Identification of a Novel Series of Anti-inflammatory and Anti-oxidative Phospholipid Oxidation Products Containing Cyclopentenone Moiety in vitro and in vivo: Implication in Atherosclerosis

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ABSTRACT

Oxidative stress and inflammation are two major contributing factors to atherosclerosis, a leading cause for cardiovascular diseases (CVDs). Oxidation of phospholipids on the surface of low density lipoprotein (LDL) particles generated...
under oxidative stress has been associated with the progression of atherosclerosis but the underlying molecular mechanisms remain poorly defined. We identified a novel series of oxidation products containing cyclopentenone moiety, termed deoxy-A \textsubscript{2}/J\textsubscript{2}-isoprostanes-phosphocholine (deoxy-A \textsubscript{2}/J\textsubscript{2}-IsoP-PC), from 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine (PAPC) in vivo using mass spectrometry and by comparison to a chemically synthesized standard. Transcriptomic analysis (RNA-seq) demonstrated that these compounds affected over two hundred genes in bone marrow-derived macrophages (BMDMs) and genes associated with inflammatory and antioxidative responses are among the top five differentially expressed. To further investigate the biological relevance of these novel oxidized phospholipids in atherosclerosis, we chemically synthesized a representative compound 1-palmitoyl-2-15-deoxy-\(\Delta\)-12,14-Prostaglandin J\textsubscript{2}-sn-glycero-3-phosphocholine (15d-PGJ\textsubscript{2}-PC) and found that it induced anti-inflammatory and antioxidant responses in macrophages through modulation of NF-κB, PPARγ and Nrf2 pathways; this compound also showed potent anti-inflammatory properties in a mice model of LPS-induced systemic inflammatory response syndrome (SIRS). Additionally, 15d-PGJ\textsubscript{2}-PC inhibited macrophage foam cell formation, suggesting a beneficial role against atherosclerosis. These properties were consistent with decreased levels of these compounds in the plasma of patients with coronary heart disease (CHD) compared to control subjects. Our findings uncovered a novel molecular mechanism for the negative regulation of inflammation and positive enhancement of antioxidative responses in macrophages by these oxidized phospholipids in LDL in the context of atherosclerosis.

Atherosclerosis is the major cause for cardiovascular diseases (CVDs); chronic inflammation and oxidative stress are two major contributing factors to the progression of atherosclerosis (1,2). Emerging evidence demonstrated that oxidation of phospholipids on LDL particles played an important role in every stage of atherosclerosis development through interactions with different cells in the vessel wall including macrophages, endothelial cells, smooth muscle cells and platelets (3,4). In particular, oxidation of one of the major phospholipids in LDL, PAPC has been extensively studied (5). Oxidized PAPC (oxPAPC) has a profound impact on the functions of macrophages and a recent study suggested that oxPAPC induced a unique phenotype of macrophages, termed Mox, characterized by abundant over-expression of nuclear factor-E2-related factor 2 (Nrf2)-mediated expression of redox-regulatory genes but the exact molecular species of the oxidation products remain to be elucidated (6,7).

PAPC is one of the major PUFA-containing PCs on the surface of LDL. In addition to be present on LDL, arachidonic acid (C20:4, \(\omega\)-6, AA) also exists on phospholipids in the membranes of most mammalian cells (Fig.1) (8). Upon stimulation of inflammatory cytokines, AA is released from cell membrane by phospholipases and converted into prostaglandins (PGs), leukotrienes, and epoxyeicosatetraenoic acids (EETs) by cyclooxygenases (COXs), lipoygenases (LOXs), and cytochrome P450s respectively (9). 15d-PGJ\textsubscript{2}, a dehydration product of prostaglandin D\textsubscript{2} (PGD\textsubscript{2}), is one of the most extensively studied cyclopentenone-containing PGs which exerts potent biological functions including anti-inflammatory and anti-oxidant responses largely through covalent modification of proteins (10-13). On the other hand, under oxidative stress AA can undergo lipid peroxidation (LPO) through free radical mechanisms (reactive oxygen species, ROS)
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to give rise to a number of oxidation products including isoprostanes (IsoPs), isolevuglandins, and isofurans (14-17). As shown in Fig.1, PAPC oxidation generates bicyclic endoperoxide IsoP-PC (or G_{2}-IsoP-PC), which can be reduced to form F_{2}-IsoP-PC or rearrange to form D_{2}-IsoP-PC, or E_{2}-IsoP-PC (not shown). These esterified isoprostanes-PC can be hydrolyzed primarily by platelet activating factor (PAF) acetyl hydrolase (AH) to generate IsoP free acid analogues (18). Overwhelming evidence demonstrates that levels of F_{2}-IsoPs have been regarded as the best marker for assessing endogenous oxidative stress (19,20). In addition, in minimally oxidized LDL (mmLDL), PAPC oxidation led to the formation of epoxide-containing isoprostanes phospholipid PEIPC and PECPC (21). These bioactive lipids modulate the inflammatory responses in endothelial cells and macrophages and thus play an important role in atherosclerosis and other inflammatory diseases (4,22,23).

Based on the free radical lipid peroxidation, we hypothesized that 15d-PGJ_{2}-like oxidation products can be formed from PAPC oxidation in LDL; specifically, dehydrosation of D_{2}-IsoP-PC will generate J_{2}-IsoP-PC and deoxy-J_{2}-IsoP-PC whereas E_{2}-IsoP-PC gives rise to A_{2}-series of analogous oxidation products. It is noteworthy that these compounds are not readily separated by chromatography and thus collective termed as deoxy-A_{2}/J_{2}-IsoP-PC. We further hypothesized that these oxidation products may possess potent biological activities due to the presence of similar structural moiety of cyclopentenone to those of 15d-PGJ_{2}. Notably, these compounds are distinct from COX-derived 15d-PGJ_{2} in which they are initially formed on phospholipids and represent different biological sources, i.e. 15d-PGJ_{2} is formed as a single compound from inflammatory responses whereas the deoxy-A_{2}/J_{2}-IsoP-PC and their free acid analogues are a mixture of structurally similar isomers and derived from oxidation of AA-containing PC under oxidative stress. These compounds are likely one of the major players bridging the two critical contributing factors in atherosclerosis: oxidative stress and inflammatory responses.

In this study, we provided evidence that deoxy-A_{2}/J_{2}-IsoP-PC were indeed generated from PAPC in LDL in significant amounts as phospholipid esters in vitro and in vivo in human atherosclerotic plaques from postmortem samples and emboiled materials captured by distal protection filter devices during uncomplicated saphenous vein graft interventions (24). To study the role of these novel oxidation products on macrophages, we chemically synthesized 15d-PGJ_{2}-PC as a representative compound for this class. Our studies showed that 15d-PGJ_{2}-PC induced anti-inflammatory and antioxidant responses and inhibited macrophage foam cell formation. Consistently, this compound also showed potent anti-inflammatory properties in a mice model of LPS-induced SIRS. We further demonstrated that the levels of deoxy-A_{2}/J_{2}-IsoP-PC and their free fatty acid analogues in plasma of patients with CHD were significantly decreased compared to control subjects. All these data uncovered a novel protective role of deoxy-A_{2}/J_{2}-IsoP-PC in atherosclerosis.

RESULTS

Identification and characterization of deoxy-A_{2}/J_{2}-IsoP-PC in vitro and in vivo— Oxidation of PAPC in LDL has been extensively studied in the context of atherosclerosis. We proposed a unified free radical mechanism (shown in Figure.1) to rationalize the formation of various oxidation products from arachidonic acid (25). F_{2}-IsoPs have been used as the best marker for oxidative stress due to their chemical stability (19). However, D_{2}/E_{2}-IsoPs are relatively less stable than F_{2}-IsoPs because they are readily dehydrated to form A_{2}/J_{2}-
IsoPs and deoxy-A<sub>3</sub>/? -IsoPs. In this work, we set out to identify these novel deoxy-A<sub>3</sub>/J<sub>2</sub>-IsoP-PC in LDL in the context of atherosclerosis. The presence of these novel oxidation products from oxPAPC was shown in Fig.2. In the positive ion mode of electrospray ionization (ESI)-MS/MS, PC species are readily ionized to give a characteristic fragment of PC head group with m/z 184. But in the negative ion mode, fragments with fatty acid carboxylate ions can be observed and used as important structural information to identify these oxidation products. Collision induced dissociation (CID) of the parent ion m/z 794.5 and m/z 838.5 (adducts with formate anion) tentatively confirmed the structure of this type of novel oxidation products (Fig.2B and C). Fragments with m/z 315 and 271 are indicative of the deoxy-A<sub>3</sub>/J<sub>2</sub> moiety in the molecules while m/z 255 corresponds to a palmitate ion. Loss of methyl group from the parent ion gives rise to a characteristic fragment of PC m/z 778.5.

PAPC is one of the major phospholipids in LDL whose oxidative modification and retention in subendothelial space initiates the process of atherosclerosis. Using multiple reaction monitoring (MRM) technique in a triple quadrupole mass spectrometer, we confirmed the formation of deoxy-A<sub>3</sub>/J<sub>2</sub>-IsoP-PC in oxLDL in vitro, human plaques from postmortem samples and embolized materials captured by distal protection filter devices during uncomplicated saphenous vein interventions (Fig.2D). Table 1 represents a brief clinical background of plaques and filter materials used in this study (24). In addition to a series of other known oxidation products like PEIPC, F<sub>2</sub>-IsoP-PC and D<sub>2</sub>/E<sub>2</sub>-IsoP-PC (5,26,27), we tentatively identified the novel PL oxidation products. Similar patterns of multiple peaks were observed in different clinical samples, consisting with our free radical mechanisms and the formation of different structural isomers.

Synthesis and characterization of a model compound 15d-PGJ<sub>2</sub>-PC—As mentioned previously, oxidation of PAPC generates a complex mixture of oxidation products and a majority of previous studies on the biological activities of oxPAPC have been using oxidation mixtures, which makes it difficult to assign specific biological activity to a specific oxidation product and sometimes even generates conflicting observations primarily due to the different oxidation conditions. Thus independent chemical synthesis of a defined oxidation product enables us to overcome the limitations and elucidate the molecular mechanisms and pathophysiological relevance of a specific class of PAPC oxidation products. To investigate the biological activities of deoxy-A<sub>3</sub>/J<sub>2</sub>-IsoP-PC, we chemically synthesized a representative compound 15d-PGJ<sub>2</sub>-PC from the commercially available 15d-PGJ<sub>2</sub> and 16:0 lyso-PC (Fig.3A). The chemical structure of purified 15d-PGJ<sub>2</sub>-PC was characterized by LC-MS/MS. As shown in Fig.3B, the first panel represented the full scan spectrum of [M+H]<sup>+</sup>. The second and third panels represented the CID spectra in positive and negative ion mode respectively. In positive ion mode, 15d-PGJ<sub>2</sub>-PC lost a characteristic fragment with m/z 184, and in negative ion mode, the specific fragments with m/z 255 and m/z 315 represented the side chain in sn-1 and sn-2 position respectively. An ion with m/z 271 is a fragmentation ion derived from 15d-PGJ<sub>2</sub> ([M-CO<sub>2</sub>]<sup>+</sup>). All these indicated that the structure of 15d-PGJ<sub>2</sub>-PC we synthesized was similar to the novel oxidation products observed in oxLDL, plaques, and filter materials.

15d-PGJ<sub>2</sub>-PC induced anti-oxidant and anti-inflammatory responses in vivo and in vitro—Our successful chemical synthesis of 15d-PGJ<sub>2</sub>-PC enables us to dissect the biological functions of these novel electrophilic oxidation products from other compounds. Because of the presence of an α,β-unsaturated cyclopentenone moiety, we hypothesized that 15d-PGJ<sub>2</sub>-PC would exert...
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similar biological effects to those of 15d-PGJ\(_2\). A whole transcriptomic analysis by RNA-seq on BMDM revealed that 15d-PGJ\(_2\) and 15d-PGJ\(_2\)-PC indeed induced a similar pattern of gene expression, but fold changes in 15d-PGJ\(_2\) group seemed more significant than those in 15d-PGJ\(_2\)-PC (Fig.4A). Moreover, compared with 15d-PGJ\(_2\)-PC, 15d-PGJ\(_2\) treatment regulated more specific genes expressions (Fig.4B). Gene ontology (GO) enrichment analysis of overlapping differentially expressed genes (DEGs) demonstrated that both 15d-PGJ\(_2\) and 15d-PGJ\(_2\)-PC treatment were associated with immune and oxidative responses (Fig.4C, D). Among up-regulated genes, we found a cluster of redox regulated genes mediated by transcription factor Nrf2 (Fig.5A). Then we confirmed that 15d-PGJ\(_2\) and 15d-PGJ\(_2\)-PC increased the expression of HO-1 and GCLM1 in a dose-dependent manner by real-time PCR (Fig.5B). In addition, HO-1 protein level was also significantly increased with 15d-PGJ\(_2\) and 15d-PGJ\(_2\)-PC treatment (Fig.5C).

Previous evidence showed that 15d-PGJ\(_2\) and other cyclopentenone lipid mediators exerts anti-inflammatory activity through inhibition of NF-\(\kappa\)B-induced pro-inflammatory genes expression (28,29). As shown in Fig.6, 15d-PGJ\(_2\)-PC and 15d-PGJ\(_2\) reduced the transcription activity of NF-\(\kappa\)B and inhibited pro-inflammatory cytokine IL1\(\beta\) and chemokine CCL2 expression in both basal and LPS stimulated condition. Furthermore, 15d-PGJ\(_2\) and 15d-PGJ\(_2\)-PC decreased LPS induced IL1\(\beta\) secretion, suggesting that 15d-PGJ\(_2\)-PC is also an antagonist of NF-\(\kappa\)B pathway (Fig.6C). In LPS-induced SIRS model, 15d-PGJ\(_2\)-PC at a low dose of 0.04mg/kg significantly decreased LPS-induced inflammatory genes IL1\(\beta\), IL6, CCL2 and CCL4 expression in multiple tissues including lung, liver and kidney (Fig.6D-F). In addition, IL1\(\beta\) levels in serum were significantly lower in the presence of 15d-PGJ\(_2\)-PC (Fig.6G), suggesting that this novel class of oxidation products possess potent anti-inflammatory properties in vivo. 15d-PGJ\(_2\) is a natural ligand of PPAR\(\gamma\) (30,31). Here we confirmed that 15d-PGJ\(_2\)-PC was a slightly less potent agonist for PPAR\(\gamma\) than 15d-PGJ\(_2\) (Fig.6H).

All these data suggested that deoxy-\(A_2/J_2\)-IsoP-PC may induce anti-inflammatory and anti-oxidant responses through NF-\(\kappa\)B, PPAR\(\gamma\) and Nrf2 pathways.

\textit{15d-PGJ\(_2\)-PC inhibited macrophage foam cell formation}—Uptake of oxLDL by macrophages generates foam cells, which initiates fatty streak formation, an early hallmark of atherosclerosis. A series of OxPLs named oxPL\(_{CD6}\) have been found to bind to CD36, contributing to foam cell formation (32). To study the functional consequences of exposure of macrophages to deoxy-\(A_2/J_2\)-IsoP-PC in oxLDL, we examined the effects of 15d-PGJ\(_2\)-PC on foam cell formation in macrophages. As shown in Fig.7, 15d-PGJ\(_2\)-PC treatment not only decreased Dil-oxLDL uptake by macrophages, but also inhibited intracellular lipid droplets accumulation, suggesting that deoxy-\(A_2/J_2\)-IsoP-PC inhibit macrophage foam cell formation and potentially play a protective role in atherosclerosis. Lectin-like oxidizd low-density lipoprotein receptor-1 (LOX-1), scavenger receptor class A (SR-A) and CD36 are the major receptors responsible for oxLDL uptake in macrophages. Here, we found 15d-PGJ\(_2\)-PC strikingly caused a decreased in mRNA level of LOX-1 and SR-A without affecting CD36 expression (Fig.7E).

Previous studies demonstrated LOX-1 and SR-A expression were regulated by adenosine monophosphate-activated protein kinase (AMPK) and extracellular signal regulated kinase (ERK) pathway respectively (33-35). As shown in Fig.7F, 15d-PGJ\(_2\)-PC slightly increased p-AMPK level, but significantly reduced p-ERK level, suggesting that deoxy-\(A_2/J_2\)-IsoP-PC inhibit foam cell formation presumably through modulation of SR-A pathways.
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The levels of circulating deoxy-A2/J2-IsoP-PC and their free fatty acid analogues are associated with cardiovascular diseases in humans—After characterization of these novel cyclopentenone-containing phospholipid oxidation products in human atherosclerotic plaques, we further investigated the association of the levels of these compounds in plasma with CVDs. To investigate this relationship, we analyzed deoxy-A2/J2-IsoP-PC and their free fatty acids analogues in human plasma from relatively healthy subjects and patients with CHDs. Table 2 represents a brief clinical background of the human subjects enrolled in this study. Fig. 8A showed the MRM spectrogram of deoxy-A2/J2-IsoP-PC identified in human plasma. Because there are several enzymes such as PAF-AH (18) in plasma that can hydrolyze the fatty acid side chain at sn-2 position, we also detected free fatty acid oxidation products in human plasma. Deoxy-A2/J2-IsoPs, sn-2 side chain of deoxy-A2/J2-IsoP-PC, have been identified and their MRM chromatograms showed multiple peaks (Fig. 8B), consistent with the patterns of lipid peroxidation instead of the COX-derived 15d-PGJ2. The levels of the total amounts of deoxy-A2/J2-IsoP-PC and deoxy-A2/J2-IsoPs were decreased in plasma of CHD patients comparing with the healthy subjects, consistent with a protective role of these compounds in cardiovascular diseases.

DISCUSSION

Atherosclerosis is the main cause of morbidity and mortality of CVDs, which are the leading cause of death in the world, responsible for over 17.5 million deaths per year (36). High level of LDL-C is one of the major risk factors for CVDs and overwhelming evidence suggests that oxidized LDL caused by oxidative stress is the culprit for atherosclerosis, which interacts with different vascular cells and plays an important role in all stages of atherosclerosis (37). However, OxPLs are a highly heterogeneous mixture and both pro-atherosclerotic and anti-atherosclerotic roles of OxPLs have been reported (23,38,39). It is essential to identify individual species and explore the biological activities of these products. In the current study, we identified and characterized a novel series of 15d-PGJ2-like compounds, termed deoxy-A2/J2-IsoP-PC, which formed as intact phospholipids via free radical-catalyzed peroxidation of PAPC. Then we confirmed the formation of deoxy-A2/J2-IsoP-PC in oxLDL and human clinical samples including atherosclerotic plaques and filter materials. Furthermore, we quantified these novel oxidation products and their hydrolyzed forms in human plasma using LC-MS/MS and found that the levels of these compounds were reversely correlated with CHD, suggesting a protective role of these oxidation products against atherosclerosis and CVDs.

To further confirm the chemical identity and investigate the biological activities of deoxy-A2/J2-IsoP-PC, we chemically synthesized a representative compound 15d-PGJ2-PC from 15d-PGJ2 and 16:0 lyso-PC. 15d-PGJ2 is one of the most extensively studied PGs containing cyclopentenone, which regulated a plethora of signaling pathways through covalent modification of critical amino acids resides (10). Kawamoto et al. (40) reported that 15d-PGJ2 was an inducer of anti-oxidant response. Later studies by Itoh et al. found that 15d-PGJ2 induced anti-oxidant responses through Keap1-Nrf2 pathway (13). Furthermore, 15d-PGJ2 regulated acute and chronic inflammation by inhibition of NF-κB-induced pro-inflammatory genes transcription (41). Besides, Kawahito et al observed that 15d-PGJ2 reduced the atherosclerotic lesions formation (42). Previous studies suggested A2/J2-IsoPs can be formed from free radical pathways and similar biological properties were observed (28,43). Our current study showed that oxidation of PAPC in LDL
primary underwent free radical pathways and deoxy-A<sub>2</sub>/J<sub>2</sub>-IsoP-PC are formed primarily as intact phospholipids. Exposure of macrophages to these oxidation products may have a profound impact on the biological activities and functions of these important immune cells (6). Based on the transcriptomic profile of BMDM, 15d-PGJ<sub>2</sub>-PC at a biologically relevant concentration induced a cluster of Nrf2-dependent redox regulated genes expression. In addition, 15d-PGJ<sub>2</sub>-PC inhibited NF-κB transcription activity, down-regulated pro-inflammatory genes expression, and inhibited cytokines secretion. Previous studies demonstrated that cys-179 in IKKβ subunit of IKK, cys-38 and cys-62 in the DNA binding domain of the p65 and p50 subunit respectively, cys-273 in Keap1 were the targets of 15d-PGJ<sub>2</sub> covalent modifications (44-48). However, 15d-PGJ<sub>2</sub> and deoxy-A<sub>2</sub>/J<sub>2</sub>-IsoP-PC represent distinct biological pathways, i.e. the former is derived from COX-mediated inflammatory response while the latter is generated from free radical-induced lipid peroxidation under oxidative stress. Furthermore, deoxy-A<sub>2</sub>/J<sub>2</sub>-IsoP-PC and 15d-PGJ<sub>2</sub> may partition into different cellular compartment due to their different polarities. Thus it remains to be studied whether deoxy-A<sub>2</sub>/J<sub>2</sub>-IsoP-PC target different proteins from those identified for 15d-PGJ<sub>2</sub> in the future. Moreover, as a representative of deoxy-A<sub>2</sub>/J<sub>2</sub>-IsoP-PC, 15d-PGJ<sub>2</sub>-PC inhibited macrophage foam cell formation, suggesting an anti-atherosclerotic role of these novel oxidation products.

Macrophages are key immune cells in the initiation and progression of atherosclerosis and they polarize to different phenotypes in response to a multitude of stimuli from their microenvironment (6). Recent evidence indicated that oxPAPC induced a distinct Mox phenotype characterized by up-regulation of Nrf2-mediated redox genes expression and Mox macrophages comprised 30% of all macrophages in advanced atherosclerotic lesions in addition to classically activated M1 and alternatively activated M2 macrophages (7). However, the exact molecular species in the mixture of oxPAPC remain to be elucidated. Based on our transcriptomic RNA-seq analysis, Nrf2-regulated genes are among the top up-regulated ones after both 15d-PGJ<sub>2</sub> and 15d-PGJ<sub>2</sub>-PC treatment, reminiscent of Mox phenotype. These data suggested that the novel deoxy-A<sub>2</sub>/J<sub>2</sub>-IsoP-PC may be one of the major classes of molecular species in the PAPC oxidation mixture responsible for the induction of Mox phenotype because 15d-PGJ<sub>2</sub> is not present in LDL. It is conceivable that 15d-PGJ<sub>2</sub> can be generated by COX-mediated formation of PGD<sub>2</sub> and subsequent dehydration in macrophages. Thus these two chemical similar bioactive lipids are important mediators in macrophage functions and metabolism in the context of atherosclerosis, bridging the two important pathological factors of immune responses and oxidative stress. Besides these similar biological functions, 15d-PGJ<sub>2</sub> exclusively down-regulated 269 genes and up-regulated 217 genes while 15d-PGJ<sub>2</sub>-PC increased 31 genes and decreased 22 genes expression. GO analysis demonstrated these genes exclusively regulated by 15d-PGJ<sub>2</sub> were associated with cell proliferation and activation including lymphocytes, mononuclear cells and leukocytes.

In summary, we provided evidence that deoxy-A<sub>2</sub>/J<sub>2</sub>-IsoP-PC were formed in vitro and in vivo from free radical-induced oxidation of PAPC in LDL. The levels of deoxy-A<sub>2</sub>/J<sub>2</sub>-IsoP-PC and their free acid analogues were significantly decreased in plasma of CHD patients compared with the healthy control. As shown in Fig 8D, deoxy-A<sub>2</sub>/J<sub>2</sub>-IsoP-PC is a class of new bioactive lipid mediators that exert anti-inflammatory and anti-oxidant properties through modulation of PPARγ, NF-κB, and Nrf2 pathways. In addition, deoxy-A<sub>2</sub>/J<sub>2</sub>-IsoP-PC inhibits foam cell formation by reducing oxLDL
uptake and lipid droplets accumulation. Together, these findings highlight a protective role of deoxy-A\textsubscript{2}/J\textsubscript{2}-IsoP-PC in cardiovascular diseases, which warrants further studies of these novel compounds as potential circulating markers for CHD or therapeutic targets for prevention/treatment of atherosclerosis.

**EXPERIMENTAL PROCEDURES**

*Materials*—PAPC and 16:0-lysoPC were purchased from Avanti Polar Lipids (Alabaster, AL, USA). 15d-PGJ\textsubscript{2} was purchased from Cayman Chemical Company (Ann Arbor, MI, USA). 4-(Dimethylamino) pyridine (DMAP), N,N’-Dicyclohexylcarbodiimide (DCC) were from Sigma (St Louis, MO, USA). Normal phase high performance liquid chromatography column was purchased from Agilent (Santa Clara, CA, USA) and reverse phase high performance liquid chromatography columns were from Phenomenex (Torrance, CA, USA). All solvents were HPLC quality and purchased from Honeywell (Gibbstown, NJ, USA). oxLDL induced by copper and 1,19-dioctadecyl-3,3,39,39-tetramethylindocarbocyaninel perchlorate labeled oxidized LDL (Dil-oxLDL) were purchased from Yiyuan Biotechnologies (Guangzhou, China).

*Cell culture*—Bone marrow cells collected from femurs and tibias from male C57BL6 mice were cultured in RPMI medium supplemented with 20% FBS and 30% L929 supernatant as source of M-CSF to induce bone marrow derived macrophages (BMDMs). COS-7 were cultured in DMEM supplemented with 10% FBS.

*Oxidation of PAPC in vitro*—PAPC was exposed to air at 37\(^\circ\)C for 72 hours to produce oxPAPC.

*Human samples collections*—Human plaques (full-thick aortic walls) obtained at autopsy from postmortem were kindly provided by Dr. Aiming Xue (Fudan University, Shanghai). The collection was approved by the Institutional Review Board at the Fudan University. Four recovered filter materials from the distal protection devices were collected from 4 patients undergoing saphenous vein graft (SVG) interventions. At the end of the procedure, the recovered filter was cut off from the wire and the entire filter with the retained biological material was placed in ice-cold PBS containing EDTA/BHT (4\(\mu\)M/20\(\mu\)M) and frozen immediately. This process was approved by the UCSD Human Research Subjects Protection Program. Human plasma samples were obtained from Beijing Tian Tan Hospital. The Institutional Review Board at Tian Tan Hospital approved the study and all participants provided written informed consent.

*Lipid extraction in oxLDL, human atherosclerotic plaques, filter materials and human plasma*—Lipids were extracted using the Folch method as referenced (49). Before extraction, human plaques were pulverized with a chilled mortar and pestle. Recovered filter materials in ice-cold phosphate buffered solution containing EDTA/BHT (4 \(\mu\)M/20 \(\mu\)M) were sonicated for 5 minutes. 100 ng PC internal standard (14:1-14:1 PC) and 5 ng prostaglandin internal standard (15d-PGJ\textsubscript{2}-d\textsubscript{4}) were added during extraction.

*Detection of oxidized products by normal phase LC/ESI-MS/MS*—Lipid extracts were re-dissolved in chloroform and analyzed by using an Agilent 1260 Quad pump coupled to a Thermo Fisher TSQ Vantage triple quadrupole mass spectrometer. It was carried out using an Agilent Zorbax RX-silica column (150 \(\times\) 2.1 mm, 5 \(\mu\)m) at a flow rate of 500 \(\mu\)l/min starting from 100\% A (chloroform/methanol/30\% ammonium hydroxide, 80/19.5/0.5, by vol) to 100\% B (chloroform/methanol/water/30\% ammonium hydroxide, 60/34/5.5/0.5, by vol) in 14 min, holding at B for 6 min and returning to A in 2 min. Deoxy-A\textsubscript{2}/J\textsubscript{2}-IsoP-PC were determined by four characteristic transitions including m/z 794.5 to 184 in positive
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Ion mode and m/z 778.5 to 255, m/z 778.5 to 315 as well as m/z 778.5 to 271 in negative ion mode. Internal standard (14:1-14:1 PC) was determined by m/z 674.5 to 184 in positive ion mode and m/z 658.5 to 225 in negative ion mode. Furthermore, the levels of deoxy-A2/J2-IsoP-PC in human plasma were quantified using transition m/z 778.5-315 while IS was quantified using transition m/z 658.5-225.

Synthesis purification and characterization of 15d-PGJ2-PC—15d-PGJ2-PC was synthesized according to a previously published method (50). In brief, 15d-PGJ2, 16:0-lysoPC, DCC and DMAP were added in a glass bottle, dissolved in chloroform and sonicated at 4°C under nitrogen for 6 hours. The reaction mixture was extracted over a C18 Sep-Pak cartridge that had been equilibrated by rinsing with methanol and deionized water. The methanol elution was further purified by reverse phase HPLC. Sample was injected onto a Phenomenex C18 column (250 × 4.6 mm, 5 μm), with a flow rate of 1 ml/min which was eluted with a linear gradient of 85% A (water/formic acid, 99.9/0.1, by vol) to 100% B (methanol/formic acid, 99.9/0.1, by vol) in 15 min, holding at 100% B for 15 min, returning to 100% A in 1 min. The fraction from 16 to 18 min was collected from the column and dried to a residue under nitrogen. Purified 15d-PGJ2-PC was dissolved in methanol and characterized by tandem mass spectrometer in full scan and CID mode.

Detection of metabolites of free fatty acid by reverse phase LC/ESI-MS/MS—Lipid extracts were dissolved in mobile phase A (water/acetonitrile/formic acid, 63/37/0.02, by vol) and analyzed by using an AB Sciex 5500 QTrap hybrid quadrupole linear ion trap mass spectrometer in negative ion mode. Samples were injected onto a phenomenex C18 column (100 × 2.1 mm, 2.6 μm) at a flow rate of 400 μl/min starting with 100% A to 92% A in 6 min, holding at 45% A from 6.5 to 10 min, turning to 100% B (acetonitrile/isopropanol, 50/50, by vol) at 13 min, and returning to A from 14 to 14.5 min. Deoxy-A2/J2-IsoPs and IS (15d-PGJ2-d6) were determined and quantified by using transition m/z 315-271 and m/z 319-275 in negative ion mode respectively.

Transcriptome microarray analysis—BMDMs incubated with 1 μM 15d-PGJ2 or 15d-PGJ2-PC for 6 hours were lysed with TRIzol reagent (Invitrogen). The library preparation and sequencing was carried out by Beijing Genomics Institute BGI (Wuhan, China). The sample library was qualified and quantified by Agilent 2100 Bioanalyzer (Agilent Technologies, San Diego, CA) and ABI StepOnePlus Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA). Then, the library products were sequenced via Illumina HiSeq™ 4000 (Illumina Inc., San Diego, CA).

Raw single-end reads were aligned to the mouse reference genome mm10 downloaded from the Illumina iGenomes website using BWA. All the sequence reads of this study were deposited in the Sequence Reads Archive at the NCBI database under accession number GSE85931. We used RSEM to quantify the expression of the transcripts isoforms and NOISeq method to screen differentially expressed genes (DEGs) between two groups. Heat maps were generated using the statistical tools provided by the R projects. Gene ontology (GO) functional annotation analysis was performed using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) v6.7 online tool.

Real-time PCR—BMDMs after stimulation were washed with PBS and lysed with TRIzol reagent. RNA was purified, reverse transcribed into cDNA and quantitative PCR analysis was performed. The genes expression were normalized to L32 expression. The primer sequences used were L32: Forward: 5'-TTA AGC GAA ACT GGC GGA AAC-3', Reverse: 5'-TTG TTG CTC CCA TAA
CCG ATG-3'; IL1β: Forward: 5'-AAG AGC TTC AGG CAG GCA GTA TCA-3'; Reverse: 5'-TGC AGC TGT CTA ATG GGA ACG TCA-3'; IL6: Forward: 5'-GGA TAC CAC TCC CAA CAG ACC T-3'; Reverse: 5'-GCC ATT GCA CAA CTC TTT TCT C-3'; CCL2: Forward: 5'-TGC CCT AAG GTC TTC AGC TAC-3'; Reverse: 5'-GCC ATT GCA CAA CTC TTT TCT C-3'; CCL4: Forward: 5'-TCT GTG CAA ACC TAA CCC CG-3'; Reverse: 5'-GAG GGT CAG AGC CCA TTG GT-3'; HO-1: Forward: 5'-GCC ACC AAG GAG GTA CAC AT-3'; Reverse: 5'-GCT TGT TGC GCT TCA TCT CC-3'; GCLM1: Forward: 5'-GCT TCG GGA CGT TAT CCA AA-3'; Reverse: 5'-AGC TGT GCA ACT CCA AGG AC-3'; LOX-1: Forward: 5'-ACA AGA TGA AGC CTG CGA AT-3'; Reverse: 5'-GCT GAG TAA GGT TCG CTT GG-3'; SR-A: Forward: 5'-TCA CTG GAT GCA ATC TCC AA-3'; Reverse: 5'-ACG TGC GCT TGT TCT TCT TT-3'; CD36: Forward: 5'-TGC TGG AGC TGT TAT TGG TG-3'; Reverse: 5'-TGG TTG TTG CAC ATC AAA GA-3'.

Luciferase assay of PPARγ Activation—According to the previous study (51), a mixture containing PPRE-tk-luc (PPAR reporter plasmid expressing firefly luciferase), pRL-SV40 (control plasmid expressing Renilla luciferase), and PPARγ expression plasmid were transfected into COS-7 cells by using FuGENE 6 (Roche Molecular Biochemicals, Indianapolis, IN). After treated with 1 μM 15d-PGJ2 and 15d-PGJ2-PC for 36h, cells were harvested and the luciferase activity was determined. All plasmids used here were kindly provided by Dr. Raymond N. DuBois (Vanderbilt University, TN).

Luciferase assay of NF-κB inhibition—NF-κB transcription activity was determined according to a previously published method (28). In brief, BMDMs obtained from transgenic mice expressing a luciferase plasmid containing NF-κB binding sites were incubated with different concentrations of 15d-PGJ2 and 15d-PGJ2-PC for 30 min, then cells were treated with 100 ng/ml LPS. Luciferase activity of NF-κB was determined after 4 hours.

IL1β expression analysis—BMDMs were pretreated with 1 μM 15d-PGJ2 and 15d-PGJ2-PC for 1 hour and following cultured with 100 ng/ml LPS for 18 hours. Subsequently, media was harvested and IL1β secretion was measured using ELISA according to the protocol of the manufacturer (eBioscience, San Diego, CA).

LPS induced systemic inflammatory response syndrome (SIRS)—Male C57BL6 mice (8 weeks old) were randomly divided into four groups: control group, 15d-PGJ2-PC group, LPS group and 15d-PGJ2-PC plus LPS group. Mice were treated with 0.04 mg/kg 15d-PGJ2-PC or 0.9% saline by an intraperitoneal injection 3h before a 1 mg/kg LPS (Escherichia coli 055:B5, Sigma-Aldrich, L2880) injection. Then, mice were sacrificed by CO2 inhalation 6 h after LPS injection. Blood, lungs, livers and kidneys were collected for analyses. All Animal experiments were approved by the review committee from the Institute for Nutritional Science (Shanghai Institutes for Biologic Sciences, Chinese Academy of Sciences) and performed in accordance with the institutional guidelines.

Western blot analysis—BMDMs were treated with 1 μM 15d-PGJ2 and 15d-PGJ2-PC for 18 hours, then cells were harvested. Western blot was performed on whole cell lysates. Primary antibodies used were anti-HO-1 (1:1000, Proteintech, catalog number 10701-1-AP) and anti-β-actin (1:5000, Abcam, catalog number ab8226). Total protein loading was assessed by staining with Coomassie blue (Invitrogen, catalog number LC6060). To analyze AMPK and ERK activity, BMDMs were treated with 1 μM 15d-PGJ2-PC for different time after 12h starvation. Primary antibodies anti-AMPK (1:1000, catalog number 5832), anti-p-AMPK (1:1000, catalog number 2535), anti-ERK1/2 (1:1000, catalog number 4695)
and anti-p-ERK1/2 (1:2000, catalog number 4370) were from Cell Signaling Technology.

Foam cell formation—BMDMs were cultured in RPMI 1640 containing 0.2% (v/v) fatty-acid free BSA (Sigma-Aldrich) in the presence/absence of 1 μM 15d-PGJ2-PC for 12h. Then cells were incubated with 10 μg/ml Dil-oxLDL. After 12 hours, cells were washed with PBS and fixed with 4% paraformaldehyde (PFA). Cell nuclei were stained with Hoechst (1 μg/ml, Sigma-Aldrich) for 10 min at room temperature. Then, cells were imaged and assessed by Thermo Fisher Cellomics ArrayScan Infinity HCS system (Thermo Fisher Scientific, Waltham, MA) including an incorporated fully automated the Carl Zeiss™ Axiovert Z1 microscope and Hamamatsu ORCA-ER2 grayscale digital CCD camera. The images were analyzed by Thermo Scientific™ Store™ Express Image and Database Management Software. For quantification of intracellular lipid droplets, BMDMs were cultured with 50 μg/ml oxLDL in the presence/absence of 1 μM 15d-PGJ2-PC for 18h. Subsequently, cells were fixed and stained with Nile red (0.25 μg/ml, N121291, Aladdin) for 30 min at room temperature. The fluorescence was measured on Thermo Fisher Cellomics ArrayScan Infinity HCS system.

Statistical analysis—Results are expressed as mean ± SEM. Statistical analysis was performed using Student’s t test. A probability value of < 0.05 was considered statistically significant.

ACKNOWLEDGEMENT
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Conflict of interests: The authors declare that they have no conflicts of interest with the contents of this article.

Author contributions: JL designed, conducted most of the experiments, analyzed the data, and wrote the manuscript. SG, XX, QC, HZ and MZ performed MS analysis. JG and YZ performed animal experiments. XL performed synthesis of 15d-PGJ2-PC. BC, MZ, LZ performed the clinical studies. WH, TS, CS, TSB helped with some of the cell experiments. ST performed the filter analysis and revision of the manuscript. NAP helped the chemical mechanisms and synthesis. HY conceived the idea, design and oversaw the research, and revised the manuscript.

REFERENCES
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TABLES

Table 1 Brief clinical background of human plaques and filter materials

<table>
<thead>
<tr>
<th>Sample</th>
<th>Device</th>
<th>Vascular bed</th>
<th>Age, yrs</th>
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<tr>
<td>1</td>
<td>Plaque</td>
<td>abdominal aorta</td>
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</tr>
<tr>
<td>2</td>
<td>Plaque</td>
<td>thoracic aorta</td>
<td>60</td>
</tr>
<tr>
<td>3</td>
<td>FilterWire EZ</td>
<td>saphenous vein graft</td>
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<td>FilterWire EZ</td>
<td>saphenous vein graft</td>
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<td>6</td>
<td>FilterWire EZ</td>
<td>saphenous vein graft</td>
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Table 2 Baseline clinical background of human subjects

<table>
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<th>Control (n=30)</th>
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<tr>
<td>Age (years)</td>
<td>55.60±10.87</td>
<td>66.73±9.90</td>
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<td>Male gender (%)</td>
<td>50.00</td>
<td>76.67</td>
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<tr>
<td>BMI (kg/m(^2))</td>
<td>25.91±3.53</td>
<td>25.87±3.07</td>
<td>0.48</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>33.33</td>
<td>56.70</td>
<td></td>
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<tr>
<td>Drinking (%)</td>
<td>23.33</td>
<td>43.30</td>
<td></td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>83.30</td>
<td>90.00</td>
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</tr>
<tr>
<td>Diabetes (%)</td>
<td>36.70</td>
<td>43.30</td>
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</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>1.77±0.94</td>
<td>1.60±0.96</td>
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</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>3.86±0.73</td>
<td>4.06±0.84</td>
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</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>2.28±0.69</td>
<td>2.42±0.76</td>
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</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>1.06±0.29</td>
<td>1.01±0.21</td>
<td>0.24</td>
</tr>
<tr>
<td>apo-A1 (g/L)</td>
<td>1.25±0.28</td>
<td>1.22±0.21</td>
<td>0.29</td>
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<tr>
<td>apo-B (g/L)</td>
<td>0.81±0.18</td>
<td>0.86±0.21</td>
<td>0.18</td>
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</table>

Angiography was performed to identify vessel narrowing. CHD patients were defined when the narrowing was more than 50% in the arteries of the heart. Values are mean ± SD or %. BMI, body mass index; LDL-C, LDL cholesterol.

FIGURE LEGENDS

Figure 1 Major Free radical pathways of PAPC oxidation and biosynthetic route of 15d-PGJ\(_2\). Formation of deoxy-\(\text{A}_2/\text{J}_2\)-IsoP-PC (Shown in the red box) is hypothesized to be formed from the dehydration of \(\text{D}_2/\text{E}_2\)-IsoP-PC. PLA\(_2\), phospholipase A\(_2\); HETES, hydroxyeicotretraenoic acid, with different regioisomers; EET, epoxyeicosatrienoic acid; Di-HETE, dihydroxyeicosatrienoic acid; LOX, lipoxygenases; COX, cyclooxygenases; PGH\(_2\), prostaglandin H\(_2\); PGD\(_2\), prostaglandin D\(_2\); PGJ\(_2\), prostaglandin J\(_2\); ROS, reactive oxygen species; LPO, lipid peroxidation; PEIPC, 1-palmitoyl-2-( epoxyisoprostane E\(_2\))-sn-glycero-3-phosphocholine; PECPC, 1-palmitoyl-2-(epoxycyclopentenone)-sn-glycero-3-phosphorylcholine. PAF-AH, platelet activating factor-acetyl hydrolase.
Figure 2 Identification and characterization of deoxy-A₂/J₂-IsoP-PC in oxPAPC, oxLDL, human plaques and filter materials. (A) The full scan EIC (extracted ion chromatogram) at m/z 794.5 from oxPAPC in the positive ion mode. (B and C) CID spectrgram at m/z 794.5 in positive (B) and m/z 838.5 in negative ion mode (C) from oxPAPC respectively. The structure shown here was used as a representative. (D) SRM chromatogram of deoxy-A₂/J₂-IsoP-PC (m/z 778.5-315) identified in oxPAPC, oxLDL, human plaques, and filter materials respectively.

Figure 3 Synthesis and characterization of a model compound 15d-PGJ₂-PC. (A) Synthetic route of 15d-PGJ₂-PC from commercial available 15d-PGJ₂ and 16:0-lysoPC. (B) Characterization of synthetic 15d-PGJ₂-PC by LC-MS/MS. The first panel represents full scan spectrum in the positive ion mode. The second and third panels show the CID spectra in positive and negative ion mode respectively.

Figure 4 Transcriptomic profile (RNA-seq) of 15d-PGJ₂ and 15d-PGJ₂-PC on genes expression in BMDMs. (A) The heat map showed the clustering of transcripts expression of DEGs in BMDMs. DEGs here were screened by NOISeq method between 15d-PGJ₂/15d-PGJ₂-PC and control group. The color scale (right) illustrates the relative expression level across all samples: red color represents an expression level above mean, green color represents expression lower than the mean. Each treatment is represented by three independent replicates. The dendrogram on the left of the heat map showed the clustering of the transcripts. (B) Genes down-regulated (left) and up-regulated (right) by 15d-PGJ₂ or 15d-PGJ₂-PC treatment. Numbers in Venn diagram represented the number of DEGs. (C) GO functional classifications on overlapping DEGs in 15d-PGJ₂ and 15d-PGJ₂-PC treatment. X axis means number of DEGs, Y axis represents GO terms. All GO terms are grouped into three ontologies: blue is for biological process (BP), green is for cellular component (CC) and pink is for molecular function (MF). Here shows top 5 GO terms in each ontology. (D) Detailed DEGs in each category enriched in the screen.

Figure 5 15d-PGJ₂ and 15d-PGJ₂-PC induced anti-oxidative responses. (A) The heat map showed the clustering of transcripts expression of Nrf2 regulated genes. The color scale (right) illustrates the relative expression level across all samples: red color represents an expression level above mean, green color represents expression lower than the mean. Each treatment is represented by three independent replicates. The dendrogram on the left of the heat map showed the clustering of the transcripts. (B) BMDMs were stimulated with different concentrations (µM) of 15d-PGJ₂ or 15d-PGJ₂-PC for 6 hours. Nrf2 target genes including HO-1 and GCLM1 expression were analyzed by real-time PCR. (C) BMDMs were cultured with 1 µM 15d-PGJ₂ or 15d-PGJ₂-PC for 18 hours. Cell lysates were analyzed by Western blot. Total protein loading was assessed by staining with Coomassie blue. Results are shown as the mean ± SEM (3 biological replicates, 3 technical replicates per group per experiment). *p <0.05, **p <0.01, ***p <0.001 versus control group based on Student’s t-test.

Figure 6 15d-PGJ₂ and 15d-PGJ₂-PC induced anti-inflammatory responses in vitro and in vivo. (A) Macrophages from transgenic mice expressing a luciferase plasmid containing NF-κB binding sites were pretreated with different concentrations of 15d-PGJ₂ and 15d-PGJ₂-PC 30 min before addition of 100 ng/ml.
LPS. Luciferase activity was determined after 4 hours. (B) BMDMs were pretreated with 1 µM 15d-PGJ2 or 15d-PGJ2-PC for 3 hours and then cultured with/without 100 ng/ml LPS for another 3 hours. NF-κB target genes IL1β and CCL2 expression were analyzed by real-time PCR. (C) BMDMs were pretreated with 1 µM 15d-PGJ2 or 15d-PGJ2-PC for 1 hour and incubated with 100 ng/ml LPS for another 18 hours. IL1β secretion in cell media were detected by ELISA. Results are shown as the mean ± SEM (3 biological replicates, 3 technical replicates per group per experiment). (D-F) 15d-PGJ2-PC decreased LPS induced pro-inflammatory genes expression in lung (D), liver (E) and kidney (F) tissues. (G) 15d-PGJ2-PC reduced serum levels of IL1β from LPS induced SIRS model. Results are shown as the mean ± SEM (2 biological replicates, 5 mice per group per experiment). (H) COS-7 cells transfected with PPARγ luciferase reporters, were treated with 1 µM 15d-PGJ2 and 15d-PGJ2-PC for 36 hours, and then PPARγ activity were determined. Results are shown as the mean ± SEM (3 biological replicates, 3 technical replicates per group per experiment). *p <0.05, **p <0.01, ***p <0.001 versus control group by Student’s t-test.

Figure 7 15d-PGJ2-PC inhibited macrophage foam cell formation. BMDMs were cultured with 1 µM 15d-PGJ2-PC 12 hours before addition of 10 µg/ml Dil-oxLDL. 12 hours later, cells were washed, fixed and stained nuclei with Hoechst. We randomly chose 20 fields of vision and imaged in each sample by using Thermo Fisher Cellomics ArrayScan Infinity HCS system. The Dil-oxLDL signals were shown in red, Hoechst signals were blue. (A) Representative images at 200× and (B) is the quantification results. The total intensities of Dil-oxLDL (red signals) in cells were normalized to total cell numbers in total fields. BMDMs were cultured with 50 µg/ml oxLDL in the presence/absence of 1 µM 15d-PGJ2-PC for 18h. (C) Representative images at 200× of Nile red staining, and (D) is the quantification results. (E) BMDMs were cultured with 1 µM 15d-PGJ2-PC for 6h, LOX-1, SR-A and CD36 expression were analyzed by real-time PCR. (E) Protein level of p-AMPK, AMPK, p-ERK1/2 and ERK1/2 in BMDMs treated with 1 µM 15d-PGJ2-PC for different time. Results are shown as the mean ± SEM (3 biological replicates, 3 technical replicates per group per experiment). **p <0.01, versus control group by Student’s t-test.

Figure 8 Identification and quantification of deoxy-A2/J2-IsoP-PC in human plasma. (A) SRM chromatogram (m/z 778.5-315) of deoxy-A2/J2-IsoP-PC detected in human plasma (top) and 15d-PGJ2-PC chemically synthesized (bottom). (B) SRM chromatogram of free fatty acid deoxy-A2/J2-IsoPs (top) and internal standard (IS) of 15d-PGJ2-d4 (bottom). (C) Quantification results of deoxy-A2/J2-IsoP-PC level in human plasma. (D) Schematic model shows formation and biological activities of deoxy-A2/J2-IsoP-PC level in human plasma. Results are shown as the mean ± SEM. *p <0.05 versus control group by Student’s t-test.
Oxidized phospholipids deoxy-A$_2$/J$_2$-IsoP-PC and macrophages

Figure 1
Oxidized phospholipids deoxy-A$_2$/J$_2$-IsoP-PC and macrophages

Figure 2
Figure 3

Oxidized phospholipids deoxy-A$_2$/J$_2$-IsoP-PC and macrophages
Oxidized phospholipids deoxy-A2/J2-IsoP-PC and macrophages

Figure 4
Oxidized phospholipids deoxy-A$_2$/J$_2$-IsoP-PC and macrophages

Figure 5
Figure 6

Oxidized phospholipids deoxy-A2/J2-IsoP-PC and macrophages
Oxidized phospholipids deoxy-A2/J2-IsoP-PC and macrophages

Figure 7
Oxidized phospholipids deoxy-A$_2$/J$_2$-IsoP-PC and macrophages

Figure 8
Identification of a Novel Series of Anti-inflammatory and Anti-oxidative Phospholipid Oxidation Products Containing Cyclopentenone Moiety in vitro and in vivo: Implication in Atherosclerosis

Jianhong Lu, Shuyuan Guo, Xinli Xue, Qun Chen, Jing Ge, Yujuan Zhuo, Huiqin Zhong, Buxing Chen, Mingming Zhao, Wei Han, Takashi Suzuki, Mingjiang Zhu, Lin Xia, Claus Schneider, Timothy S. Blackwell, Ned A Porter, Lemin Zheng, Sotirios Tsimikas and Huiyong Yin

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