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PII: S0167-4889(12)00295-9
Reference: BBAMCR 16775

To appear in: *BBA - Molecular Cell Research*

Received date: 13 June 2012
Revised date: 12 October 2012
Accepted date: 12 October 2012

Please cite this article as: Wei-dong Qin, Shu-jian Wei, Xu-ping Wang, Juan Wang, Wen-ke Wang, Fuqiang Liu, Lei Gong, Fei Yan, Yun Zhang, Mingxiang Zhang, Poly(ADP-ribose) Polymerase 1 Inhibition Protects Against Low Shear Stress Induced Inflammation, *BBA - Molecular Cell Research* (2012), doi: 10.1016/j.bbamcr.2012.10.013

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Poly(ADP-ribose) Polymerase 1 Inhibition Protects Against Low Shear Stress Induced Inflammation

Running title: PARP-1 Inhibition Attenuates Inflammation

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Abstract

**Background:** Atherosclerosis begins as local inflammation of vessels at sites of disturbed flow, where low shear stress (LSS) leads to mechanical irritation and plaque development and progression. Nuclear enzyme poly(ADP-ribose) polymerase 1 (PARP-1) is associated with the inflammation response during atherosclerosis. We investigated the role and underlying mechanism of PARP-1 in LSS-induced inflammation in human umbilical vein endothelial cells (HUVECs).

**Methods and Results:** HUVECs were simulating by LSS (0.4 Pa). PARP-1 expression was inhibited by ABT888 or siRNA. The iNOS and ICAM-1 expression was regulated by LSS in a time dependent manner. LSS could increase superoxide production and 3-nitrotyrosine formation. LSS induced DNA damage as assessed by H2A.X phosphorylation and comet assay. Compared with cells under static, LSS increased PARP-1 expression and PAR formation via MEK/ERK signaling pathway. PARP-1 inhibition increased Sirt1 activity through an increased intracellular NAD⁺ level. Moreover, PARP-1 inhibition attenuated LSS-induced iNOS and ICAM-1 upregulation by inhibiting NF-κB nuclear translocation and activity, with a reduced NF-κB phosphorylation.

**Conclusions:** LSS induced oxidative damage and PARP-1 activation via MEK/ERK pathway. PARP-1 inhibition restored Sirt1 activity by increasing NAD⁺ level and decreased iNOS and ICAM-1 expression by inhibiting NF-κB nuclear translocation and activity as well as NF-κB phosphorylation. PARP-1 played a fundamental role in LSS induced inflammation. Inhibition of PARP-1 might be a mechanism for treatment of inflammation response during atherosclerosis.

**Key Words:** atherosclerosis; wall shear stress; poly(ADP-ribose) polymerase 1; Sirt1; inflammatory factors; NF-κB
1. Introduction

Atherosclerosis is a complicated chronic inflammation in arteries resulting from interaction between immune mechanisms and metabolic risk factors.[1] It primarily affects large and medium-sized arteries in a site-specific manner, with a predilection to the inner wall of curvatures and outer wall of bifurcations.[2] Numerous studies suggest that local hemodynamic forces play a major role in regulating the site-specific predilection of atherosclerosis, and the most important one is called wall shear stress.[3]

Wall shear stress is a frictional force exerted parallel to the arterial wall by the wall shear rate and viscosity of blood.[4] Undisturbed shear stress (USS) ranges from 0.5 to 1.2 Pa, and values below and above this range are thought to indicate low shear stress (LSS) and increased shear stress, respectively.[5] Wall shear stress acts on endothelial cells and leads to activation of various cell signaling molecules, such as protein kinase C (PKC), mitogen-activated protein kinase (MAPK) and so on.[6] LSS promotes mass transport of atherogenetic substances between the lumen and the arterial wall to induce atherosclerosis and predicts plaque development.[7]

Poly(ADP-ribose) polymerase 1 (PARP-1) is a highly conserved DNA-binding nuclear enzyme that can be activated by oxidative DNA damage mainly by ROS.[8] Upon DNA damage, PARP-1 binds to DNA strand breaks and catalyses the addition of long branched chains of poly(ADP-ribose) (PAR, the product of PARP-1) to a number of nuclear proteins. Excessive activation of PARP-1 leads to intracellular depletion of NAD and ATP, thus resulting in cellular energy crisis, irreversible cytotoxicity and cell death.[9] Moreover, PARP-1 is a co-activator of nuclear factor-κB (NF-κB) and can regulate the expression of various key inflammatory genes, including inducible nitric oxide synthase (iNOS), intercellular adhesion molecule 1 (ICAM-1), and vascular cell adhesion molecule 1, all of which are regulated by NF-κB.[10] Excessive activation of PARP-1 is closely associated with the pathogenesis of cardiovascular diseases. Pharmacological inhibition or genetic deletion of PARP-1 can protect against
endothelial dysfunction, reduce inflammation and atherosclerotic plaque size, and promote factors of plaque stability.

Class III histone deacetylases (HDACs), also called sirtuins or Sirts, constitute a special group of enzymes that require NAD in their deacetylation reaction.[11] Sirt1 is a prototype member of the sirtuins family, which has been implicated in transcriptional silencing, genetic control of aging, cell metabolism, and calorie restriction-mediated longevity of the organism.[12] Studies have suggested that Sirt1 can promote cell survival and may protect cells against oxidative stress.[13,14]

However, previous studies focused on the role of PARP-1 in ox-LDL induced atherosclerosis, little was known about the relationship between PARP-1 and low shear stress, we aimed to investigate the role and underlying mechanism of PARP-1 in LSS-induced inflammation in HUVECs.
2. Materials and Methods

2.1. Cell culture, flow system and genes inhibition

HUVECs (ATCC, USA) were cultured in endothelial cell medium (ECM, ScienCell, CA, USA) supplemented with 5% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 ug/ml streptomycin at 37℃ up to passage 4 and seeded onto gelatin-coated slides. A parallel-plate flow system was used to impose LSS (0.4 Pa).[15] We used SP600125, SB203580, and U0126 (Cell Signaling Technology, MA, USA) to inhibit the activity of JNK, p38, and ERK. To inhibit PARP-1 expression, HUVECs were treated with ABT888[16] or transiently transfected with siRNA negative control (si-NC) or si-PARP-1 (GenePharma) in Optimem Medium (Invitrogen, CA, USA) with use of Lipofectamine 2000 (Invitrogen). Experiments were performed 24 hrs after transfection.

2.2. RT-PCR

RNA was extracted from HUVECs by use of Trizol (Invitrogen). cDNA generated by RT-PCR was analyzed by real-time RT-PCR with iQ™ SYBR Green Supermix (Bio-Rad Laboratories, CA, USA). Each sample was analyzed in triplicate, and expression was normalized to that of GAPDH. The primers for iNOS were as follows: forward, 5'-GTTCTCAGCCCAACAATACAAGA-3'; reverse, 5'-GTGGACGGGTCGATGTCAC-3'; for ICAM-1: forward, 5'-TTGGAAGCCTCATCCG-3'; reverse, 5'-CAATGTGGTGAGACCC-3'; and for GAPDH: forward, 5'-AGGTGGATGATGACCAAACAAGA-3'; reverse, 5'-TGTGACCATGTAGTTGAGGTT-3'. Amplification, detection, and data analysis involved use of the iCycler real-time PCR system (Bio-Rad Laboratories).

2.3. Western blot analysis

Protein was extracted from HUVECs. Equal amounts of protein were separated on 10% SDS-PAGE and electro-transferred onto nitrocellulose membrane (Amersham Biosciences, NJ, USA). After being blocked with 5% non-fat milk for 2 hrs at room
temperature, blots were washed in TBS-T 3 times for 10 min and incubated with primary antibodies at 4°C overnight. The primary antibodies were as follows: rabbit monoclonal anti-β-actin (1:2000, Cell Signaling Technology), anti-PAR (1:2000, BD Biosciences, NJ, USA), anti-IkBα (1:1000, Cell Signaling Technology), anti-c-Jun/phospho-c-Jun (1:1000, Cell Signaling Technology), anti-MAPKAPK-2/phospho-MAPKAPK-2 (1:1000, Cell Signaling Technology), anti-ERK/phospho-ERK (1:1000, Cell Signaling Technology), anti-H2A.X/phospho-H2A.X (1:1000, Cell Signaling Technology) and anti-Sirt1 (1:2000, Abcam, MA, USA); rabbit polyclonal anti-PARP-1 (1:500, Sigma-Aldrich), anti-iNOS (1:200, Abcam, MA, USA), and anti-NF-κB p65/phospho-NF-κB p65 (Ser536, 1:1000, Cell Signaling Technology); mouse monoclonal anti-ICAM-1 (1:500; Santa Cruz Biotechnology) and anti-3-nitrotyrosine (1:1000, abcam). After being washed in TBS-T, membranes were incubated with horseradish peroxidase-conjugated secondary antibody for 2 hrs at room temperature. Signals were detected by enhanced chemiluminescence (Millipore) and analyzed by use of Image-Pro Plus 6.0.

2.4. Immunofluorescence

HUVECs were fixed in 4% paraformaldehyde and permeabilized in PBS containing 0.1% Triton X-100. After being blocked with BSA for 30 min, samples were incubated with the antibodies rabbit anti-NF-κB p65 (1:100, Cell Signaling Technology) overnight at 4°C Alexa 488-conjugated goat anti-rabbit IgG (1:500; Jackson immunoresearch) was used as secondary antibodies. A drop of Prolong Gold antifade reagent with DAPI (Vector Laboratories, CA, USA) was used to seal the coverslip. Images were acquired by laser scanning confocal microscopy (LSM 710, Zeiss, Germany). Data were analyzed by use of Image-Pro Plus 6.0.

2.5. O$_2^-$ production

To measure O$_2^-$ production in HUVECs, we used dihydroethidium (DHE) (Beyotime, Beijing) as described.[17] Shear-preconditioned and static cells were incubated in 5
µM DHE for 30 min at 37°C in a light-protected environment, then washed with ECM without FBS for 3 times. Fluorescence was acquired by use of Zeiss LSM 710 confocal microscope. Static HUVECs were used as reference samples.

2.6. Sirt1 activity and NAD+ level assay

Sirt1 activity was assessed by SIRT1 Fluorescent Activity Assay Kit (Enzo Life Sciences) to the manufacturer’s instructions with minor modifications as previously described.[18] Briefly, 20 µg of cells were used in deacetylation assay, with 25µM of Fluor de Lys-SIRT1 as the substrate. The deacetylation of the substrate was measured by use of a varioskan flash (Thermo Scientific), with excitation wavelength set at 360 nm and emission at 460nm. Intracellular NAD+ levels were measured by using NAD+/NADH Assay Kit (Abcam) according to the manufacturer’s instructions. Briefly, HUVECs were washed with cold PBS and extracted with NADH/NAD Extraction Buffer by freeze/thaw two cycles (20 min on ice, then 10min at room temperature). Total NAD was detected in a 96-well plate and color was developed and read at 450nm by use of a varioskan flash (Thermo Scientific).

2.7. Comet assay

DNA damage induced by LSS was assessed by comet assay using comet assay kit (Trevigen, MD, USA). The Comet Low Melting Point agarose was prepared by heating the bottle in a 90–100°C water bath for 5 minutes. Then place the bottle in a 37°C water bath for at least 20 minutes to cool. Cells were harvested by scraping in cold PBS, centrifuged at 1000 g and the cell pellet was re-suspended in PBS to 10⁵ cells/ml. The cell suspension (50 µl) was quickly mixed with 500 µl Low Melting Point agarose, then 50 µl of the mixture was dropped and spreaded over sample area to ensure complete coverage of the sample area, and allowed to gel at 4 °C. Slides were then submerged in lysis solution for 40 minutes at 4 °C. Then the slides were placed in Alkaline Unwinding Solution and allowed to incubate for 40 minutes at room temperature. The slides were then placed under freshly prepared electrophoresis buffer composed of: 200 mM NaOH, 1 mM EDTA, and electrophoresed at 4 °C, for 30
min at 21V. The slides were then immersed in dH₂O, followed by 70% alcohol for 5 min and then allowed to air dry. The slides were stained by immersion in SYBR Green I and photographed under a 40× objective. Slides were scored using CometScore software v1.5 (TriTek Corporation, Summerduck, VA).

2.8. Electrophoretic mobility shift assay (EMSA)

EMSA was performed by using LightShift Chemiluminescent EMSA kit (Thermo Scientific) according to the manufacturer’s instructions. Briefly, HUVECs were collected and nuclear proteins were extracted by nuclear extraction kit (active motif, CA, USA). Single-stranded oligonucleotides containing binding sites of NF-κB (5′-AGTTGAGGGGACTTTCCCAGGC-3′) were synthesized and endlabeled with biotin (Biosune, Shanghai, China). Single-stranded oligonucleotides were annealed to double-strand probes for EMSA. Nuclear protein (5 μg) was incubated with 20 fmol biotin-labeled oligonucleotides for 20 min at room temperature in binding buffer consisting of 50mM KCl, 10mM EDTA, 2.5% glycerol, 5mM MgCl₂, 50ng poly(dI·dC), and 0.05% NP-40. The specificity of the NF-κB–DNA binding was determined in competition reactions in which a 200-fold molar excess (4pmol) of unlabeled oligonucleotide was added. After electrophoresis in 6.5% PAGE using 0.5× TBE buffer, protein–oligonucleotide complex was electroblotted to a positively charged nylon membrane. Then transferred DNA was crosslinked to membrane with a UV lamp for 10 min. After incubation in blocking buffer for 15 min at room temperature, the membrane was washed and incubated with Substrate Equilibration Buffer for 10 min at room temperature. The membrane was then incubated with chemiluminescent substrate for 5 min before analyzed in ImageQuant LAS 4000 mini (GE healthcare).

2.9. Statistical analysis

Data are expressed as mean±SD. SPSS for Windows v16.0 (SPSS Inc, Chicago, IL, USA) was used for statistical analysis. Intergroup comparisons involved 2-tailed Student t test or one-way ANOVA followed by Tukey test (with equal variances assumed) or Dunnett T3 test (with equal variances not assumed). A p value less than
0.05 was considered to indicate statistical significance.
3. Results

3.1. Time-dependent regulation of inflammatory factors by LSS in HUVECs

HUVECs were stimulated by LSS (0.4 Pa) at various times. LSS had a
time-dependent regulation of iNOS and ICAM-1 expression. With LSS, the mRNA and
protein expression of iNOS began to elevate at 4 hr and peaked at 12 hr (p<0.05 vs. control),
and the expression of ICAM-1 increased at 12 hr to 235.84±15.29% and
175.13±17.13% (p<0.05 vs. control, Figure 1). We used HUVECs stimulated by LSS
for 12 hr for further experiments.

3.2. LSS increased PARP-1 expression and activity in HUVECs

We used PARP-1 siRNA and PARP-1 inhibitor ABT888 in following experiment. To
ensure that effective PARP-1 inhibition was achieved in target cells, we analyzed
PARP-1 expression after addition of siRNA. Compared with the control, PARP-1
expression was significantly reduced by siRNA (p<0.05, Figure 2A and 2B). Then we
examined the effect of LSS on PARP-1 expression and activity. Compared with the
static cells, PARP-1 expression was significantly increased by LSS (p<0.05, Figure
2C and 2D). Moreover, LSS could significantly increase PAR formation, while ABT888
or si-PARP-1 attenuated it (p<0.05, Figure 2C and 2E).

3.3. O$_2^-$ production and 3-NT formation were upregulated by LSS

As O$_2^-$ and 3-NT can damage DNA which leads to PAR formation, we detected O$_2^-$
production and 3-NT formation with LSS stimulation in HUVECs for 12 hr. O$_2^-$
production and 3-NT formation were significantly increased in LSS-stimulated
HUVECs, which suggested that LSS could induce oxidative stress (p<0.05, Figure 3A
to 3C).

3.4. DNA damage was induced by LSS

DNA damage induced by LSS was determined by H2A.X phosphorylation and comet
assay. Histone H2A.X is a variant histone which is required for checkpoint-mediated
cell cycle arrest and DNA repair following double-stranded DNA breaks. Various
stimulus induced DNA damage can result in rapid phosphorylation of H2A.X. LSS
significantly increased H2A.X phosphorylation ($p<0.05$, Figure 3D and 3E). Comet assay showed that there was little DNA in tail of static cells, while LSS could increased the percentage of DNA in tail ($p<0.05$, Figure 3F and 3G).

3.5. PARP-1 was activated by LSS via MEK/ERK pathway

We then investigated the mechanism of PARP-1 activation (PAR formation) induced by LSS. LSS could increase the protein expression of p-c-Jun, p-MAPKAPK-2, and p-ERK compared with static ($p<0.05$, Figure 4A to 4F). Addition of SP600125 (JNK inhibitor), SB203580 (P38 inhibitor), and U0126 (ERK inhibitor) significantly reduced them, but only MEK/ERK inhibition reduced PAR formation ($p<0.05$, vs. low, Figure 4). The results suggested that the MEK/ERK pathway is involved in LSS induced PARP-1 activation.

3.6. Inhibition of PARP-1 attenuated inflammatory factors expression

Considering the critical role of the inflammatory factors in atherogenesis, we measured the effect of PARP-1 inhibition on iNOS and ICAM-1 expression in HUVECs. LSS stimulation markedly increased iNOS and ICAM-1 expression as compared with static cells ($p<0.05$, Figure 5A to 5C). After PARP-1 inhibition, iNOS and ICAM-1 expression was significantly reduced ($p<0.05$, vs. low).

3.7. PARP-1 inhibition increased Sirt1 activity by upregulation of NAD$^+$ level

Compared with static cells, LSS decreased the Sirt1 expression and activity, while PARP-1 inhibition had no significant effect on Sirt1 expression but increased its activity (Figure 5A, 5D and 5E). As the activity of Sirt1 depended on NAD$^+$ concentration, the effect of PARP-1 on NAD$^+$ was determined. Compared with the control, LSS reduced the NAD$^+$ concentration, while PARP-1 inhibition by inhibitor or siRNA restored it ($p<0.05$, Figure 5F).

3.8. PARP-1 inhibition reduced LSS-induced inflammatory factors expression by inhibiting NF-κB nuclear translocation and activity as well as NF-κB phosphorylation

Given that iNOS and ICAM-1 expression depend on NF-κB activation,[19] we
wondered whether the negative effect of PARP-1 inhibition was associated with defective NF-κB activation. Figure 6A shows that NF-κB p65 was mostly cytoplasmic before LSS stimulation, but its localization quickly changed to the nucleus with LSS stimulation. After PARP-1 inhibition, p65 remained primarily cytoplasmic after LSS treatment. EMSA result showed that LSS could increase NF-κB activity, while PARP-1 inhibition reduced it (p<0.05, Figure 6B). Then we investigated the underlying mechanism. Western blot analysis showed that LSS reduced the IκBα expression, while PARP-1 inhibition had no effect on it (Figure 6C and 6D), which suggested that the defect might reside in the actual translocation of the transcription factor to the nucleus rather than its association with IκBα. Then we measured p-p65 level. LSS could significantly increase p-p65 level compared with static controls, and PARP-1 inhibition reduced it (p<0.05, Figure 6E and 6F).
4. Discussion

Atherosclerosis begins with fatty streak formation in an atherogenic background that is caused by the interaction between systemic cardiovascular risk factors and local hemodynamic forces such as LSS.[20] The precise interplay between LSS, vessels and plaque development is still obscure. We focused on the potential role and underlying mechanism of PARP-1 in LSS-induced atherogenesis. In HUVECs, LSS increased superoxide production and nitrotyrosine formation as well as DNA damage. PARP-1 could be activated by LSS via MEK/ERK-dependent pathway, which resulted in an increased NF-κB activation and iNOS and ICAM-1 expression. PARP-1 plays a critical role in LSS-induced inflammation through a pathway involving a decreased Sirt1 activity and increased inflammatory factors expression.

PARP-1 is a DNA repair-associated nuclear enzyme that is activated in response to DNA damage.[21] It is closely related to various pathological conditions, including heart failure, ischemia-reperfusion injury, hypertension, atherosclerosis, and aging.[22,23,24] Accumulating evidence suggests that the endothelial dysfunction in atherosclerosis is related to the local formation of ROS near the vascular endothelium and PARP-1 activation.[25] In our experiment, we found that LSS increased PARP-1 expression and PAR formation. Inhibition of PARP-1 reduced LSS induced inflammation, which meant PARP-1 played a critical role in LSS induced inflammation.

As far as we know, PARP-1 is a DNA damage sensor and can be activated by damaged DNA, but few reports have described the connection between LSS and PARP-1. In our experiment, we demonstrated that LSS could activate a variety of cell signaling transduction molecules, including ERK, JNK, and p38. The expression and/or activity of these signaling molecules were significantly decreased after application of siRNA or inhibitor, but only inhibition of MEK/ERK lead to decreased PARP-1 activation induced by LSS. We firstly showed that LSS could induce PAR formation through MEK/ERK dependent pathway.

We further investigated the potential mechanisms of PARP-1 in LSS-induced
inflammation. As an NAD\(^+\)-dependent class III histone deacetylase, Sirt1 functions as a key regulator of stress response and energy homeostasis by regulating various molecules that were involved in cell survival and energy metabolism. Several studies have demonstrated the beneficial effects of Sirt1 on endothelial cell biology. Ota et al. showed that overexpression of Sirt1 prevented oxidative stress-induced endothelial senescence, whereas inhibition of Sirt1 had an adverse effect.\[^{26}\] Mattagajasingh and colleagues demonstrated that inhibition of Sirt1 attenuated endothelium-dependent vasodilation in rat arteries because of the enhanced acetylation of endothelial nitric oxide synthase (eNOS).\[^{27}\] Furthermore, endothelial cell-specific overexpression of Sirt1 decreased atherosclerosis in apolipoprotein E-deficient mice.\[^{28}\] Our experiments demonstrated that PARP-1 inhibition had no significant effect on Sirt1 expression, but it remarkably increased Sirt1 activity. Considering the protective effect of Sirt1, this might be a probable mechanism of protection against inflammation by PARP-1 inhibition. Then we investigated the underlying mechanism. As the activity of Sirt1 depended on the intracellular NAD\(^+\) concentration, the effect of PARP-1 on NAD\(^+\) was determined. As we expected, compared with the static cells, LSS could decrease the NAD\(^+\) concentration, while PARP-1 inhibition alleviated the effect of LSS, which suggested that PARP-1 could increase the Sirt1 activity by upregulation the NAD\(^+\) concentration.

Inflammation and inflammatory factors are implicated in the pathogenesis of atherosclerosis, from initiation through progression and ultimate complications.\[^{29}\] Many indications suggest a role for NF-κB in atherosclerosis. The transcription factor NF-κB may play a critical role in guarding the balance of the atherosclerotic process as a direct regulator of proinflammatory and anti-inflammatory genes and as a regulator of cell survival and proliferation.\[^{30}\] Hypercholesterolemia was shown to induce activated NF-κB associated with a decrease in NO bioavailability in the initial stages of atherosclerosis in the coronary vasculature.\[^{31}\] In our experiment, PARP-1
inhibition decreased iNOS and ICAM-1 expression, which may be another mechanism of anti-atherosclerosis by PARP-1 inhibition. We speculated that the reduced expression of iNOS and ICAM-1 may be associated with NF-κB. As we expected, PARP-1 inhibition decreased NF-κB nuclear translocation and activity. These findings may support in part the relationship between PARP-1 and NF-κB. Then we investigated the mechanism(s) by which PARP-1 inhibition reduced the signal transduction of NF-κB. Hassa found that PARP-1 acted synergistically with p300 and played an essential regulatory role in NF-κB-dependent gene expression.[32] The active binding form of NF-κB is composed of various combinations of members of the NF-κB/Rel family. The NF-κB complex remains inactive in the cytoplasm through interaction with the inhibitory protein inhibitor κB (IκB) that sterically hinders binding of import proteins to the nuclear localization sequence of the NF-κB subunits. Activation of NF-κB requires phosphorylation of IκBα by IκBα kinase and subsequent degradation through a common ubiquitin–proteasome pathway.[33] As shown in figure 6, LSS stimulation reduced the protein expression of IκBα. To our surprise, PARP-1 inhibition had no significant effect on IκBα expression. Accumulating evidences have demonstrated that after the release of NF-κB from inhibition by IκBα, the phosphorylation of the p65 subunit is critical for binding to its target sites on DNA.[34] It was important to determine whether PARP-1 played a role in events leading to p65 NF-κB phosphorylation. Our results clearly suggested that PARP-1 inhibition significantly decreased the p-p65 level. However, the mechanism by which PARP-1 influences these phosphorylation events needs further investigation.

In conclusion, we firstly report that PARP-1 plays a critical role in LSS induced inflammation. PARP-1 can be activated by LSS via MEK/ERK pathway, and inhibition of PARP-1 pharmacologically or genetically can protect against LSS-induced inflammation by increasing Sirt1 activity and decreasing inflammatory factors expression. PARP-1 inhibition shed light on new interventions to prevent inflammation during atherogenesis.
Sources of Funding:
This work was supported by the National 973 Basic Research Program of China (No. 2009CB521904), the State Program of National Natural Science Foundation of China for Innovative Research Group (No. 81021001), the State Key Program of National Natural Science of China (No. 60831003), and the National Natural Science Foundation of China (Nos., 81170275, 81000127, 30871037, 30873325).

Disclosures
None
References


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**Figure legends:**

**Figure 1:** Relative mRNA and protein expression of iNOS and ICAM-1 with LSS (0.4 Pa) at various times in HUVECs.

After HUVECs were stimulated by LSS for 0, 4h, 8h, 12h, 16h, 24h and 36h, mRNA and protein expression of iNOS and ICAM-1 were assessed by RT-PCR and western blot analysis. *(A, B)* Quantification of RT-PCR results of iNOS and ICAM-1 mRNA expression. *(C, D, E)* Western blot analysis of iNOS and ICAM-1 protein expression. Values are expressed as means ± SD from three independent experiments. *p* < 0.05 vs. control. Control: cells under static.

**Figure 2:** Low shear stress increased PARP-1 expression and PAR formation.

HUVECs were stimulated by LSS for 12h after PARP-1 inhibition by siRNA, PARP-1 expression and PAR formation were determined by western blot analysis. *(A, B)* Western blot analysis of PARP-1 expression. *(C, D, E)* Western blot analysis of PARP-1 expression and PAR formation. si-NC: negative siRNA control; si-PARP-1: PARP-1 siRNA. Values are means ± SD. *p* < 0.05 vs. control; #p < 0.05 vs. low.

**Figure 3:** LSS increased O$_2^-$ production and 3-nitrotyrosine (3-NT) formation as well as DNA damage.

After LSS stimulation for 12h, superoxide production was measured by dihydroethidium (DHE) and nitrotyrosine formation was assessed by western blot analysis. DNA damage was determined by H2A.X phosphorylation and comet assay. *(A, B)* Quantification of O$_2^-$ production (red). Nuclei were labelled with 4’,6-diamidino-2-phenylindole (DAPI) (blue). *(C)* 3-NT expression was measured by western blot analysis. *(D, E)* Western blot analysis of H2A.X phosphorylation. *(F, G)* The content of DNA in tail was assessed by comet assay. Values are means ± SD. *p* < 0.05 vs. control.

**Figure 4:** LSS induced PAR formation via MEK/ERK signaling pathway.

PAR formation was determined by western blot analysis after MAPK inhibition before LSS stimulation. *(A, B, C)* Protein expression of p-c-Jun, p-MAPKAPK-2, p-ERK, and
PARP. SP600125: JNK inhibitor; SB203580: P38 inhibitor; U0126: MEK/ERK inhibitor.

(D, E, F) Quantification of p-c-Jun, p-MAPKAPK-2 and p-ERK protein expression. (G, H, I) Quantification of PAR formation. Values are means ± SD from three separate experiments. * p<0.05 vs. static; # p<0.05 vs. low.

Figure 5: PARP-1 inhibition reduced iNOS and ICAM-1 expression and increased Sirt1 activity.

HUVECs were stimulated by LSS for 12h after PARP-1 inhibition by siRNA, iNOS, ICAM-1 and Sirt1 expression were determined by western blot analysis, Sirt1 activity and NAD⁺ were determined. (A, B, C, D) Western blot analysis of iNOS, ICAM-1, and Sirt1 expression. (E) Sirt1 activity of HUVECs. (F) NAD⁺ level in HUVECs. si-NC: negative siRNA control; si-PARP-1: PARP-1 siRNA. Values are expressed as means ± SD. * p<0.05 vs. static; # p<0.05 vs. low.

Figure 6: PARP-1 inhibition suppressed LSS induced NF-κB nuclear translocation and activity as well as phosphorylation. (A) Immunofluorescence analysis of NF-κB p65 (green) and DAPI (blue). Nuclei were labelled with 4′,6-diamidino-2-phenylindole (DAPI) (blue); p65 was stained with rabbit anti-p65 primary antibody and Alexa 488-conjugated goat anti-rabbit second antibody (green). (B) NF-κB activity was assessed by EMSA. (C, D) Western blot analysis of IκBα. (E, F) Western blot analysis of phospho-NF-κB p65 (p-p65) level. Values are means ± SD. * p<0.05 vs. static control, # p<0.05 vs. low.
Figure 1
Figure 2
Figure 3
Figure 4
Figure 5
Figure 6
Highlights

Δ We firstly demonstrated the critical role of PARP-1 in low shear stress induced inflammation. Δ Low shear stress increased superoxide production and nitrotyrosine expression. Δ Low shear stress increased PARP-1 expression and PAR formation. Δ PARP-1 can be activated by LSS via ERK dependent pathway. Δ PARP-1 inhibition decreased LSS-induced inflammatory cytokines upregulation by preventing NF-κB nuclear translocation.