug development for its role in DNA repair, cell death, and transcription of proinflammatory genes. Recent studies have established a novel concept that PARP1 is critically involved in the formation and destabilization of atherosclerotic plaques in experimental animal models and in humans. Reduction of PARP1 activity by pharmacological or molecular approaches attenuates atherosclerotic plaque development and enhances plaque stability as well as promotes the regression of pre-established atherosclerotic plaques. Mechanistically, PARP1 inhibition significantly reduces monocyte differentiation, macrophage recruitment, Sirtuin 1 (SIRT1) inactivation, endothelial dysfunction, neointima formation, foam cell death, and inflammatory responses within plaques, all of

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which are central to the pathogenesis of atherosclerosis. This article presents an overview of the multiple roles and underlying mechanisms of PARP1 activation (poly(ADP-ribose) accumulation) in atherosclerosis and emphasizes the therapeutic potential of PARP1 inhibition in preventing or reversing atherosclerosis and its cardiovascular clinical sequalae. Med. Res. Rev., 00, No. 00, 1–32, 2013

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1. INTRODUCTION

The major sources of human illness in the first part of the 21st century are cardiovascular diseases, mental illness, and cancer. There are overlaps in these areas as, for example, state of well-being is a contributing factor to cardiovascular disease[1] and there are interactions between depression and cardiovascular disease.[2] Cardiovascular disease is manifested as heart attacks and strokes and their clinical consequences include heart failure and neurological disorders as well as other manifestations, such as limb amputations and renal and ophthalmic diseases. The major underlying pathology of most cardiovascular diseases is atherosclerosis[3–5]—the development of plaques in medium-sized vessels, followed by the rupture of these plaques and thrombosis, which precipitates the tissue ischemia producing the clinical consequences.[6,7] The major drivers of atherosclerosis are cigarette smoking, hypertension, mental status, hyperlipidemia, insulin resistance, hyperglycemia, and nonmodifiable factors, such as age and genetics.[1] Atherosclerosis in humans commences with a preinflammatory stage involving the trapping of lipids in the vessel wall by modified proteoglycans,[8–10] followed by an inflammatory stage involving multiple immune cells resulting in the formation of atherosclerotic plaques.[4,11] These plaques can be stable or labile where the latter are vulnerable to rupture and the acute precipitation of adverse clinical events.[6,7] Treatments for atherosclerosis are directed at the above risk factors most prominently systemically directed treatments, such as antihypertensives and lipid-lowering medications, but even in clinical trials these strategies only prevent one-third of the cardiovascular events.[12,13] What is required is a greater understanding of the mechanisms of plaque formation and determinants of stability and liability in the vessel wall and the generation of novel therapeutic agents that address these drivers of the atherosclerotic process.[8,11,14] Among the therapeutic agents tested preclinically, pharmacological inhibitors of poly(ADP-ribose) polymerase 1 (PARP1, also named as ARTD1[15]) are of therapeutic efficacy in experimental models of atherosclerosis.[16] Therefore, this review focuses on, PARP1, one such novel potential target, its role in plaque development, and stability and its potential as a therapeutic target.

2. BRIEF OVERVIEW OF POLY(ADP-RIBOSYL)ATION

Poly(ADP-ribosyl)ation (PARylation), catalyzed by PARPs, is an ancient, reversible posttranslational modification that regulates DNA repair, gene transcription, metabolism, and immune functions. It was discovered in 1963 by Chambon et al.[17] PARylation commences with the nucleophilic attack of the glycosidic bond between nicotinamide (NAM) and ADP-ribose (ADPR) portion of NAD^+ by positively (e.g., lysine residue[18]) and negatively (e.g., glutamic and aspartic acid residues) charged amino acids, or the carboxyl terminus of proteins (Fig. 1).[19] ADPR moieties bind to the aforementioned positively and negatively charged groups by forming an ester bond.[18,20] Further ADPR units can be joined to the 2′ or 3′ hydroxyl groups of ribose leading to the formation of large, branched PAR chains on proteins consisting of up to 200 ADPR units.[21]

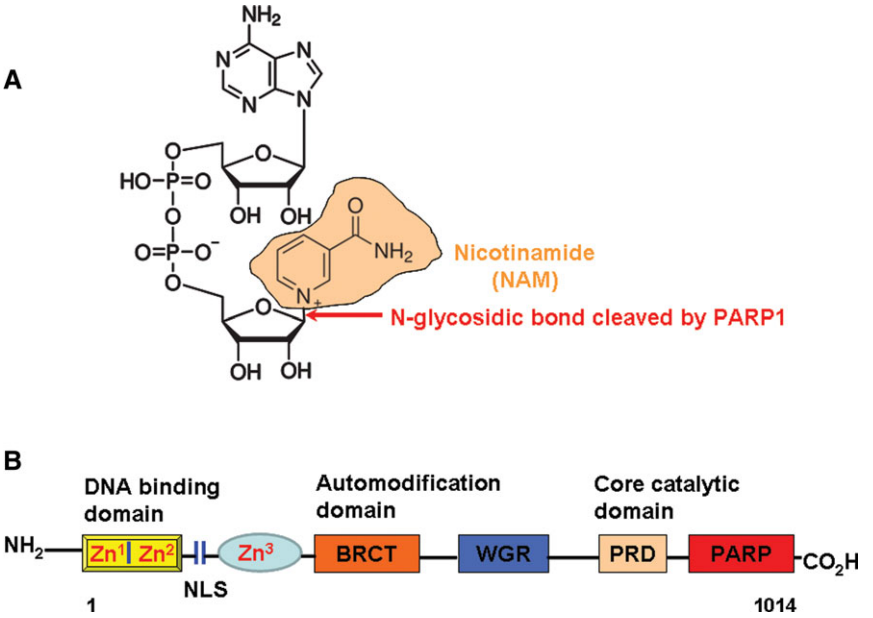

Figure 1. The chemical structure of NAD^+ and domain structure of PARP1. (A) PARP1 cleaves NAD^+ by attacking the *N*-glycosidic bond, thereby releasing nicotinamide (NAM, highlighted in brown) and ADP-ribose. (B) Schematic representation of human PARP1 domains. The PARP1 protein has multiple functional domains: the DNA-binding domain (DBD) in the N-terminus including two Zinc-finger motifs (Zn^1, Zn^2), followed by a nuclear localization signal (NLS) and Zn^3 motif (a dimerization interface); the automodification domain including a breast cancer suppressor protein 1 domain (BRCT), a WGR domain (named after a conserved Trp-Gly-Arg sequence motif), and the catalytic PARP domain in the C-terminus followed by a PARP regulatory domain (PRD). NAM, nicotinamide; PARP, poly(ADP-ribose) polymerase.

The first identified PARP was PARP1 (EC 2.4.2.30)[22] followed by the identification of several novel enzymes sharing similar catalytic domains to PARP1 in different species constituting the PARP enzyme family.[23,24] PARP1 is a large, multidomain protein, which consists of several functional domains, three N-terminal zinc-binding domains (Zn^1, Zn^2, Zn^3) containing the nuclear localization signal (NLS), automodification domain (AD) that bears the major sites of automodification and contains a BRCT (breast cancer type 1 susceptibility protein (BRCA1) C-terminal region) motif, WGR domain (conserved residues tryptophan [W], glycine [G], and arginine [R]), and C-terminal PARP catalytic domain (PARP)[25,26] (Fig. 1). PARP1, considered the prototypical member of PARPs, is activated by breaks in DNA and abnormal DNA forms.[27–29] Activated PARP1 is responsible for 85–90% of total cellular PARP activity,[30] and the rest is mostly covered by PARP2.[30,31] A plethora of proteins are PARylated upon the induction of PARPs.[32,33] PARylation of PARP1 is termed auto-PARylation that inhibits the catalytic activity of PARP1.[34,35] PAR degradation, catalyzed mainly by poly (ADPR) glycohydrolase (PARG), ADP-ribosyl hydrolase-3 (ARH3), and newly identified macrodomain-containing proteins (including human MacroD1, MacroD2, C6orf130) as terminal ARHs (which cleave the terminal ADPR attached to glutamic acid residue)[26,36,37] (Fig. 2), is a rapid process: the PAR half-life is estimated to be less than 1 min in cells.[38] Due to such a rapid turnover rate, sustained activation of PARP1 leads to a substantial decrease in cellular NAD^+ levels. Consequently, the attempt to resynthesize NAD^+ depletes cellular ATP levels leading to cell death.[39] Besides cell death, PARP1 and PARylation have been linked to several other, partly overlapping biological functions, including transcription,[40] metabolism, DNA repair,[41] and immune functions[42] (Fig. 2).

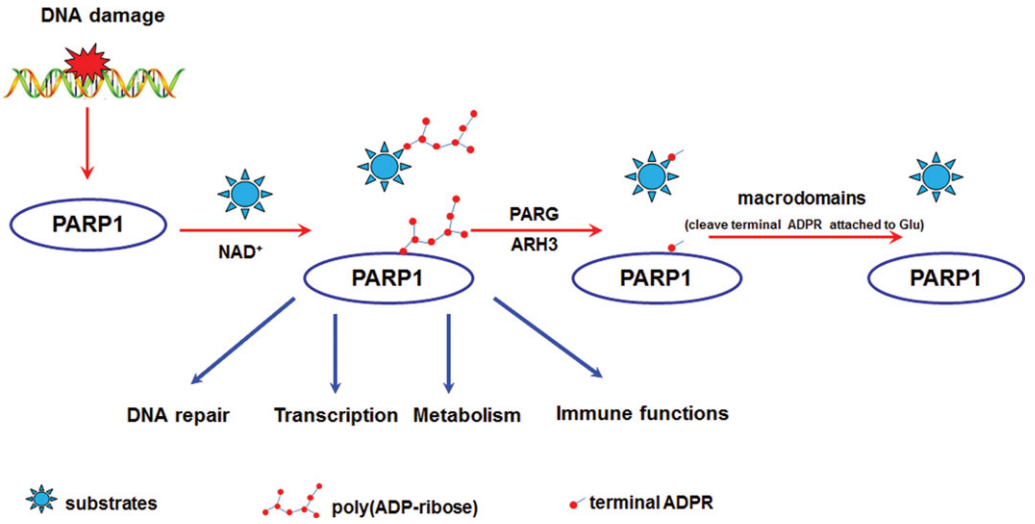

Figure 2. The regulation and functions of PARP1 activation. DNA strand breaks recruit PARP1. PARP1 binds to the sites of DNA damage via the DNA-binding domain (DBD) and initiates the poly(ADP-ribosyl)ation (transfer of ADP-ribose units from intracellular NAD^+ to acceptor proteins) of histones, transcription factors, DNA repair proteins, unidentified substrates, and PARP1 itself, leading to the formation of long and highly branched ADP-ribose polymers. These negatively charged poly(ADP-ribosyl)ated substrates modulate a wide range of important cellular processes including single and double strand DNA repair responses, gene transcription, metabolism, and immune functions. The polymer attached to PARP1 and substrates can be rapidly hydrolyzed by PARG and ARH3, leaving the terminal ADP-ribose attached to substrates intact. Recently identified macrodomain-containing proteins are terminal ADP-ribosyl glycohydrolases that can cleave the terminal ADPR attached to glutamic acid residue, but not lysine residue (by other hydrolases to be identified). The concerted action of these enzymes removes poly(ADP-ribose) and terminal ADP-ribose from PARylated PARP1, restoring its ability to recognize DNA strand breaks and initiate a new round of damage repair. Although not shown to simplify the scheme, both PARG and ARH3 cleave the ribose-ribose bond in the poly(ADP-ribose) polymer, generating monomeric ADP-ribose. ADPR, ADP-ribose; ARH3, ADP-ribosyl-hydrolase-3; PARG, poly(ADP-ribose) glycohydrolase; PARP, poly(ADP-ribose) polymerase.

There are several PARP inhibitors available for the modulation of tissular PARP activity.[43] Most inhibitors primarily bind to the NAD^+-binding pocket on the PARP catalytic domain,[25,44] as such current PARP inhibitors are therefore pan-PARP inhibitors.[45] Substituted NAM inhibitors, used as early PARP inhibitors, were shown to have unspecific targets.[46,47] More potent inhibitors seem to have less off-target effects, although less data are available on the specificity of these compounds (specificity of PARP inhibitors and their applicability in atherosclerosis treatment is detailed at Chapter 5). Considerable research effort has been dedicated to design selective PARP inhibitors. The best selectivity attained for PARP2 versus PARP1 is 60-fold, or for PARP1 versus PARP2 is 10-fold, a degree that probably cannot provide adequate selectivity in cells or in vivo.[48] Selective inhibitors could have practical importance in reducing isoform-specific adverse effects (e.g., the detrimental effects of PARP2 ablation on the pancreas[49] could be avoided by the application of a PARP1-specific inhibitor that protects the pancreas). There are several clinical trials testing PARP inhibitors (see Chapter 6).

3. THE ROLE OF PARP1 IN ATHEROGENESIS: CLINICAL AND EXPERIMENTAL EVIDENCE

There is accumulating evidence showing augmented oxidative DNA damage and PARP1 activation in human atherosclerotic plaques[50,51] and in experimental animal models of

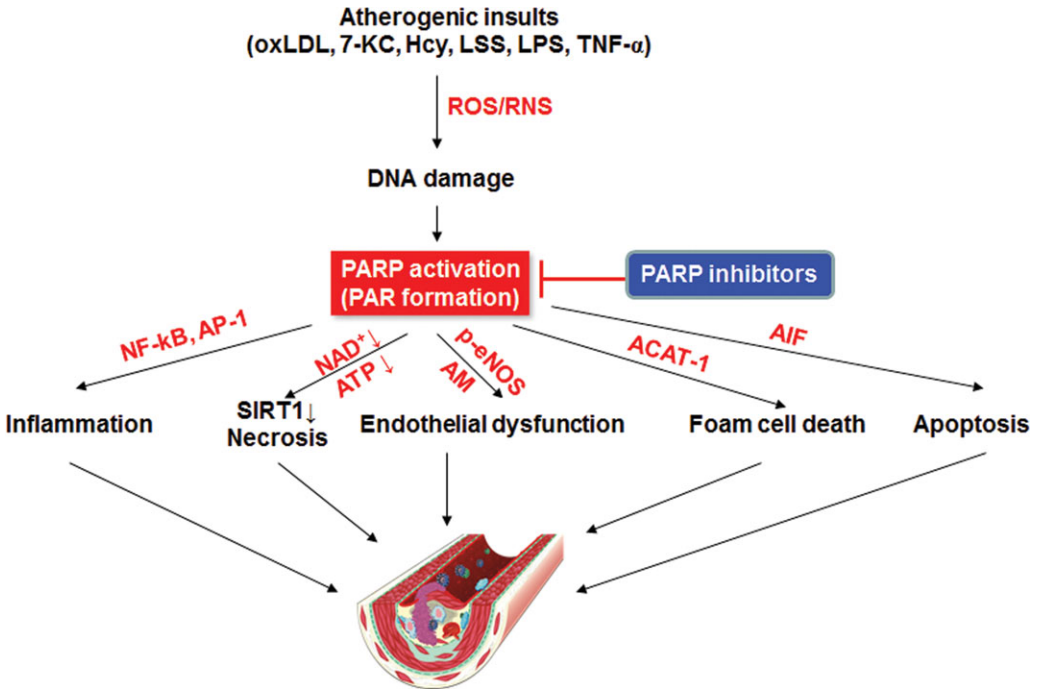

Figure 3. The central role of PARP activation in atherosclerotic plaque formation. Proatherogenic insults (such as oxLDL, 7-KC, Hcy, LSS, LPS, TNF-α) induce PARP1 hyperactivity (PAR accumulation) by oxidative and nitrosative stress, leading to the following events: (i) redox-sensitive transcription factors (NF-κB, AP-1, etc.) mediated proinflammatory response (ICAM-1, VCAM-1, P-selectin, E-selectin, iNOS, MCP-1); (ii) depletion of NAD^+ and ATP, causing cellular energy crisis (necrosis), also the downregulation of atheroprotective SIRT1; (iii) reducing eNOS activity and increasing the expression of adhesion molecules, leading to endothelial dysfunction; (iv) ACAT-1 mediated foam cell death; (v) caspase-independent activation of parthanatos by triggering mitochondrial release of AIF and translocation to nucleus. All these events promote the initiation and progression of atherosclerosis. PARP inhibitors inhibit atherosclerotic plaque formation and enhance plaque stability by inhibiting PARP activation and PAR accumulation. ACAT-1, acetyl-coenzyme A acetyltransferase-1; AIF, apoptosis inducing factor; AM, adhesion molecules; AP-1, activator protein-1; eNOS, endothelial NO synthase; Hcy, homocysteine; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; LSS, low-shear stress; NF-κB, nuclear factor kappa B; oxLDL, oxidized LDL; PARP, poly(ADP-ribose) polymerase; PAR, poly(ADP-ribose); SIRT1, sirtuin 1; TNF-α, tumor necrosis factor-α; 7-KC, 7-ketocholesterol.

atherosclerosis.[16] Proatherogenic conditions that can produce free radicals and oxidants within vascular cells, including oxidized low-density lipoprotein (oxLDL),[52,53] oxysterols within oxLDL (such as 7-ketocholesterol [7-KC]),[54] immunogenic lipopolysaccharides (LPS),[55] homocysteine (Hcy),[56,57] hyperglycemia,[58] angiotensin II (Ang-II),[59] low-shear stress (LSS),[60] myeloperoxidase-derived hypochlorite,[61] H_2O_2,[62] and peroxynitrite,[63] have been identified as activators of PARP1. Depending on the severity of DNA damage, three relevant events may be triggered in atherosclerosis:[25,64–66] (i) activation of PARP1 by mild DNA damage is physiologically relevant by promoting DNA repair, which prevents the formation of atherosclerotic plaques; (ii) more severe DNA damage induces caspase-dependent apoptosis as well as caspase-independent cell death (mediated by apoptosis inducing factor [AIF]); (iii) the most severe DNA damage causes excessive activation of PARP1, which induces the intracellular depletion of its substrate NAD^+ and of the precursor ATP stores, thereby causing a cellular energy crisis and necrotic cell death[65] (PARP activation-coupled cell death has been recently renamed as parthanatos[67]). In general, PARP1 participates in the development of atherosclerotic plaques at multiple steps (Fig. 3).

A. PARP1 in Inflammatory Conditions

Inflammatory responses are implicated in the pathogenesis of atherosclerosis from initiation through the phase of progression to ultimate complications.[68] The observation that PARP inhibitors reduce inflammation was made almost 20 years ago[69] and subsequent research efforts have uncovered multiple molecular processes behind this phenomenon.[42] Most knowledge in conjunction with inflammation has been gathered on PARP1, though it must be noted that there is also emerging evidence supporting the involvement of other PARPs in inflammatory regulation: PARP2,[48,70,71] PARP3,[71] tankyrases (PARP5a, PARP5b),[72] PARP9,[73] and PARP14.[74,75] Furthermore, it seems there are PARP enzymes (tankyrases or PARP14) that, in contrast to PARP1 or PARP2, are anti-inflammatory.[72,74,75]

PARP1 influences inflammatory processes at multiple levels. The deletion or inhibition of PARP1 may influence the differentiation and maturation of immune cells, however, hampered cell maturation does not seem to be the determining cause of reduced inflammation in PARP1$^{-/-}$ mice.[42] Specific transcriptional rearrangements provide a plausible explanation for immunmodulation by PARP1. PARP1 interacts with numerous proinflammatory transcription factors. The first identified transcription factor was nuclear factor-kappaB (NF-κB)[76] followed by nuclear factor of activated T-cells (NF-AT),[77,78] activator protein 1 (AP-1),[79–81] Yin Yang 1 (YY1),[82] and specificity protein 1 (sp1).[83]

Genetic or pharmacological deactivation of PARP1 reduces the activation of the abovementioned transcription factors that modify gene expression. Analysis of these rearrangements revealed key processes contributing to the proinflammatory properties of PARP1. In terms of the inflammatory aspect of atherosclerosis, PARP1 is required for the expression of proinflammatory cytokines (IL-1, IL-6, IL-12, TNF-α, MIP-1, MIP-2 [where MIP is macrophage inflammatory protein]),[42] adhesion molecules (intercellular adhesion molecule 1 [ICAM-1], vascular cell adhesion molecule 1 [VCAM-1], liver cell adhesion molecule [LCAM], etc.),[84] inducible nitric oxide synthase (iNOS),[85] cyclooxygenase-2 (COX-2),[86–88] therefore, deletion or inhibition of PARP1 results in decreased expression of these proinflammatory mediators via an inhibitory effect on NF-κB activation.[89] Along the same line, PARP1 is also critical in the assembly of the inflammasome.[90] In inflammation, the ratio between matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) changes in favor of MMPs. PARP inhibition restores the balance: PARP inhibition, or the deletion of PARP1 restores the appropriate MMP-9/TIMP-2 ratio.[16,80,91,92]

The induction of the PARP1-dependent genes (those detailed in the previous paragraph) support tissue infiltration and activation of immune cells therefore sustaining and enhancing inflammation, and increasing oxidative stress. Oxidative stress, on the one hand, enhances redox-sensitive transcription factors (NF-κB, e.g.),[93] while on the other, induces PARP-mediated cell death.[39] PARP1 hyperactivation diverts cell fate toward parthanatos.[66,67,94] From the perspective of inflammation, it is important to note that parthanatos leads to cellular disintegration that aggravates inflammation.[65]

Available human data indicate that the findings observed in mice seem transferable to humans. Administration of a potent PARP inhibitor-INO-1001 showed a tendency to reduce the level of IL-6 and C reactive protein (CRP), suggesting that INO-1001 is capable of reducing the expression of inflammatory mediators in patients.[95] However, it must be noted that functional redundancy among PARPs (e.g., PARP1 vs. PARP2[96,97]) may lead to the underestimation of the role of PARP1 in inflammatory processes and in particular in atherosclerosis.

B. PARP1 and SIRT1

Sirtuins (SIRTs) were identified as essential regulators of metabolism.[98–101] Yeast Sir2 protein, the prototypical enzyme of the family was identified decades ago,[102,103] however, true interest in

these enzymes arose when Imai et al. identified Sir2 as an NAD^+ dependent deacetylase.[104] In the course of the deacetylation reaction, Sir2 cleaves NAD^+ and acetyl groups are transferred onto the ADPR moiety of NAD^+.[104] In mammals the SIRT family constitutes seven members (SIRT1–7), our review focuses on SIRT1 (for detailed information on SIRTs refer to recent reviews[98,99,105]).

Several signal transduction pathways converge on SIRT1, however, the most intriguing feature for controlling SIRT1 activity relies on its NAD^+-dependence. The K_m value of SIRT1 (100–300 μM) is close to the normal cellular NAD^+ concentration (200–500 μM)[106] strongly suggesting that fluctuations in the cellular NAD^+ regulate SIRT1 activity (the understanding of SIRT1 regulation through NAD^+ is still incomplete, we refer the reader to specific reviews[106–108] for details). Cellular NAD^+/NADH ratio reflects cellular metabolism, whereby higher levels of NAD^+ indicate insufficient energy production. Higher NAD^+ levels induce SIRT1 that through deacetylating certain transcription factors (e.g., peroxisome proliferator activated receptor-γ coactivator-1α [PGC-1α], forkhead box O1 [FOXO1], p53, sterol regulatory element-binding proteins [SREBPs]) alters gene expression (for comprehensive review on SIRT1 targets see[107,109]). Altered gene expression fine-tunes mitochondrial activity, glucose and lipid metabolism to meet the needs of the organism.[107,110] SIRT1 activation seems to be beneficial in metabolic, cardiovascular, and neurodegenerative pathologies, cancer, aging, and inflammation.[105]

The NAD^+-dependence of SIRT1 is further strengthened by the observation that physiological processes involving energy stress (exercise, fasting, caloric restriction, etc.) elevate NAD^+ levels and induce SIRT1 activity.[111,112] Furthermore, NAD^+ precursors (e.g., NAM-riboside, or NAM) or increased NAD^+ salvage (e.g., NAM phosphoribosyltransferase [NAMPT] overexpression) enhance SIRT1 activity.[108,113,114] Importantly, SIRT1 activity can be also induced by inhibiting NAD^+ degradation (e.g., CD38,[115] or PARPs).[49,116–118]

The idea that SIRT1 and PARP1 may compete for the common NAD^+ substrate arose 10 years ago;[118] however, it must be noted that the interconnection between SIRT1 and PARP1 is more intricate (for detailed review see[108]). When comparing the enzymatic properties of PARP1 and SIRT1, it is evident that PARP1 has a higher affinity for NAD^+ compared to SIRT1 (K_m 20–60 μM vs. 100–300 μM, respectively).[106,119,120] Furthermore, PARP1 has a higher catalytic turnover rate than SIRT1.[117] Both PARP1 and SIRT1 are present in the nuclear compartment, therefore may compete for nuclear NAD^+, whereby PARP1 can easily limit NAD^+ availability for SIRT1 as evidenced by PARP1 deletion studies.[60,116,121–125] SIRT1 does not reciprocally limit NAD^+ for PARP1, but deacetylates and inactivates PARP1.[125] To date PARylation of SIRT1 has not been detected.[116] Other PARPs, such as PARP2 and PARP7, were also shown to influence SIRT1.[49,108,126]

The balance between PARP1 and SIRT1 has been shown to modulate several physiological and pathophysiological processes, such as oxidative stress-mediated pathologies, metabolism, genomic stability, and aging (detailed review in[108]), however, it is very likely that the extent of such processes will increase (e.g., inflammatory diseases) as there is a large overlap between SIRT1- and PARP1-mediated pathologies that likely involves atherosclerosis.[65,100,127,128]

Importantly, a recent study showed that SIRT1 activation in smooth muscle cells (SMCs) is a crucial protective factor against atherosclerosis.[129] The ablation or inhibition of PARP1 is antiatherogenic[16,130] similarly to SIRT1 activation,[129] therefore, it is likely that the interplay between SIRT1 and PARP1 could have prime importance in atherosclerosis. There are several key points where disturbances in the balance between SIRT1 and PARP1 may hypothetically contribute to atherosclerosis. (i) Enhanced PARP1 activation may lead to the inhibition of SIRT1 hampering feeding behavior[131] and nutrient storage[108,116] in a proatherogenic fashion. (ii) PARP1 activation is proinflammatory[42,132–135] and therefore proatherogenic, while

SIRT1 opposes inflammation,[136] therefore, it is likely that the balance of the two proteins sets the inflammatory tone. Furthermore, it has been recently demonstrated that the joint action of SIRT1 and PARP1 is required for the appropriate functioning of NF-κB,[137] which is a proatherogenic transcription factor. (iii) Cholesterol homeostasis might be influenced by the PARP1-SIRT1 balance as SIRT1 activation reduces SREBP activity,[109,138] while the inhibition or genetic deletion of PARP1 has beneficial effects on the HDL/LDL ratio.[139] (iv) PARP1 and SIRT1 regulate each other's activity in vascular oxidative stress-mediated events[108,125] and atherosclerosis is characterized by oxidative stress. (v) In aging, a risk factor for atherosclerosis, PARP1-SIRT1, activities are unbalanced.[140–143] (vi) PARP enzymes, other than PARP1, also interact with SIRT1[108] and regulate proatherogenic processes (e.g., inflammation or fat storage).[42,48,108,144] It should be stressed that these points connecting PARP1–SIRT1 balance to different risk factors of atherosclerosis are speculative and warrant further investigation.

C. PARP1 and Endothelial Dysfunction

Endothelial dysfunction, in which the barrier and signal-transduction function of endothelial cells (ECs) are impaired, is a hallmark of early atherosclerosis.[145] The activation of ECs leads to increased expression of proinflammatory cytokines and adhesion molecules (including E-selectin, P-selectin, VCAM-1, ICAM-1) that promote leukocyte adhesion, and transmigration into the inflamed subendothelium.[145] These cellular processes are fundamental to initiation, progression, and destabilization of atherosclerotic plaques.[145]

Recent evidence suggests that PARP1 is involved in the endothelial dysfunction observed in various pathophysiological conditions, such as atherosclerosis,[62] ischemia reperfusion injury,[146] hypertension,[147] diabetes,[58] chronic heart failure,[148] and aging.[149,150] An important milestone in establishing the critical role of PARPs in endothelial dysfunction stems from two reports from Szabo's laboratory.[130,151] These investigators showed that the activation of PARP1 contributes to the development of endothelial dysfunction in peroxynitrite-induced cytotoxicity of human ECs as well as in a rat model of endotoxemia. Also, pharmacological inhibition of PARP by INO-1001 restores the endothelium-dependent vasorelaxant responses in the aortic rings of ApoE$^{-/-}$ mice fed with a high-fat diet (HFD).[151] Long-term pharmacological inhibition of PARP (by PJ-34) or genetic deletion of PARP1 inhibits atherosclerotic plaque formation in ApoE$^{-/-}$ mice by decreasing the expression of adhesion molecules (such as VCAM-1, P-selectin, and E-selectin).[133] In cultured ECs, pharmacological inhibition of PARP by 3-aminobenzamide (3-AB) reduces peroxynitrite induced P-selectin expression and TNF-α induced ICAM-1 expression.[146] Moreover, lymphocyte adhesion to a monolayer of TNF-α-activated ECs was higher in PARP1$^{+/+}$ than PARP1$^{-/-}$ ECs,[152] suggesting that PARP1 is a critical determinant of the expression of adhesion molecules in vivo and in vitro.

PARP1 also mediates vasorelaxation as long-term treatment with the PARP inhibitor PJ-34 or INO-1001 significantly improves endothelium-dependent relaxation, suggesting the involvement of free radical production-induced PARP activation in the pathogenesis of atherosclerosis.[149] The burst of reactive oxygen species (ROS) including H_2O_2, superoxide anion ($^{\bullet}O_2^-$), and hydroxyl radical ($^{\bullet}$HO; generated by mitochondria, NADPH oxidases, xanthine oxidase, uncoupled endothelial NO synthase [eNOS] activity, as well as exposure to inflammatory cytokines and growth factors), is another important factor causing endothelial dysfunction.[145,153] Superoxide interacts with vasodilatory nitric oxide (NO) in a rapid and diffusion-controlled manner to form the oxidant peroxynitrite ($ONOO^-$) that can cross cell membranes, enter the nucleus, and trigger breakage in the strands of DNA.[154] DNA breakage triggers PARP1 activation, resulting in rapid depletion of the intracellular NAD^+ and ATP levels, contributing to sustained endothelial dysfunction and inflammation.[153,155] The

most probable explanation for PARP1-induced endothelial dysfunction is a reduction in the phosphorylation of eNOS and therefore suppressed NO bioavailability. eNOS is an NADPH-dependent enzyme[156] and Garcia Soriano et al.[58] suggested that PARP1 mediates eNOS activity through depleting and hence limiting NADPH in ECs exposed to high glucose. There is also evidence showing that phospho-eNOS immunoreactivity (the phosphorylation site is not specified by the authors) is significantly enhanced in ApoE$^{-/-}$ PARP1$^{-/-}$ mice compared to ApoE$^{-/-}$ mice, following either normal diet or HFD, without affecting total eNOS mRNA and protein expression.[139] Although the effect of PARP1 activation on endothelial dysfunction is well recognized, the effect of PARP1 on oxLDL uptake by ECs (mainly via lectin-like oxLDL receptor 1 [LOX-1]) remains incompletely understood. This aspect needs to be examined in further studies.

D. PARP1, Foam Cell Formation, and Foam Cell Death

PARP1 activation caused by proatherogenic stimuli can induce nuclear translocation of NF-κB and subsequent upregulation of inflammatory mediators, including ICAM-1, VCAM-1, and monocyte chemoattractant protein 1 (MCP-1).[157] These events drive the recruitment and adhesion of monocytes to the diseased endothelium and their differentiation into macrophages, being a prerequisite step for macrophages to become lipid-laden foam cells. Multiple scavenger receptors (SR; such as SR-A, CD36, and LOX-1)[158] and transporters (such as ATP-binding cassette transporter [ABC] A-1, ABCG-1, and SR-BI) are involved in the uptake and efflux of oxLDL and subsequent foam cell formation. PARP1 deletion does not affect fluorescently labeled acetyl-LDL (ac-LDL) uptake in foam cells.[159] In agreement with this finding, PARP inhibition by thieno[2,3-c]isoquinolin-5-one (TIQ-A) had no significant impact on the expression of ABCA-1 or SR-A, but it markedly reduced acetyl-coenzyme A acetyltransferase 1 (ACAT-1) expression in atherosclerotic lesions of ApoE$^{-/-}$ mice and in macrophage foam cells treated with ac-LDL or 7-KC (the main oxysterol in oxLDL).[160] These data may suggest that TIQ-A does not affect cholesterol influx (by SR-A) and efflux (by ABCA-1) processes.[160] More recently, it has been reported that PARP1 activation promotes NF-κB transcriptional activity by reducing NAD^+ concentrations and thereby inhibiting SIRT1-mediated deacetylation of NF-κB p65 subunit.[161] As LOX-1 is transcriptionally regulated by NF-κB, it is plausible that PARP1 activation may aggravate atherosclerosis by enhancing LOX-1-mediated macrophage-derived foam cell formation.[162] More experiments are undoubtedly required to clarify the effect of PARP inhibition (by genetic deletion or pharmacological agents) on oxLDL uptake and Apo-AI- or HDL-mediated cholesterol efflux in quantitative assays.

PARP1 also contributes to foam cell death, which is an important determinant of plaque composition. In ex vivo–generated foam cells (stimulated with ac-LDL), PARP inhibition was highly protective against 25 μM H_2O_2-induced cytotoxicity. At a higher concentration of H_2O_2 (50 μM), PARP1 knockout not only protected against H_2O_2-induced cytotoxicity, but also switched necrotic cell death to apoptosis as assessed by AnnexinV-PI staining.[16] In a following study, Hans et al.[159] demonstrated that pharmacological inhibition of PARP protects against the death of vascular cells in response to inflammatory factors, including 7-KC. These data coincide with the fact that PARP inhibition diverts necrosis to apoptosis,[66,94] thereby reducing the likelihood of enlarged necrotic core formation, which may be of therapeutic benefit in stabilizing vulnerable plaques.

E. PARP Inhibition and Lipid Levels

The majority of atherosclerotic patients have elevated circulating cholesterol levels, which can be addressed therapeutically by cholesterol-lowering drugs, such as statins. Therefore, it is important to know whether PARP inhibition exerts its effects by altering the lipid profile.

In ApoE$^{-/-}$ mice, a strain susceptible to atherosclerosis, 16 weeks of HFD regimen dramatically increased total cholesterol (TC) and LDL-cholesterol (LDL-C) levels. The high fat regimen-induced elevation in TC and LDL-C was less pronounced in ApoE$^{-/-}$PARP1$^{-/-}$ mice. The atherogenic index (Log [triglycerides/HDL-C]) was significantly higher in ApoE$^{-/-}$ mice than in ApoE$^{-/-}$PARP1$^{-/-}$ mice on normal diet as well as on HFD.[139] Similarly, in another study of ApoE$^{-/-}$ mice with preexisting atherosclerotic plaques, TIQ-A markedly lowered serum LDL-C levels, compared with vehicle-treated mice,[160] suggesting that the protective and regressive effects of PARP1 inhibitors may be mediated partly through reduction of lipid levels. It is also observed that treatment of 10-week-old mice with PJ-34 (10 mg/kg) for 5 days can also reduce serum triglyceride and free fatty acid levels, potentially via activation of the NAD$^+$-SIRT1-oxidative metabolism pathway.[116] In contrast, there are other studies reporting that PARP1 inhibition or deletion has no significant effect on lipid profile.[16,163] These discrepancies may arise from different experimental settings, the choice of PARP inhibitor, genetic background of the animals, the duration of treatment, as well as the type of atherogenic diet.[117] It will be of more clinical relevance to examine whether PARP inhibition alters lipid levels in hyperlipidemic human subjects.

4. THE ROLE OF PARP1 IN PLAQUE DESTABILIZATION

Disruption of unstable atherosclerotic plaques (''vulnerable'' plaques) and subsequent formation of occlusive thrombi are the primary causes of acute coronary syndrome (ACS).[164] The so-called ''vulnerable'' plaques are characterized by large necrotic cores, thin fibrous caps (caused by SMCs apoptosis and matrix degradation), enhanced inflammatory state, advanced lesional macrophage apoptosis, together with defective efferocytosis (phagocytic clearance of apoptotic cells).[165,166] Activated PARP1 is present in circulating mononuclear cells of patients with unstable angina, concurrent with NF-κB activation and increased expression of TNF-α and IL-6.[167] PARP inhibitors facilitate foam cell death, but protect against the death of SMCs and ECs, which is favorable for enhancing plaque stability and regression.[159] This finding suggests that PARP inhibition confers a prosurvival, a neutral, or a prodeath effect in the plaque dynamics dependent on the vascular cell types (macrophage foam cells, ECs, or SMCs) and type and duration of proatherogenic stimuli (7-KC, H_2O_2, or TNF-α). The molecular mechanisms by which PARP inhibitors or PARP1 genetic deletion stimulate the death of foam cells are not fully characterized. ACAT-1 is the principal enzyme that converts cytotoxic free cholesterol to esterified cholesterol in macrophage foam cells, thereby contributing to the lowering of cytotoxicity.[168] Genetic deletion of PARP1 or treatment with the PARP inhibitor TIQ-A in ApoE$^{-/-}$ mice downregulates ACAT-1 mRNA and protein expression in vivo and in 7-KC-treated macrophage foam cells. This observation suggests that PARP1 inhibitors may promote free cholesterol-mediated cell death by inhibiting ACAT-1 expression.[159] Moreover, PARP1 gene deletion significantly reduces prodeath caspase-3 and c-Jun N-terminal kinase (JNK) activation in SMCs stimulated with TNF-α or 7-KC, and also induces the prosurvival extracellular signal-regulated kinases (ERKs) signaling pathway, resulting in the net decrease of SMC apoptosis. This effect might contribute to the reversal of the thinning of the fibrous cap in vulnerable plaques.[159] TIQ-A treatment also resulted in a significant decrease in nitrotyrosine and 8-oxo-2′-deoxyguanosine (8-oxo-dG) immunoreactivity, suggesting that PARP inhibitors promote plaque stability by modulating nitrosative stress and oxidative stress.[159] In addition, in the plaques of ApoE$^{-/-}$ mice receiving TIQ-A treatment or those that are heterogeneous for PARP1, SMCs and collagen content was increased, fibrous caps were thicker, and lipid cores were well contained. These protective effects result from increased expression of TIMP-2

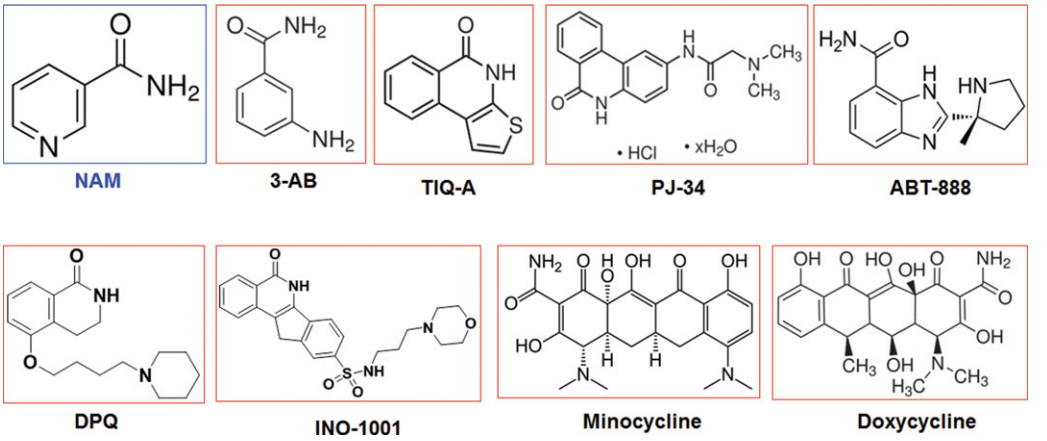

Figure 4. The chemical structures of PARP inhibitors with demonstrated antiatherosclerotic effects. Nicotinamide (NAM), which is released in the poly(ADP-ribosyl)ation process, was the first PARP inhibitor identified. All of the current classes of PARP inhibitors are based on the NAM/benzamide pharmacophore. PARP, poly(ADP-ribose) polymerase.

and TIMP-3, without a significant effect on collagen I mRNA expression.[16,160] Moreover, in ApoE$^{-/-}$ mice kept on a high cholesterol diet, treatment with the PARP inhibitor PJ-34 increased the thickness of the fibrous cap and collagen content, while reducing the necrotic core diameter and apoptotic cell death, thus, favoring features of plaque stability. However, PJ-34 did not affect cell proliferation.[133] These data suggest that PARP1 is critically involved in plaque destabilization by modulating plaque composition, which can be prevented by PARP inhibitors.

The ratio of MMPs to TIMPs is another important determinant of plaque instability. Genetic ablation of PARP1 or pharmacological inhibition of PARP by PJ-34 also restores the original MMP-9/TIMP-2 ratio in oxazolone-induced contact hypersensitivity in mice[80,92] and an apparently similar rebalancing may be induced by PARP inhibitors in atherosclerosis as shown by Oumouna-Benachour et al.[16] All these data underscore the potential involvement of PARP1 in plaque instability. It remains to be determined, however, whether PARP inhibition exerts antiatherosclerotic effects by promoting efferocytosis, a process that clears apoptotic cells. Despite consistent reports on the inhibitory effects of PARP inhibitors on lesion progression, reports on the plaque-stabilizing effects of PARP inhibitors are inconsistent.[163] For example, Erbel et al.[163] recently observed no difference in collagen and SMC content between vehicle and INO-1001-treated ApoE$^{-/-}$ mice. These contradictory reports might be explained by differences in PARP inhibitors, mouse strains, as well as the type of atherogenic diet.

5. PARP INHIBITORS AS ANTI-ATHEROSCLEROTIC AGENTS

Based on the structure of NAM/benzamide, several PARP inhibitors, including 3-AB,[57,157,169] PJ-34,[58,133,170] TIQ-A,[16,160] ABT-888 (Veliparib),[60] 3,4-dihydro-5-[4-(1-piperidinyl)butoxyl]-1(2H)-isoquinolinone (DPQ),[52,53,171,172] INO-1001,[59,62,163,173,174] and the tetracycline derivates minocycline[175,176] and doxycycline[168,177,178] demonstrate atheroprotective effects by decreasing PARP activation, inflammatory markers, macrophage recruitment, endothelial dysfunction, foam cell death, and by promoting plaque stability. The chemical structures of these inhibitors show that they share the NAM/benzamide pharmacophore (Fig. 4). However, it is important to note that these PARP inhibitors may not only inhibit members of the PARP family,[45] but also,

presumably at higher concentrations, inhibit other completely unrelated targets, such as PJ-34 that inhibits Pim kinases[179] and 3-AB that inhibits the generation of oxidant species[180] and MMP-2 activity.[181] However, the fact that PARP1 knockout animals are also protected against atherosclerosis suggests that the antiatherogenic activity of PARP inhibitors is indeed related to the inhibition of PARP1. The PARP inhibitors used in the experimental and clinical therapeutics for atherosclerosis are summarized in Table I and II. However, it remains elusive whether other PARP inhibitors in clinical development will reduce plaque burden in experimental animals and patients with ACS.

A. 3-AB

3-AB is a prototypical PARP inhibitor that has been used in multiple studies.[65] A recent study has shown that 3-AB substantially reduced atherosclerotic plaque area (by 40%) in Hcy-induced atherosclerosis, but it has no significant effect on plaque area from chow-diet fed ApoE$^{-/-}$ mice. Interestingly, the plasma Hcy level and lipid contents are not affected by 3-AB.[157] The atheroprotective effect afforded by the 3-AB was due to an inhibitory effect on PARP activation, and inhibitory effects on NF-κB-mediated production of inflammatory factors, such as VCAM-1 and MCP-1.[157] In a rat model of hyperhomocysteinemia, 3-AB improves acetylcholine-induced, NO-mediated vasodilation by decreasing the levels of nitrite/nitrate and endothelin-1. These findings indicate that 3-AB may be helpful in preventing endothelial dysfunction in the setting of hyperhomocysteinemia.[57] In isolated rat aortic arteries stimulated with Hcy, 3-AB not only prevents, but also reverses Hcy-induced endothelial dysfunction.[56] 3-AB (10 mg/kg, i.v.) also attenuates myocardial ischemia/reperfusion injury in rats by decreasing the activity of creatine phosphokinase and myeloperoxidase, peroxynitrite-induced cytotoxicity, and by preserving myocardial ATP levels in the infarcted hearts.[182] In parallel, in cultured ECs, 3-AB decreases peroxynitrite-induced P-selectin expression and TNF-α-induced ICAM-1 expression.[146] Although 3-AB has served as the "benchmark" inhibitor of PARP for a long time, one must bear in mind that this compound has additional, non-PARP-related pharmacological effects, such as antioxidant and MMP-2 inhibitory activities, and therefore, some of the protective effects observed with 3-AB may be mediated by direct oxidant scavenging and MMP-2 inhibition, rather than direct catalytic inhibition of PARP activation.[65,180,181] Therefore, it is advisable to confirm the atheroprotective effects of 3-AB by using more potent and specific PARP inhibitors (see below) and/or PARP1 deficient cells or animals.

B. TIQ-A

TIQ-A reduces plaque burden in ApoE$^{-/-}$ mice fed with a HFD without affecting lipid levels.[16] These findings are corroborated genetically with the use of ApoE$^{-/-}$ mice that are heterozygous for PARP1.[133] TIQ-A also promotes an increase in collagen content, potentially through an increase in TIMP-2, and transmigration of SMCs to the intima of atherosclerotic plaques.[16] In a subsequent study with preestablished atherosclerotic plaques in ApoE$^{-/-}$ mice, TIQ-A, administered with a normal chow diet, promoted the regression of established plaques, concurrent with a reduction in TC and LDL-C. Furthermore, increased collagen and SMC content together with decreased macrophage content, and thicker fibrous caps were observed in atherosclerotic plaques of TIQ-A-treated mice, suggesting enhanced plaque stability.[160] These changes are associated with diminished expression of MCP-1, ICAM-1, TNF-α, as well as ACAT-1, rather than ABCA-1 and SR-A.[159,160] However, the effect of TIQ-A on CD36-mediated oxLDL uptake and HDL-mediated macrophage cholesterol efflux was not quantitatively analyzed.

Table I. Beneficial Effects of PARP Inhibition/Deletion in Animal Models of Atherosclerotic Cardiovascular Diseases

Mode of PARP inhibition	Disease model/patients	Effect of PARP inhibition	References
3-AB	1. $ApoE^{-/-}$ mice + Hcy 2. Rats fed with a high-methionine diet 3. Myocardial I/R in rats	↓AIF nuclear translocation, ↑vasorelaxation –Hcy, –lipid profile, ↓NF-κB, ↓VCAM-1, ↓MCP-1 ↓Infarct size, ↑endothelium-dependent vascular relaxation ↓Serum creatine phosphokinase, ↓MPO activity ↓neutrophil infiltration, ↓nitrotyrosine, ↑ ATP ↓MDA, ↑NO, ↓ET-1	57, 157, 182
TIQ-A	$ApoE^{-/-}$ mice + HFD	↓Plaque number and size, –serum lipid profile ↑SMCs and collagen content, ↑TIMP-2 ↑SMCs migration to intima ↓NF-κB DNA binding activity, ↓MCP-1, ↓ICAM-1, ↓TNF-α ↓nitrotyrosine, ↓8-oxo-dG ↓Foam cell death, ↓macrophage recruitment ↓TC, ↓VLDL-C+LDL-C –ABCA-1, –SR-A, ↓ACAT-1, ↓caspase-3	16, 159, 160
PJ-34	1. $ApoE^{-/-}$ mice + HCD 2. Streptozotocin-induced diabetic mice 3. Ang-II-infused rats 4. Isolated rat aortic rings 5. Balloon-injured rat carotid artery	↓Plaque area, ↓apoptosis, -proliferation ↓E- and P-selectin, ↓VCAM-1, ↓iNOS ↓Macrophage and T cell content, ↑fibrous cap thickness ↓Necrotic core area, ↑collagen content ↑Endothelium-dependent relaxation –plasma glucose, –TC, ↑endothelial dysfunction ↑ATP, ↑NAD^+, ↑NADPH, ↓neointima formation ↓$CD45^+$ leukocyte infiltration, ↑endothelial cell recovery ↑Ach-stimulated cGMP content	59, 133, 183, 184

Table I. Continued

Mode of PARP inhibition	Disease model/patients	Effect of PARP inhibition	References
ABT-888	Diabetic db^{-}/db^{-} mice	↓Myogenic tone, ↑Endothelium-dependent relaxation ↑p-eNOS, ↑cGMP, ↑cleaved PARP1	147
DPQ	Myocardial I/R in rats	↓Myocardial infarct size, ↑cardiac function ↓$TUNEL^{+}$ apoptotic cells, ↓p-JNK ↓AIF translocation from mitochondria to nucleus ↓NF-κB DNA binding activity, ↓ICAM-1, ↓COX-2, ↓MMP-9, ↑p-Akt, ↑p-GSK-3β, ↑p-FOXO3a	52, 171
INO-1001	1. $ApoE^{-/-}$ mice + HFD 2. Carotid endarterectomy in rats 3. Patients with ST-elevation myocardial infarction	–Lipid profile, ↓dendritic cells, ↓T lymphocytes ↓macrophages, ↓oxLDL auto-antibody, –collagen content ↓apoptosis, –SMCs content, ↓MIP-3α, ↓CD83, ↓IL-12 ↓iNOS, ↓VCAM-1, ↓caspase-3 ↑Endothelium-dependent relaxation ↓Neointima formation, ↓neutrophil infiltration ↓AIF nuclear translocation, ↓nitrotyrosine Trend toward blunted CRP, IL-6	95, 130, 163, 186
Minocycline	1. $ApoE^{-/-}$ mice + HFD 2. New Zealand white rabbits + HCD	↓Lesion size and stenosis, ↓SMCs proliferation, ↓$p27^{Kip1}$ ↓Macrophage content, ↓ MMP-2, ↓ MMP-9 activities	168, 175
Doxycycline	1. $ApoE^{-/-}$ mice + chow diet 2. Patients with ACS 3. Ang-II infused $LDL\text{-}R^{-/-}$mice 4. Balloon catheter denudation of rat carotid artery	–TC, –TG, ↓lesion size, ↓CRP, ↓IL-6, ↓MMP-9, ↑HDL ↓TNF-α, ↓MCP-1, ↓p-NF-κB –Systolic blood pressure ↓Ang-II-Induced AAAs incidence and severity –Ang-II-Induced atherosclerosis ↓MMP-2, ↓MMP-9 activity, ↓Intima/media ratio ↓SMCs migration and proliferation	189–192

Table I. Continued

Mode of PARP inhibition	Disease model/patients	Effect of PARP inhibition	References
PARP1$^{-/-}$	1. ApoE$^{-/-}$ mice + HFD 2. Myocardial I/R in mice	↓TC, ↓LDL-C, ↓atherogenic index, –heart rate ↑Baroreflex sensitivity, ↓p-eNOS, ↓iNOS, ↓nitrotyrosine, ↓dilated cardiomyopathy ↓MMP-9 activity, ↑TIMP-2 and TIMP-3 expression ↓Serum creatine phosphokinase ↓MPO activity, ↓neutrophil infiltration ↓Nitrotyrosine, ↓P-selectin, ↓ICAM-1 ↓Disruption of the myocardial structure	91, 139, 146

AAAs, abdominal aortic aneurysms; ABCA-1, ATP-binding cassette transporter-1; ACAT-1, acetyl-coA cholesterol acyltransferase-1; Ach, acetylcholine; ac-LDL, acetyl-LDL; ACS, acute coronary syndrome; AIF, apoptosis inducing factor; Ang-II, angiotensin-II; ApoE$^{-/-}$, apolipoprotein E knock-out; CE, cholesterol ester; cGMP, cyclic guanosine monophosphate; COX-2, cyclooxygenase-2; CRP, C reactive protein; DPQ, 3,4-dihydro-5-[4-(1-piperidinyl)butoxyl]-1 (2H)-isoquinolinone; ET-1, endothelin-1; FC, free cholesterol; FOXO-3a, forkhead box O-3a; GSK-3β, glycogen synthase kinase-3β; Hcy, homocysteine; HCD, high-cholesterol diet; HDL-C, high-density lipoprotein-cholesterol; HFD, high-fat diet; ICAM-1, intercellular adhesion molecule-1; IL-6, interleukin-6; iNOS, inducible nitric oxide synthase; I/R, ischemia/reperfusion; LDL-C, low-density lipoprotein; LDL-R, LDL receptor; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein-1; MDA, malondialdehyde; MIP-3α, macrophage inflammatory protein-3α; MMP-9, matrix metalloproteinase-9; MPO, myeloperoxidase; p-JNK, phosphorylated c-Jun NH_2-terminal kinase; p-NF-κB, phosphorylated nuclear factor kappa B; oxLDL, oxidized LDL; PAR, poly(ADP-ribose); PARP1, poly(ADP-ribose) polymerase 1; p-eNOS, phosphorylated endothelial nitric oxide synthase; SIRT1, sirtuin 1; SMCs, smooth muscle cells; SR-A, scavenger receptor-A; TC, total cholesterol; TG, triglyceride; TIMP, tissue inhibitor of metalloproteinases; TIQ-A, thieno[2,3-c]isoquinolin-5-one; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling; VCAM-1, vascular cell adhesion molecule-1; VLDL-C, very-low density lipoprotein; 3-AB, 3-aminobenzamide; 7-KC, 7-ketocholesterol; 8-oxo-dG, 8-oxo-2′-deoxyguanosine; ↓ denotes "decrease", ↑ denotes "increase", – denotes "no significant effect."

C. DPQ

DPQ is a highly potent and selective inhibitor of PARP1. It was demonstrated that DPQ inhibits PARP activation in oxLDL-stimulated human aortic ECs in vitro and in myocardial ischemia–reperfusion injury.[53] PARP inhibition by DPQ partially restored aldehyde dehydrogenase 2 (ALDH2) activity in oxLDL treated human aortic ECs and ischemia–reperfusion rat hearts by preventing SIRT3-mediated deacetylation.[53] These data suggest that DPQ may be of great benefit in the therapy of atherosclerosis by promoting ALDH2-catalyzed metabolism of aldehydes (into less reactive chemical species), the major end products of lipid peroxidation. DPQ also reduces heart ischemia/reperfusion injury by suppressing the PARP1/JNK/AIF pathway.[52] More recently, the same group showed that DPQ protects against oxLDL-induced

Table II. Atheroprotection Conferred by PARP Inhibition/Deletion in vitro

Mode of PARP inhibition	Stimuli	Target cell	Effect of PARP inhibition	References
3-AB	TNF-α, Peroxynitrite, TGF-β1	1. ECs, 2. SMCs	↓P-selectin, ↓ICAM-1, ↑mitochondrial respiration	146, 169, 182
			↓Peroxynitrite induced cytotoxicity	
			↓p-Smad3, ↓PARylation and DNA binding of Smad3	
			↓Collagen Iα1, ↓collagen IIIα1, ↓TIMP-1	
TIQ-A	7-KC	Foam cells	–ABCA-1, –SR-A, ↓ACAT-1, ↑H_2O_2-induced apoptosis	159, 160
			↓Necrosis, –ac-LDL uptake, ↑Sensitization to 7-KC	
			↑TC, ↑FC, ↓CE, ↓Caspase-3 activation	
PJ-34	LPS	1. SMCs 2. Macrophages	↓PARP1, ↓$p27^{Kip1}$, ↓MIP-1α, MIP-2	168, 170
			↓NF-κB DNA binding and transcriptional activity, –MAPK	
ABT-888	Low-shear stress, High glucose	ECs	↑NAD, ↑SIRT1 activity, ↓p-NF-κB	60, 147
			↓NF-κB nuclear translocation and DNA binding activity	
			↓iNOS, ↓ICAM-1, ↓$O_2^{\cdot -}$, ↓nitrotyrosine, ↓ p-H2A.X	
			↓cleaved PARP1, ↓DNA-binding activity of PARP1, ↓DNA tails	
DPQ	oxLDL	ECs	↑ALDH2 activity, ↓cellular NAD^+, –mt NAD^+, ↓SIRT3	172
			↑cell viability, ↓PARP1, ↓iNOS, ↓nitrotyrosine, ↓NO	
			↓$TUNEL^+$ apoptotic cells	
INO-1001	Hypoxia and reoxygenation, TNF-α	1. Macrophages 2. ECs	↓TNF-α, ↓MIP-1α, ↓NF-κB expression	173, 174
			↓NF-κB nuclear translocation, ↓ICAM-1	

Table II. Continued

Mode of PARP inhibition	Stimuli	Target cell	Effect of PARP inhibition	References
Minocycline	LDL, Chlamydia pneumoniae infection	1. SMCs 2. *C. pneumonia* infected human monocytes	↓ Proliferation, –migration, –Apoptosis ↓PARP1, ↓$p27^{Kip1}$ ↓Monocytes differentiation to macrophages ↓Phagocytic activity	168,176
Doxycycline	CRP/oxLDL, LPS	PBMCs	↓TNF-α, ↓IL-6, ↓MCP-1, ↓CRP, ↓MMP-9, ↓p-NF-κB ↑apoA-I, ↑HDL-C	178
$PARP1^{-/-}$	LPS	1. SMCs and ECs 2. Macrophages	↓p-NF-κB, ↓NF-κB nuclear Translocation ↓NF-κB DNA binding and transcriptional activity ↓iNOS, ↓ICAM-1, ↑TIMP-2, ↑TIMP-3, ↓MIP-1α and MIP-2 ↓NF-κB DNA binding and transcriptional activity, –MAPK	89,91
$PARP2^{-/-}$	DOX	SMCs	↑SIRT1, ↑ mitochondrial biogenesis, –PARP activity, ↑ DOX- induced O^{2-}, –apoptosis, –NAD^+ depletion	31

ABCA-1, ATP-binding cassette transporter-1; ACAT-1, acetyl-coA cholesterol acyltransferase-1; ac-LDL, acetyl-LDL; ALDH2, aldehyde dehydrogenase 2; ApoA-I, apolipoprotein A-I; CE, cholesterol ester; DOX, doxorubicin; ECs, endothelial cells; FC, free cholesterol; ICAM-1, intracellular adhesion molecule-1; iNOS, inducible nitric oxide synthase; LDL, low-density lipoprotein; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MIP, macrophage inflammatory protein; MMP, matrix metalloproteinase; mt NAD^+, mitochondrial NAD^+; oxLDL, oxidized LDL; PAR, poly(ADP-ribose); p-H2A.X, phosphorylated histone H2A.X; p- NF-κB, phosphorylated nuclear factor-kappa B; PARP, poly(ADP-ribose) polymerase; PBMCs, peripheral blood mononuclear cells; SIRT1, sirtuin 1; SMCs, smooth muscle cells; SR-A, scavenger receptor-A; TC, total cholesterol; TGF-β1, transforming growth factor- β1; TIMP, tissue inhibitor of metalloproteinases; TNF-α, tumor necrosis factor-α; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling; 7-KC, 7-ketocholesterol. ↓ denotes "decrease", ↑ denotes "increase", – denotes "no significant effect."

apoptosis in microvascular ECs by inactivating the PARP1/iNOS/NO pathway that led to inhibition of peroxynitrite (nitrotyrosine) formation.[172]

D. PJ-34

Hcy and high glucose, two independent risk factors for patients with atherosclerosis, trigger the production of ROS, causing DNA strand breaks and impairing endothelium-dependent relaxation. Pharmacological PARP inhibition by PJ-34 not only prevents, but also rapidly

reverses the development of endothelial dysfunction under diabetic conditions[183] and in hyperhomocysteinemia.[56] In ApoE$^{-/-}$ mice fed with a high-cholesterol diet, treatment with PJ-34 decreased atherosclerotic plaque formation by 46% via diminishing the expression level of adhesion molecules.[133] Furthermore, PJ-34 reduces the content of macrophages and T-cells, while increasing the thickness of the fibrous cap, favoring features of plaque stability.[133] PJ-34 was also able to suppress the pathogenesis of chronic heart failure,[148] aging,[148] and neointima formation after balloon injury.[184] However, recent studies have shown that PJ-34 inhibits not only PARP activity, but also other completely unrelated targets (as discussed earlier), such as Pim-1 ($IC_{50} = 3.7\ \mu M$) and Pim-2 ($IC_{50} = 16\ \mu M$) serine/threonine kinases[179] as well as MMP-2 activity ($IC_{50} = 56\ \mu M$).[181] These findings raise concerns on the appropriateness of using PJ-34 as a chemical tool for PARP biology studies at concentrations higher than 10 μM in future studies.

E. INO-1001

Inotek Pharmaceutical Inc. (Beverly, MA, USA) has developed a potent lead compound INO-1001 that exerts protective effects against atherosclerosis, myocardial infarction, stroke, and chronic heart failure.[25,185] INO-1001 is the most extensively studied PARP inhibitor for the treatment of cardiovascular diseases. For example, in isolated aortic rings of ApoE$^{-/-}$ mice kept on a HFD, Benko et al.[130] provide the first line of experimental evidence showing the endothelial protective and regressive effects of INO-1001. Subsequent evidence[61,62] indicates that INO-1001 improves endothelial dysfunction induced by myeloperoxidase-derived hypochlorite and H_2O_2 in isolated normal aortic rings. A more recent study shows that INO-1001 reduces atherosclerotic lesion development by modulating the activation of dendritic cells, T lymphocytes, and macrophages, concurrent with the reduction of the inflammatory responses within the plaques.[163] Pharmacological inhibition of PARP with INO-1001 prevents neointimal hyperplasia after endarterectomy in rats by reducing the neointima area, neutrophil infiltration, nitrosative stress, and AIF nuclear translocation.[186]

F. Tetracycline Antibiotic and Bacteriostatic Agents

Tetracyclines are a group of naturally occurring and chemically synthesized pluripotent antimicrobial agents, the actions of which include potent inhibition of PARP1 enzymatic activity (occurs at submicromolar concentrations), broad-spectrum MMPs inhibitory activity, and anti-inflammatory activity.[177] These activities contribute to the plaque inhibitory and stabilizing effect of tetracyclines. The rank order of potencies for these compounds for inhibiting recombinant PARP1 activity in a cell-free assay was minocycline > doxycycline > demeclocycline > chlortetracycline.[187] By comparison of the chemical structures, all the tetracycline derivatives with demonstrated PARP1 inhibitory activity have a carboxamide and aromatic ring structure (similar to the NAM moiety of NAD^+), the pharmacophore shared by established competitive PARP inhibitors.[187] Among the tetracycline class, minocycline and doxycycline have been evaluated extensively, in clinical as well as knockout and transgenic mouse studies, as a possible therapy for atherosclerosis, ischemia-reperfusion injury, left ventricular remodeling, restenosis, hypertension, heart failure, abdominal aortic aneurysms, and most importantly, for patients with ACS.[188–192] Mechanistically, selective inhibition of MMPs and PARP1 activity conferred by tetracycline derivatives reduces inflammatory responses in atherosclerotic lesions, prevents fibrous cap thinning, and, therefore, prevents the rupture of unstable atherosclerotic plaques. Further studies are warranted to examine whether the MMPs inhibitory effect of these

agents stems from direct PARP1 inhibition, and whether other tetracycline derivatives can stabilize the unstable plaques and prevent the occurrence of ACS.[193]

6. PARP INHIBITORS IN CLINICAL TRIALS

PARP inhibitors have attracted intense attention as an effective therapeutic strategy for cancer patients.[43,194] Based on the structure–activity relationship of benzamide parent drugs, third generation PARP inhibitors were developed through screening of chemical libraries and structural refining. Among these inhibitors, several compounds are promising entities, including ABT-888 (Veliparib, Abbott Laboratories), AZD-2281 (Olaparib, KuDOS/AstraZeneca Pharmaceuticals), INO-1001 (Inotek Pharmaceuticals), AG-014699 (Rucaparib, Pfizer), MK-4827 (Niraparib, Merck), and CEP-9722 (Cephalon). These inhibitors are now in different stages of clinical development either as single therapy for homologous recombination repair-deficient (for example, BRCA1- or BRCA2-deficient) cancers and sporadic cancers; or in combination therapy with standard DNA-damaging chemotherapy and radiotherapy.[43,194,195] In 2005, the US Food and Drug Administration granted the request of Inotek Pharmaceuticals for orphan drug designation for INO-1001, for the prevention of the postoperative complications of aortic aneurysm repair surgery. In addition to the aortic aneurysm repair indication, INO-1001 has been evaluated in Phase II clinical trials as a drug to protect the heart during cardiopulmonary bypass surgery as well as for angioplasty procedures after myocardial infarction (ClinicalTrials.gov identifier: NCT00271765, NCT00271167). It remains, however, elusive whether other PARP inhibitors in clinical development will reduce plaque burden in patients with atherosclerosis.

7. CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Over the past decade, great strides have been made toward characterizing the underlying molecular mechanisms of PARP1 activation. Emerging evidence demonstrates that PARP activation (PARP1 in particular) is critically involved in atherosclerotic plaque formation and destabilization. The use of PARP inhibitors is beneficial not only in preventing atherogenesis, but also in promoting the regression of previously established atherosclerotic plaques. However, many questions remain to be addressed: (i) What are the diverse roles of the less well-characterized PARP family members in atherosclerosis? Cell-type-specific knockout mice for particular PARPs are required to clarify this issue. (ii) What are the substrates of PARP1 in the context of atherosclerosis and how will the PARylation of these substrates affect the development of atherosclerosis? PARylated substrates identified in pull-down experiments using macrodomain proteins[196,197] coupled with phosphoproteomics-based mass spectrometry[33,198] will yield valuable information on this issue. (iii) How to design effective therapeutic agents aiming to remove PAR from target proteins in atherosclerosis? (iv) Is it possible to regulate PARP1 activity via their interactors (e.g., resetting the PARP1–SIRT1 activity balance)? (v) Will PARP inhibitors exhibit therapeutic utility in combination with other therapeutic modalities of atherosclerosis (such as statins, Ang-converting enzyme inhibitors, Ang-II receptor blockers)? (vi) When planning the application of PARP inhibitors in patients with atherosclerosis, the risk/benefit ratio of the long-term administration of PARP inhibitors must be considered. This is particularly important in the setting of atherosclerosis—a chronic inflammatory disease that typically requires long-term therapeutic administration, but it represents a significantly higher drug development/toxicology challenge than short-term administration of PARP inhibitors for acute indications.[25,177] The safety and risks associated with long-term administration of

PARP inhibitors are associated with the regulatory role of PARPs in DNA repair and genomic integrity.[96,195] Taken together, the discoveries reviewed here provide novel insights into the rational design of PARP-targeting drugs, and depict an upcoming translational era of PARP inhibitors in the clinical management of atherosclerotic cardiovascular diseases.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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