Computational Investigation of Darapladib and Rilapladib Binding to Platelet Activating Factor Receptor.  
A Possible Mechanism of Their Involvement in Atherosclerosis

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Abstract
Platelet Activating Factor (PAF), the most potent inflammatory mediator, is involved in a wide range of pathophysiological actions. PAF signal transduction is mediated through PAF receptors (PAFR) that are coupled with several isoforms of G-proteins. PAF hydrolysis is mediated through specific enzymes clustered as PAF acetylhydrolases (PAF-AH). The plasma isoform is known as lipoprotein-associated PLA2 (Lp-PLA2), and is considered a marker, or a mediator in the mechanism of atherosclerosis. Darapladib and rilapladib are selective Lp-PLA2 inhibitors. They are, thus, proposed as a novel therapeutic approach for cardiovascular disease (CVD). The data derived from the computational methods used in this paper suggest that darapladib and rilapladib are potential PAFR antagonists, predicted to bind inside the PAF-binding site with a comparable binding affinity to the endogenous agonist ($\Delta G = -11.1$ Kcal mol$^{-1}$). Darapladib ($\Delta G = -10.6$ Kcal mol$^{-1}$) exhibited a higher affinity than rilapladib ($\Delta G = -8.2$ Kcal mol$^{-1}$). The fact that darapladib down-regulates PAFR expression, while PAFR inhibitors down-regulate the expression of CD36, could be the biochemical explanation in the observed necrotic core reduction, both in animals and humans. The reported results in conjunction with bibliographical data lead to the hypothesis that the involvement of darapladib and rilapladib in atherosclerosis could be through direct inhibition of PAF activity as well as modification of PAF metabolism.

Keywords: platelet activating factor receptor, darapladib, rilapladib, atherosclerosis, molecular docking calculations

1. Introduction

1.1 Platelet Activating Factor

1.1.1 Platelet Activating Factor General Data
Platelet Activating Factor (PAF), a phosphoglycercylether lipid (Scheme 1), is the most potent inflammatory mediator involved in a wide range of pathophysiological actions. PAF is a considered a cell-to-cell messenger acting both intercellular and intracellular (Antonopoulou, Nomikos, Karantonis, Fragopoulou, & Demopoulos, 2008). Even though the majority of ether lipids have been replaced with their esterified analogues during evolution, PAF and some minor phosphoglycercylether lipids were conserved in various organisms due to their important biological roles (Kulikov & Muzya, 1997). While the term PAF was initially attributed to 1-$O$-alkyl-2-acetyl-$sn$-glycero-3-phosphocholine (Demopoulos, Pinckard, & Hanahan, 1979), today it is clear that PAF is a member of a large family or structurally related phospholipids with similar pathophysiological activities. These molecules are produced due to enzymatic and chemical oxidation and their main common structural feature is the short chain at sn-2 position (Montrucchio, Alloatti, & Camussi, 2000).

1.1.2 Platelet Activating Factor Metabolism

PAF is synthesized by two distinct pathways, namely the “remodeling” and “de novo” (Snyder, 1995), while it is hydrolyzed by PAF acetylhydrolase (PAF AH). PAF AH is a PLA2 belonging to groups VII and VIII. The plasma
isoform, also known as lipoprotein-associated PLA2 (Lp-PLA2, EC 3.1.1.47), has been classified as group VIIA PLA2 (PLA2G7), is calcium independent and circulates bound with LDL and HDL. Two intracellular PAF AHs, namely PAF AH Ib and PAF AH II, have been also characterized (Snyder, 1995; Tselepis & John Chapman, 2002). Increased Lp-PLA2 activity is associated with increased risk of cardiac events, but it is not known whether Lp-PLA2 is a causative agent.

1.1.3 Platelet Activating Factor Receptor

PAF signal transduction is mediated through PAF receptors (PAFR). PAFR is coupled with several isoforms of G-proteins. The type of cell and ligand determines the G-protein isoform(s) activated by PAFR each time. The ability of PAF to stimulate distinct signaling pathways via multiple G-proteins may explain the diverse biological responses of human cells to it (Ishii & Shimizu, 2000; Honda, Ishii, & Shimizu, 2002).

PAFR are “serpentine receptors”, with a seven a-helical domains that wave in and out of the plasma membrane seven times. PAFR have been found in all the blood cells and several tissue and organ cells. Soon after the cloning of PAFR, the expression levels of PAFR mRNA in various tissues and organs were determined.

The internalization and desensitization of PAFR is regulated through a phosphorylation site consisted of serine and threonine residues in the C-terminal cytoplasmic tail. PAFR is modified posttranslationally by disulfide bonding at C90-C173 and glycosylation at N169, and modifications are necessary for the surface exposure of PAFR (Prescott, Zimmerman, Stafforini, & McIntyre, 2000; Honda et al., 2002). PAF and PAF-like lipids bearing a short oxidized acyl chain at the sn-2 position readily bind to PAFR, but PAFR also interacts with components of the bacterial wall, such as lipopolysaccharides (LPS) (Nakamura et al., 1992) and phosphorylcholine (Cundell, Gerard, Gerard, Idanpaan-Heikkila, & Tuomanen, 1995). These interactions are thought to be an alternative recognition system for innate immunity inducing inflammatory responses to the immune cells.

In humans, the PAFR mRNA is most abundant in neutrophils, monocytes, placenta, lung, dendritic cells and endothelial cells and is the same isoform for all the cell types. The PAFR was initially cloned from the guinea-pig lung by functional expression in Xenopus laevis oocytes. Subsequently, the cloning of human, rat, mouse, porcine, bovine and caprine PAFR was reported (Ishii & Shimizu, 2000; Honda et al., 2002).

1.1.4 Platelet Activating Factor and Atherosclerosis

PAF interplays in critical stages of atherogenesis including thrombosis, inflammation and oxidation. PAF promotes oxidation by stimulating human monocytes/macrophages and neutrophils to produce superoxide anions and hydrogen peroxide that cause LDL oxidation, creating a positive feedback effect, as PAF itself is produced during LDL oxidation. Lp-PLA2 protects LDL against the production and activity of Ox-LDLs by facilitating hydrolysis of PAF-like lipids (Demopoulos, Karantonis, & Antonopoulou, 2003).

In vitro studies involving PAF-like lipids that are mostly fragmented and/or oxidized sn-2 fatty acyl groups and their hydrolysis products, have shown that these molecules can act both as pro- and anti-inflammatory mediators (Berliner, Leitinger, & Tsimikas, 2009; Feige, Mendel, George, Yacov, & Harats, 2010). These studies though do not take into consideration the fact that lyso-PC and free fatty acids are associated with lipoproteins and other plasma carriers making it difficult to calculate their exact concentration and bioavailability in plasma (Öörni & Kovanen, 2009; Rosenson & Stafforini, 2012). These results are further weakened by the fact that many of the observations for lyso-PC can be attributed to contaminating traces of PAF remaining in the lyso-PC preparations used in the studies (Marathe et al., 2001), and the fact that there is no evidence for a specific lyso-PC receptor.

Several PAF agonists/antagonists have been synthesized and isolated from natural sources (Antonopoulou et al., 2008). Some of them are isolated from Mediterranean foods and when co-administered with cholesterol in animals, were able to significantly reduce the amount of esterified cholesterol in aorta without affecting cholesterol plasma levels, and reduce PAF-induced early atherogenesis (Nomikos, Fragopoulou, & Antonopoulou, 2007), as well to cause regression of the existing plaques.

Darapladib and rilapladib (Scheme 1) are selective Lp-PLA2 inhibitors and produce sustained inhibition of plasma Lp-PLA2 activity. Darapladib is a potent, freely reversible, inhibitor of human Lp-PLA2, with an inhibition constant $K_i$ of 0.11 nM (Blackie et al., 2003). Rilapladib is also a potent and reversible inhibitor of human Lp-PLA2 with half maximal inhibitory concentration (IC$_{50}$) in the range of 0.1 to 10 nM (Patent publication numbers: WO2012080497 A2, WO 2012080497 A3 and US20130267544 A1).

Initially darapladib was tested in vivo in atherosclerosis animal models with promising results, as the medication reduced plaque and necrotic core area in swine (Wilensky et al., 2008), and plaque area and inflammatory burden in mice (Hu et al., 2011; Wang et al., 2011). These results suggest that the drug could move on to Phase II
clinical trials. Darapladib reduced plasma Lp-PLA2 activity dose dependently in patients with stable CHD on atorvastatin, and also reduced IL-6 and CRP levels by 12.3 and 13%, respectively, on the highest dose tested (160 mg) compared with placebo (Mohler III et al., 2008).

The IBIS-2 (Integrated Biomarker and Imaging Study) found an inhibition of Lp-PLA2 in the darapladib treated group by 59% while no effect was observed on coronary atheroma deformability and plasma hs-CRP levels. The most interesting result of the study was the prevention of necrotic core expansion in the darapladib treated group compared with the standard-of-care treated patients in control group (Serruys et al., 2008). Therefore, darapladib has been proposed as a novel therapeutic approach for cardiovascular diseases (CVD).

Darapladib is currently being tested in two large randomized, placebo-controlled, double-blind, international, multicenter, event-driven trials. The STABILITY trial (Stabilization of Atherosclerotic Plaque by Initiation of Darapladib Therapy) compares the effect of darapladib and placebo on cardiovascular events in 15,828 patients with chronic coronary heart disease (CHD) (White et al., 2010), while SOLID-TIMI 52 (Stabilization of plaques using darapladib thrombolysis in myocardial infarction) investigates the effects of darapladib in 13,000 subjects, randomized to darapladib or matching placebo within 30 days of hospitalization with an acute coronary syndrome (O'Donoghue et al., 2011).

This work is an attempt to explain a possible mechanism of action and the implication of darapladib and rilapladib in atherosclerosis using molecular modeling in combination to literature data.

![Scheme 1. Structures of PAF, darapladib and rilapladib](image)

2. Computational Methods

Molecular docking calculations were carried out in order to investigate the role of darapladib and rilapladib as potential PAF antagonists. The structural model of PAFR was constructed based on homology modelling following the procedure described by Gui et al. (2007). The crystallographic structure of bovine rhodopsin (PDB ID: 1L9H) (Palczewski et al., 2000) was used as template and the final model was refined as described in (Gui et al., 2007; Tsoupras, Papakryiakou, Demopoulos, & Philippopoulos, 2013). For the modelling of Lp-PLA2 complexes, the crystal structure of human plasma platelet-activating factor acetylhydrolase (PDB IDs: 3D59) were employed (Samanta & Bahnson, 2008). Hydrogen atoms were added and the parm99SB force field parameters were applied using the XLeaP module of AMBER 10 (Case et al., 2005; Hornak et al., 2006). Acidic and basic residues were modelled in their ionized state, whereas all histidines were set to their neutral state. To optimize the hydrogen atoms’ position, energy minimization was performed in implicit solvent with positional restraints of 100 Kcal mol⁻¹ Å⁻² on protein heavy atoms. For the docking calculations non-polar hydrogen atoms were removed and Gasteiger charges were applied using AutoDockTools 1.5 (Sanner, 1999). The 3D models of PAF, darapladib and rilapladib were obtained from their SMILES representation using OMEGA 2.3 (Hawkins,
Skillman, Warren, Ellingson, & Stahl, 2010) and they were also assigned the Gasteiger charge set. Docking calculations were performed using AutoDock4.2 and the Lamarckian genetic algorithm (Huey, Morris, Olson, & Goodsell, 2007). The search space for PAFR was defined by a grid box centred at Pro162 with 80 points of 0.375 Å spacing in each dimension, so that is large enough to cover both the PAF-binding site and the extracellular domain of the receptor. For the modelling of Lp-PLA2, a similar grid box in size and resolution was centred at Ser273. Each calculation comprised 100 docking runs with default parameters (Morris et al., 1998), except for the energy evaluations that were set to 5 millions. The resulting conformations were clustered within a 2.0-Å tolerance and the most populated cluster with the highest free energy of binding was selected. Calculations were performed on an x86-64 quad-core workstation running Linux kernel 2.6.29 and VMD 1.9 was used for structural analysis and preparation of the figures (Humphrey, Dalke, & Schulten, 1996).

3. Results

3.1 Molecular Modeling

The high resolution (1.5 Å) crystal structure of the ligand-free Lp-PLA2 in conjunction with x-ray structures of covalent complexes with organophosphate compounds, Samanta and Bahnson (2008) and Samanta, Kirby, Srinivasan, Cerasoli, and Bahnson (2009) provided a comprehensive working model of how plasma PAF-AH binds to lipoproteins and catalyzes the hydrolysis of PAF and oxidized phospholipids. To gain insight into the binding mode of darapladib and rilapladib, molecular docking calculations using the crystal structure of Lp-PLA2 (PDB ID: 3D59) have been employed. However, prediction of their interactions is a challenging computational task for two reasons: (a) the high flexibility of darapladib and rilapladib ligands (15 and 13 rotatable torsions, respectively), and (b) the large conformational space provided by the open channel of Lp-PLA2 that exposes its active site to the solvent (Figure 1). For this reason, such docking calculations result in a high number of distinct conformational clusters (in this case 60–80 out of 100 docking runs, clustered within a 2-Å tolerance), with free energies of binding ranging from approximately −10 to −5 Kcal mol⁻¹. The binding modes of the two inhibitors shown in Figure 1 were selected amongst the top-ranked binding modes, after visual inspection of their residue-specific interactions with the active site of Lp-PLA2. In a potential binding mode, darapladib is predicted to interact with the catalytic residues, Ser273 and His351 via two hydrogen bonds, in addition to a hydrogen bond with Tyr160 (Figure 1B). The fluorophenyl ring of darapladib is stacked with Trp298 and the N-diethylamino ethyl moiety exhibits hydrophobic interactions with Phe110 and Leu111. The trifluoromethyl biphenyl rings of darapladib follows the direction of the long alkyl chain of PAF towards the hydrophilic–hydrophobic interface as predicted for the PAF-AH model with C₁₈-PAF (Samanta & Bahnson, 2008). An analogous model of rilapladib complex with Lp-PLA2 is shown in Figure 1C, although our calculation did not exhibit specific hydrogen bonding interactions with the catalytic residues unambiguously.

Figure 1. Molecular models of Lp-PLA2 complexes with darapladib and rilapladib. (A) Darapladib docked to the crystal structure of human plasma platelet-activating factor acetylhydrolase, also referred to as lipoprotein-associated phospholipase A2 (PDB ID: 3D59). The inhibitors are shown with green sticks for C atoms, red for O, blue for N, yellow for S and pink for F. (B) Close-up view of the active site showing darapladib-interacting residues as sticks with brown C atoms. The catalytic triad of Ser273, His351 and Asp296 is highlighted with light brown color and the potential hydrogen-bonding interactions are shown with dashed lines. (C) Rilapladib–Lp-PLA2 model in the same orientation as shown in (A)
Concerning the interaction of the two inhibitors with the receptor of PAF, and in the absence of an experimental structure, a reasonable model of PAFR was prepared by homology modelling based on the x-ray crystallographic structure of bovine rhodopsin (Palczewski et al., 2000). The homology models of PAFR were refined using unconstrained molecular dynamics simulations in dipalmitoylphosphatidylcholine (DPPC) lipid bilayer, as described previously (Gui et al., 2007; Tsoupras et al., 2013). The calculated conformation of PAF bound to the ligand-binding pocket of PAFR shown in Figure 2A is consistent with that described previously, Gui et al. (2007) and Tsoupras et al. (2013) exhibiting the substrate’s polar head occupying the central core region, and the O-alkyl 16-carbon chain extending along the hydrophobic channel of the binding site (Figure 2B). In particular, the phosphate group of PAF is hydrogen bonded with His248 and His249 imidazole rings, as well as with the phenolic group of Tyr102. The positively charged trimethylethanaminium moiety of phosphocholine group exhibits electrostatic interactions with Glu175 and His188, in addition to the hydrophobic contacts with Phe98 and Phe152. The acetyl group of PAF might form a hydrogen bond with the main chain amide nitrogen of Phe98 and provide aliphatic π interactions with the sidechain of Phe174. Finally, the C-16 aliphatic chain is folded within the hydrophobic channel comprising Ile25, Phe26, Phe66, Leu71, Leu70, Ile74, Phe97, Leu279, and Leu282, as well as the side chain groups of Tyr22, Pro71, Thr171 and Arg172 that provide additional hydrophobic contacts.

![Figure 2](image-url)

**Figure 2.** (A) Molecular model of PAFR showing the ligand-binding site with docked PAF as van der Waals spheres. (B) Close-up view of the PAF-binding site indicating the interacting residues at the polar and the hydrophobic sites. (C) Predicted bound conformations of darapladib and (D) rilapladib. The ligands are shown with green sticks for C, while the interacting residues of PAFR are shown with light brown sticks. All other atom colors are blue for N, red of O, yellow for S and purple for F.

Docking of darapladib to the same site of PAFR revealed a similar binding mode, with the 4-trifluorobiphenyl moiety of the drug accommodated inside the hydrophobic channel (Figure 2C). Importantly, the darapladib exhibits contacts with all three histidine residues (His188, His248 and His249) that were shown to be critical for binding of PAF to its receptor (Ishii et al., 1997). More specifically, the side chains of His248 and His249 could potentially form hydrogen bonds with the 4-oxo-6,7-dihydro-5H-cyclopenta[d]pyrimidinyl ring of darapladib, which exhibits also hydrophobic contacts with Tyr102, Phe245 and Leu282. The 4-fluorophenyl ring and the diethylaminoethyl group of darapladib are predicted to occupy the same pocket that accommodates the choline and acetyl moieties of PAF. The 4-trifluoromethyl biphenyl group of darapladib extends inside the hydrophobic channel up to Tyr22 that provides an edge-to-face halogen aromatic interaction with the -CF₂ group (Figure 2C).

In a similar way, rilapladib is predicted to bind within the ligand-binding domain of PAFR (Figure 2D), albeit with a lower estimated free energy of binding (ΔG = −8.2 Kcal mol⁻¹) with respect to darapladib (ΔG = −10.6 Kcal mol⁻¹). For comparison, the equilibrium dissociation constant for PAF, which was determined using tritiated PAF on rabbit platelet plasma membranes (Hwang, Lee, Cheah, & Shen, 1983), is K_d = 1.36 × 10⁻⁸ M at 0 ºC. Therefore, the corresponding free energy of binding for its receptor is ΔG = −11.1 Kcal mol⁻¹, using the equation:
\[ \Delta G = R \ln K_d, \]  
where \( R = 1.987 \text{ cal K}^{-1} \text{ mol}^{-1} \) and \( T = 273 \text{ K} \). Visual inspection of the lowest energy and highest populated model of PAFR–rilapladib complex (Figure 2D) revealed that its lower affinity could be attributed to the lack of interaction between the oxoquinolinyl ring and His248/His249 residues. In such a configuration, the position of the phosphate group of PAF is occupied by the methoxyethyl piperidinyl moiety of rilapladib, which provides no hydrogen bonding interactions with either His248 or His249. The oxoquinolinyl ring is hydrogen bonded with Tyr177 and is buried inside the same site of PAF’s choline moiety. The 2,3-difluorophenyl ring of rilapladib is stacked above the oxoquinolinyl moiety interacting with Phe98 and Phe152. Finally, the 4-trifluoromethyl biphenyl group is buried a bit deeper inside the hydrophobic channel with respect to the corresponding moiety of darapladib, with the -CF₃ group at the same position of C-10 of PAF’s aliphatic chain.

4. Discussion

4.1 Interaction of Darapladib and Rilapladib With Lp-PLA2 and PAFR

Taken together, the above results suggest that darapladib may be a potential PAFR antagonist, which is predicted to bind inside the PAF-binding site with a comparable binding affinity (\( \Delta G = -10.6 \text{ Kcal mol}^{-1} \)) with respect to the endogenous agonist (\( \Delta G = -11.1 \text{ Kcal mol}^{-1} \)). Similarly, rilapladib could also serve as a PAFR antagonist, albeit with lower binding affinity (\( \Delta G = -8.2 \text{ Kcal mol}^{-1} \)). The predicted binding modes of both inhibitors with either Lp-PLA2 or PAFR indicate that their trifluoromethyl biphenyl moiety is probably interacting within the same binding sites that accommodate the long alkyl chain of PAF, whereas the pyrimidinyl and oxoquinolinyl rings (of darapladib and rilapladib, respectively) interact with the catalytic residues of Lp-PLA2 and the hydrophilic PAF-binding site.

4.2 Lp-PLA2 and PAF Involvement in Atherosclerosis

There is a considerable amount of data reporting Lp-PLA2 as a marker of the risk of coronary heart disease (CHD), with controversial results. Even though Lp-PLA2 tends to be considered an independent marker for CVD, recent studies fail to establish its association with CVD event in apparently health subjects and in patients treated with statins that have their cholesterol levels managed (Rosenson & Stafforini, 2012).

According to our proposed theory where PAF is the initial cause of atherosclerosis and plaque formation (Demopoulos et al., 2003), and the literature data, darapladib and rilapladib are likely to inhibit/reduce atherosclerosis and its development process (therefore all its harmful consequences) also through the inhibition/reduction of PAF effects.

The deposition and binding of LDL-Cholesterol in the subintimal space is considered a key factor for the initiation and development of the atherosclerosis. After their binding to proteoglycans the LDL-Cholesterol are oxidatively modified (ox-LDL) resulting in high concentrations of really potent inflammatory molecules, like PAF and PAF-like lipids. These molecules act through the specific PAF receptor present in almost all the cells involved in atherosclerosis like smooth muscle cells, cardiomyocytes, neutrophils, monocytes-macrophages, eosinophils, and Kupffer cells. It has also been shown that endothelial cells, express PAFR not only on the cell surface, but also in the large endosomal compartment (Montrucchio et al., 2000; Antonopoulou et al., 2008).

In addition, we and others have published (Nomikos, Fragopoulou, & Antonopoulou, 2007) that the PAF-inhibitors usually inhibit the key-PAF biosynthetic enzymes, and, additionally, either inhibit or activate PAF AH, the key-PAF catabolic enzyme.

4.3 Darapladib, Rilapladib and PAF Biological Activity as Well as PAF Levels

Therefore, darapladib and rilapladib are likely to inhibit/reduce atherosclerosis and its development process (therefore all its harmful consequences) through the inhibition/reduction of PAF effects, but also through inhibition of PAF biosynthesis that leads to reduced PAF levels, as do statins (Tsantila et al., 2011). Unfortunately there are no in vitro experimental data concerning the regulation of the two major PAF biosynthetic enzymes by darapladib and rilapladib that could help us understand the mechanism of PAF levels regulation.

On the other hand darapladib and rilapladib are potent specific inhibitors of Lp-PLA₂, which is considered the main PAF degrading enzyme. The effect of darapladib on PAF levels has only been studied in mice by two different research groups. Hu et al. showed that darapladib reduced plaque area in LDL receptor (LDLR) deficient mice, without affecting the lipid profile or PAF levels of the mice. The intervention reduced as expected Lp-PLA2 activity along with CRP and IL-6 levels and the expression of the inflammatory genes of MCP-1 and VCAM-1 (Hu et al., 2011). The same results were obtained by Wang et al. in Apo-E deficient mice that also had decreased macrophages’ content and increased collagen content in the lesions of the darapladib group (Wang et al., 2011).
These controversial results can be explained by suggesting that PAF hydrolysis is not mediated only through Lp-PLA2. The PAF clearance was measured in mice and showed that the majority of the PAF molecules were hydrolyzed in liver and kidney by the intracellular PAF AH, after being transported as intact molecules. The fact that mice have an 8.6 times increased enzymatic activity relative to humans indicates that this is probably the PAF clearance pathway also in humans (Liu et al., 2011).

These observations give rise to the critical question of whether and to what extent Lp-PLA2 is responsible for the clearance of the other highly inflammatory PAF-like molecules present in oxLDL that have a smaller affinity to the enzyme compared to PAF, but are in much higher concentrations (Markakis et al., 2010).

4.4 Possible Actions of Darapladib and Rilapladib in Aorta, That Are Explained by PAFR Inhibition

One critical aspect of the in vivo darapladib administration to diabetic and hypercholesterolemic swine, which is not discussed by the authors, is the down-regulation of PAFR expression by 49% compared to control group not receiving the drug (Wilensky et al., 2008).

It is known that the expression of CD36 is directly associated with the uptake of oxLDL by macrophages (Febrario & Silverstein, 2007; Rios, Gidlund, & Jancar, 2011) and this uptake is not regulated by intracellular levels of cholesterol, leading to continuous uptake of oxLDL and the differentiation of macrophages into foam cells. The oxLDL molecules increase CD36 expression, exerting a positive feedback effect on the expression of its receptor in human and mouse monocytes/macrophages (Feng et al., 2000; Rios, Jancar, Melo, Ketelhuth, & Gidlund, 2008), an effect that is reversed by PAFR antagonists. Moreover treatment of LDL receptor-deficient mice with PAFR antagonists reduced the formation of fatty streaks lesions (Subbanagounder, Leitinger, Shih, Faull, & Berliner, 1999).

PAFR down-regulation with a possible antagonistic effect from darapladib and rilapladib can give an explanation to the reduction of the necrotic core in darapladib and rilapladib treated animals and humans. The mechanism can involve the reduction of oxLDL uptake from macrophages through CD36, thus inhibiting extensive foam cell formation and subsequently a smaller necrotic core.

4.5 PAF Levels and PAF AH, Lp-PLA2 Expression

Several studies have shown that PAF and PAF inhibitors levels can affect PAF AH expression. LPS and PAF stimulate expression of PAF AH via distinct signaling pathways (Howard, Abdel-Al, Ditmyer, & Patel, 2011). In another study involving human non-adherent monocyte–macrophage cells (Mono–Mac 6; MM6) it was found that both PAF and LPS were able to up-regulate the expression of PAF AH in a dose dependent manner. The specific PAFR inhibitor WEB2170 was able to completely block the PAF stimulated up-regulation of PAF AH and also inhibited the PAF AH production in the tested cell line but also in rats (Howard & Olson, 2000), after LPS stimulation. The p38 MAPK inhibitor, SB203580 inhibited by 60% the up-regulation of PAF AH after LPS stimulation while PAF stimulation was not affected. The co-administration of WEB2170 and SB203580 completely abolished PAF AH expression, indicating that the LPS-induced PAF AH mRNA levels present after SB203580 administration are the result of autocrine activation of the PAF receptor due to LPS-stimulated production of PAF, or the fact that LPS acts through the PAFR.

4.6 The PAFR Inhibition by Darapladib and Rilapladib Hypothesis, Concerning Hydrolysis Products of Lp-PLA2

From the above it could be formulated in more detail the aforementioned hypothesis concerning the harmful consequences of the hydrolysis products of Lp-PLA2 as follows:

It is well-known that the general body’s response is to increase the levels of Lp-PLA2 when the levels of PAF are increased (i.e. PAF affects the gene expression of Lp-PLA2), as shown in a recent study involving 150 patients with CHD and 120 controls that found a strong positive relationship between elevated plasma PAF or Lp-PLA2 levels and the risk of CHD. This study also provided evidence that there was a strong correlation between plasma PAF and Lp-PLA2 levels, and between plasma PAF and Lp-PLA2 and inflammatory factors IL-6 and hs-CRP levels (Zheng et al., 2012).

So it could thus be suggested that:

a) Darapladib and rilapladib through reduction of the PAF biological activity (as PAF inhibitors) and PAF levels (as possible inhibitors of PAF biosynthesis) could reduce Lp-PLA2 biosynthesis and prevent the possible adverse effects of Lp-PLA2 (the pro-inflammatory molecules, such as LPC and oxNEFA), and

b) In addition, darapladib and rilapladib inhibit also the effects of the existing Lp-PLA2 and so the harmful consequences of the LPC and the oxNEFA.
5. Conclusion

In conclusion, the combination of computational modeling data presented in this paper with the bibliography on PAFR inhibitors and their effect on atherosclerosis, recommend that more experiments should be done in order to clarify our hypothesis that the implication of darapladib and rilapladib in atherosclerosis is through the inhibition of PAF actions, but moreover through the modification of PAF metabolism. These experiments must include measurement with basic commonly accepted methodology of the effect of darapladib and rilapladib first on 

\textit{ex-vivo} models of washed platelet aggregation and second on the \textit{in vitro} PAF biosynthetic enzymes.

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