Platelet-Activating Factor Inhibitors as Therapeutics and Preventatives

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Platelet-activating factor (PAF) refers to the classical structure reported in 1979, which is a pro-inflammatory phospholipid mediator. PAF mediates a wide variety of cellular functions and cell–cell interactions.

platelet-activating factor | inflammation | cardiovascular disease | cell signalling | phospholipids

1. Platelet-Activating Factor

Since its discovery, the structure of platelet-activating factor (PAF) also known as PAF-acether or AGEPC (acetyl-glyceryl-ether-phosphorylcholine) has been identified as a phosphoglycerylether lipid mediator involved in diverse physiological and pathophysiological processes. It seems apparent that PAF has different physiological roles in animals, plants, and monocellular organisms. It is considered the most potent lipid mediator known to date [1][2]. Previous to the 1970s, lipid mediators were thought to be generally derived from phospholipids. However, PAF was the first intact phospholipid mediator to demonstrate autacoid or messenger functions [3]. PAF was initially considered one molecule, which is commonly referred to as the classical PAF. However, now it is understood that there are a large number of structurally related phospholipids or PAF analogues that are dissimilar in structure to PAF that interact with the PAF-receptor (PAF-R) and belong to the ‘PAF family’, collectively known as PAF-like lipids (PAFLL). For the purpose of this review, PAF refers to the classical structure reported in 1979, which is responsible for most of the known biological effects and is thought to be the most potent PAF molecule. PAF mediates a wide variety of cellular functions and cell–cell interactions. Therefore, PAF is involved in several physiological processes including apoptosis, physiological inflammation, wound healing, reproduction, angiogenesis, long-term potentiation, and potentially retrograde signaling [4][5][6][7]. However, PAF is also a potent pro-inflammatory mediator that is implicated in a variety of conditions and chronic diseases such as cancer, renal diseases, cerebrovascular and central nervous system disorders, allergies, asthma, infections, and cardiovascular diseases (CVD) [5][8][9][10][11][12][13]. PAF is known to carry out its broad pathophysiological actions at concentrations as low as 10^{-12} M and almost always by 10^{-9}M as an intercellular messenger [14]. In evolutionary terms, many ether lipids were replaced over time by their esterified analogues; however, PAF and other minor phosphoglycerylether molecules were conserved in various organisms due to their important biological roles [15].

2. The Discovery and Structural Elucidation of the Platelet-Activating Factor

2.1. The Discovery of the Platelet-Activating Factor

PAF was first introduced into the literature in 1966 when Barbaro and Zvaifler described a substance that caused antigen induced histamine release from rabbit platelets producing antibodies in passive cutaneous anaphylaxis [16]. Almost four years later, Henson described a 'soluble factor' released from leukocytes that induced vasoactive amine release in platelets. Further observations by Siraganuan and Osler [17] described the existence of a diluted substance that had the capacity to cause platelet activation. A year later Jacques Benveniste and colleagues elaborated on the findings of the previous two studies and described a novel factor that induced aggregation and secretion of platelets, which participated in a leukocyte-dependent histamine release from rabbit platelets [18]. Hence, the term platelet-activating factor (PAF) was coined because of the initial observations of its effects on platelets [18]. It was later discerned that PAF was a lipid-like molecule [19]. It is recalled that to study PAF Benveniste prepared a measure of PAF from 100 L of hog blood, which resulted in a 100 L solution from which 1 µL was sufficient to induce platelet aggregation, indicating its high level of potency [20]. However, this amount of PAF was too low to use techniques at the time such as mass spectrometry or magnetic resonance that might determine the structure of the bioactive compound [20]. Despite the lack of structural data, Benveniste and others had determined several of the physical characteristics of PAF. They determined that it was a lipid compound, it could bind to albumin, and it migrated between lysolecithin and sphingomyelin in thin-layer chromatography separation, all properties of which were similar to that of lysophosphatidylcholine. The compound was also affected by several phospholipases (PLA_2, PLC, and PLD) but resistant to others (sphingomyelinase C and PLA_1), indicating that indeed it had a phospholipid type structure [20][21]. Studies began to discern that PAF was implicated in IgE anaphylaxis [22] and many of the properties of PAF released during IgE anaphylaxis began to be elucidated [23]. Furthermore, the role of PAF in platelet aggregation was beginning to be further understood by June 1979 [24].

2.2. Structural Elucidation of the Platelet-Activating Factor

Following several experiments with phospholipases, etc. the structure of PAF was thought to be 2-acyl-*sn*-glycero-3-phosphocholine (1-lysophosphatidylcholine) [25], but owing to acyl chain migration this molecule was known for its instability and did not demonstrate the biological properties corresponding to PAF [20][26]. Around that time, several other structures were interrogated, and many researchers were involved in discussions as reviewed by Chap [20]. However, on the 10th of October 1979, Constantinos Demopoulos, Neal Pinckard, and Donald Hanahan, from San Antonio Texas published the structure of PAF (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) under the name AcGEPC (Acetyl-glyceryl-ether-phosphocholine), which was shown to have biological activities indistinguishable from that of naturally generated rabbit PAF (**Figure 1**) [27]. The researchers realised that the AcGEPC they synthesised was indeed the same structure as naturally occurring PAF. Interestingly, nineteen days after the Demopoulos, Pinckard, and Hanahan [27] publication, the same structure was reported by a group led by Fred Snyder who were assessing the properties of an isolated compound in the kidney that was responsible for peculiar biological activity, which was known by them as the antihypertensive polar renomedullary lipid (APRL) [28]. These studies were followed by an article by Benveniste who subsequently proposed the name PAF-acether [29]. Later articles confirmed that synthetically produced PAF initiated identical biological effects to the PAF molecules responsible for IgE-induced systemic anaphylaxis [30], which also caused similar vascular, cardiovascular, and

respiratory problems associated with anaphylaxis in rabbits [31] and baboons [32]. In addition, platelets were not required to induce anaphylactic shock in rabbits when injected with synthetic PAF, indicating for the first time that PAF acts via a receptor [33].

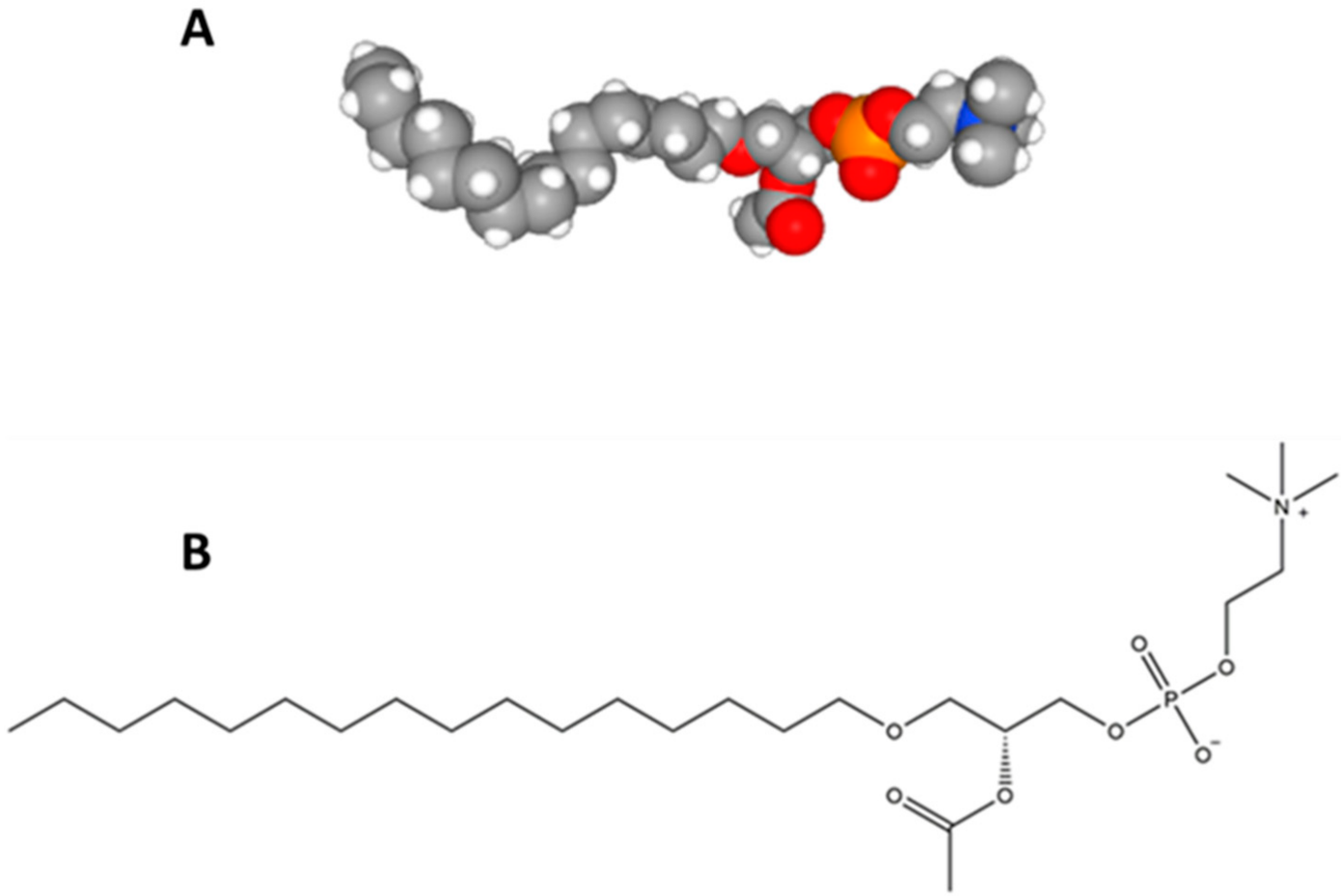

Figure 1. The structure of platelet-activating factor (PAF): (**A**) PAF space fill model data from [34] and (**B**) PAF structural model.

Hanahan and colleagues formally confirmed the structure of PAF in 1980 using mass spectrometry and simplified their abbreviation of the molecules name to AGEPC [35]. Likewise, Benveniste and colleagues simplified the name of the PAF precursor to lyso-PAF [36]. As many researchers were working with PAF at the same time, it is reported that there were conflicting attitudes between the groups with reference to what the name of the molecule should be. Furthermore, Chap described the difficulty encountered by Benveniste who was unfortunate not to have elucidated the structure of PAF previous to the other groups [20]. Considering that we now know PAF exhibits a vast diversity of actions and the fact that a myriad of other molecules can activate platelets, it seems ironic that the name PAF is a misnomer [37] that has remained in the literature.

However, that was not the end of Benveniste's role in determining some of the properties of PAF. Indeed, Benveniste and colleagues provided the first evidence that platelets synthesise PAF [38] and they determined the subcellular localisation of PAF biosynthesis in human neutrophils [39]. However, Benveniste's important role in the

discovery of PAF may be overshadowed by his later controversial research that led to major scientific scandals [20][40][41] that are not the subject of this review. The very first account of the discovery of PAF and its various properties was published in Nature in 1980 by Cusack [42]. The intensive and dedicated research of many scientists involved in the discovery and structural elucidation of PAF in the 70s and 80s set in motion a research field that is ever growing to this day, which has had profound implications to medical research.

3. The Importance of Platelet-Activating Factor Research

PAF is implicated in various physiological processes and a multitude of pathophysiological processes. However, the critical feature of PAF physiologically and in disease is that the biological effects of PAF can be modulated by diet, lifestyle, and environmental factors [5][43][44][45][46]. This means that PAF could be a potential therapeutic target for many chronic diseases [5][8][10] and thus PAF is of significant importance and value to researchers across several disciplines. While this review discusses many of these events, not all of PAF's roles are discussed due to the vast accumulation of research published around PAF in the last forty years. In the last two years alone there has been over 2000 articles published in relation to PAF. This review specifically focuses on some of the emerging PAF-related research trends over the last decade. In particular, this article discusses the most contentious issues of PAF research such as the role of the PAF metabolic enzymes in physiological and inflammatory processes and the role of PAF in various chronic diseases, such as disorders of the central nervous system (CNS), CVD, and cancer. These diseases have major health implications for patients and are an enormous burden to healthcare globally. Indeed, some of the research highlighted in this article may lead to ground-breaking discoveries that enhance our understanding of cell signaling, inflammation, and disease.

After the elucidation of the structure of PAF in 1979, there was much motivation in the development of research in the field that from 1983 lead to several congresses being organised entirely focused on PAF research. These congresses were held every three years worldwide until 2004 (**Table 1**). After 21 years, PAF research became interdisciplinary and grew and expanded to virtually all areas of biochemistry and medicine. The congresses stopped being organised as much of the research surrounding PAF were disseminated at various international conferences. However, attempts have been made to reignite these congresses as recent as February 2015 in Tokyo Japan, where PAF communications were presented in special sessions at the '6th International Conference on Phospholipase A_2 and Lipid Mediators' [47].

Table 1. International conferences of platelet-activating factor (PAF).

Title	Date	Location
1st International Symposium on Platelet-Activating Factor and Structurally Related Ether-Lipids	26–29 June 1983	Paris, France

Title	Date	Location
2nd International Conference on Platelet-Activating Factor and Structurally Related Ether-Lipids	26–29 October 1986	Gatlinburg, Tennessee, USA
3rd International Conference on Platelet-Activating Factor and Structurally Related Ether-Lipids	8–12 May 1989	Tokyo, Japan
4th International Congress on Platelet-Activating Factor and Related Lipid Mediators	22–25 September 1992	Snowbird, Utah, USA
5th International Congress on Platelet-Activating Factor and Related Lipid Mediators	12–16 September 1995	Berlin, Germany
6th International Congress on Platelet-Activating Factor and Related Lipid Mediators	21–24 September 1998	New Orleans, Louisiana, USA
7th International Congress on Platelet-Activating Factor and Related Lipid Mediators	24–27 September 2001	Tokyo, Japan
8th International Congress on Platelet-Activating Factor and Related Lipid Mediators	6–9 October 2004	Berlin, Germany
6th International Conference on Phospholipase A_2 and Lipid Mediators	10–12 February 2015	Tokyo, Japan

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2. Demopoulos, C.A. Biological activity of lipids of pine pollen on platelet aggregation in correlation with the platelet activating factor. In Proceedings of the second international conference on platelet activating factor and structurally related alkyl ether lipids, Gatlinburg, TN, USA, 26–29 October 1986.
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4. The Potential Use of Platelet-Activating Factor Inhibitors as Therapeutics and Preventatives of Disease

Research into potential physiological and therapeutic ways of suppressing PAF activity demonstrated that endogenous or ingested PAF inhibitors could inhibit the actions of PAF [10][48]. Endogenous inhibitors of PAF have been identified in humans [49], many of which were identified as cardiolipins [50][51]. As a consequence of discovering that the body circulated PAF antagonists, it was thought that the absence of circulating antagonists could result in increased PAF activity [5]. Therefore, the potential role of PAF inhibitors in disease prevention and

treatment has been of significant interest over the last three decades. Indications in the early 1980s demonstrated that PAF release from leukocytes could be modulated pharmacologically [52]. This was followed by studies using pharmacological compounds such as ticlopidine and calmodulin to study PAF-induced platelet aggregation [53][54]. At that time it was also shown that methanolic extracts of garlic bulbs exhibited inhibition of various platelet agonists including PAF [55]. This seems to be the first time in the literature that compounds originating from food were reported to have inhibited PAF-induced platelet aggregation. This was a significant finding as it demonstrated the existence of not only pharmacological therapeutics, but potentially dietary sources of PAF inhibitors also.

Around this period of PAF research there was a large increase in the number of published research relating to the discovery of PAF antagonists of natural and synthetic origin for which we now know of several hundred natural and synthetic PAF inhibitor molecules in existence [14]. In particular, researchers were investigating the potential use of compounds known as ginkgolides isolated from the *Ginkgo biloba* tree, a tree native to China, the existence of which dates back over 270 million years [56].

There are several ways to classify PAF inhibitors including if they are of natural of synthetic origin, they can be classified by their various chemical structures, and they can be classified by their interaction with the PAF-R, e.g., specific and non-specific inhibitors [57]. In terms of their structures, PAF inhibitors can be PAF analogues such as polar lipids, or there are molecules that are dihydropyridines, nitrogen heterocyclic compounds, phenolics, and other various natural medicinal compounds [57][58][59].

Along with being classified into compounds of natural or synthetic origin, PAF inhibitors can be characterised into two main classes according to their specificity: non-specific and specific inhibitors. Non-specific PAF inhibitors are compounds that inhibit certain processes in the PAF-induced signal transduction pathways such as calcium channel blockers, G-protein inhibitors, intracellular calcium chelators, etc. [14]. Various non-specific PAF inhibitors were crucial to identifying the individual steps of PAF-related signal transduction pathways. However, their pharmacological value is limited due to their low specificity [60][61][62][63]. By contrast, specific PAF inhibitors competitively or noncompetitively bind with the PAF-R. These types of inhibitors may have potential therapeutic value [5][14].

4.1. PAF Inhibitors of Synthetic Origin

The initial synthetic PAF inhibitor compounds such as CV-3988 [64][65], CV-6209 [66], RO 19-3704 [67], and ONO-6240 [68] were structurally similar to PAF. In fact CV-3988 a thiazolium derivative was a zwitterionic species that was the first synthetic antagonist of the PAF-R [64]. Later inhibitors replaced the glycerol backbone with cyclic structures such as SRI 63-441 [69], SRI 63-073 [70], UR-11353 [71], and CL-184,005 [72]. Subsequently, other PAF antagonists were developed that had no structural similarity to PAF. These antagonists were comprised of heterocyclic structures that were characterised by sp^2 nitrogen atom that interacted with the PAF-R as a hydrogen bond acceptor [57]. Many of these were derivatives of imidazolyl that lead to the development of lexipafant [73] and modipafant [74], thiazolidine derivatives such as SM-10661 [75], pyrrolothiazole-related antagonists such as

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Table 2. A list of some of the major synthetic PAF antagonists assessed against several conditions in clinical trials.

PAF-R Antagonist	Target Disease or Disorder	Outcome	Reference
Lexipafant	Cognitive impairment complications as a result of coronary artery bypass graft	No significant reduction in cognitive impairment	[84]
	Myocardial infarction	No significant effect on streptokinase-induced hypotension in myocardial infarction patients	[85]
	Sepsis	No significant affect in patients with severe sepsis	[86]
	Organ failure related to pancreatitis	No significant amelioration of systemic inflammatory response syndrome in pancreatitis-induced organ failure	[87]
Modipafant	Asthma	No significant effect against chronic asthma	[74]
	Asthma	No significant effect in early or late responses to allergens	[88]
	Responses to inhaled PAF	Potent inhibition of airway and neutrophil responses to PAF with a duration of up to 24 h and a reduction of secondary eicosanoid production in response to inhaled PAF	[89]

infusion of acetyl glyceryl ether phosphorylcholine (AGEPC), a synthetic platelet-activating factor (PAF), in the rabbit. J. Immunol. 1980, 124, 2919–2924.

PAF-R Antagonist	Target Disease or Disorder	Outcome	Reference
SR27417A	Asthma	Modest inhibitory effects against asthma	[90][91]
SR27417A	Ulcerative colitis	No evidence of efficacy in the treatment of acute ulcerative colitis	[92]
WEB 2086	Asthma	No attenuation of early of late allergen-induced responses or airway hyperresponsiveness	[93]
	UVB-induced dermatitis	Significant inhibition of UVB light-induced erythema	[94]
BN 50730	Rheumatoid arthritis	Ineffective in the treatment of rheumatoid arthritis	[95]
BN 52021	Pulmonary function in the early post ischaemic graft function in clinical lung transplantation	Improvement of alveoloarterial oxygen difference and a reduction of PAF levels	[96]
Ro 24-238	Psoriasis	No significant effects reported	[97]
TCV-309	Septic shock	No significant difference in adverse events or mortality. A substantial reduction of organ dysfunction and morbidity associated with septic shock was reported	[98]
Levocetirizine	Chronic idiopathic urticaria	Reduction of urticarial activity score	[99]
Rupatadine	Chronic idiopathic urticaria	Reduction of urticarial activity score but not as effective as levocetirizine	[99][100]

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PAF-R Antagonist	Target Disease or Disorder	Outcome	Reference
	Allergic rhinitis and allergies	Significant effects against both conditions as demonstrated in the comprehensive review by Mullol et al.	[101]
Y-24180	Asthma	Improvement of bronchial hyperresponsiveness in patients with asthma	[102]

...30, ...itors. For ... [103] ... LDP-392 ... [104] ... [105][106] ...retrovirals ... [107][108] ...d, various ... [109] ...mboxane ... [110] ... of various ... A2 and ...onists [57]. ...hetic and ... inorganic metal complexes with PAF-R antagonistic properties, their structures, synthesis, and biological effects [81][57][88][101].

4.2. PAF Inhibitors of Natural Origin

Extracts from *Ginkgo biloba* were some of the first PAF inhibitors of natural origin to be discovered. Several studies by Pierre Braquet and colleagues demonstrated that one compound in particular, BN 52021, was a highly specific competitive PAF antagonist. Several related ginkgolides also exhibited inhibitory properties against PAF [112][113][114][115][116][117]. Indeed, several other researchers at the time discovered anti-PAF properties in other natural isolates of Chinese medicinal herbs such as phomactin A, kadsurenone, and various xanthones [118][119][120][121][122]. In fact, the discovery that compounds from garlic bulbs possess anti-PAF activity stimulated interest in the exploration of natural compounds for anti-PAF activity [55].

By 1996, several lipid structures had been discovered with PAF-like activity as reviewed by Demopoulos [123]. Further experimentation uncovered that a neutral glycerylether lipid without an acetyl group from pine pollen exhibited biological activity against PAF [124]. Consequently, it was deduced that other lipid extracts could potentially inhibit PAF-induced platelet aggregation. This led to a series of studies investigating food lipid extracts starting around 1993, which initially lead to the discovery of PAF antagonists in the polar lipid fractions of olive oil [125], honey and wax [126], milk and yoghurt [127], mackerel (*Scomber scombrus*) [128], and wine [129] before the turn of the century. These studies deduced that mainly polar lipids such as glycerophospholipids and glycolipids exhibited potent inhibition against PAF-induced platelet aggregation through competitive binding to the PAF-R. As this research field developed, it was noted that many of the compounds discovered that exhibited anti-PAF activity were also constituents of foods of the Mediterranean diet [5][130][131]. Therefore, these constituents may be responsible for the observed beneficial effects of consuming the Mediterranean diet [5][130][131]. Indeed, later research demonstrated that polar lipid extracts of olive oil, olive pomace, and fish could also affect many of the PAF metabolic enzymes both in vitro and in vivo [132][133][134]. These extracts were able to aid in the re-equilibration of PAF levels with beneficial outcomes against models of chronic inflammation.

Research into the effect of lipids on PAF activity and PAF metabolism is still being explored today in the pursuit of finding natural ways to prevent the pro-inflammatory signaling of PAF. It is now known that many foods, beverages, and other natural sources including food industry by-products are rich in PAF antagonists [58][135]. However, there

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have been several clinical trials in vivo that suggest that PAF inhibitors of natural origin may help prevent diseases such as CVD. In studies in vivo, olive oil, olive oil polar lipids extracts, and olive oil neutral lipids extracts were administered to rabbits consuming an atherogenic diet. It was demonstrated that rabbits consuming olive oil or olive oil polar lipid extracts had more beneficial physiological and biochemical changes as a result of increased plasma levels of PAF-AH, less oxidation in the plasma, a reduction of atherosclerotic lesion thickness, and retention of vessel wall elasticity, thus impeding atherosclerosis development [136]. These results were corroborated in a subsequent study that found that polar lipid extracts of olive oil and olive pomace can impede early atherosclerosis development through reducing platelet sensitivity to PAF and reducing atherosclerotic lesion thickness [137]. A later follow-up study in rabbits demonstrated that olive pomace polar lipid extracts were equipotent to simvastatin in preventing the progression of atherogenesis [138].

It was questioned whether other polar lipid extracts of natural origin could exhibit the same effects. Therefore, two studies of similar design demonstrated anti-atherogenic effects when rabbits consumed polar lipids extracted from fish (seabream, *Sparus aurata*) in a model of hypercholesterolaemia. These studies demonstrated that fish polar lipids could also reduce platelet aggregation, reduce atherosclerotic lesion size, and increase HDL levels in rabbits [139] along with modulating PAF metabolism leading to lower PAF levels and activity in rabbit blood [134]. Representative optic micrographs (×100) of the aortic wall of these rabbits are presented in **Figure 2**. These images demonstrate that rabbits consuming an atherogenic diet supplemented with fish polar lipids leads to a reduction of atherosclerotic lesion width (b) versus a control group that consumed only an atherogenic diet (a) [139].

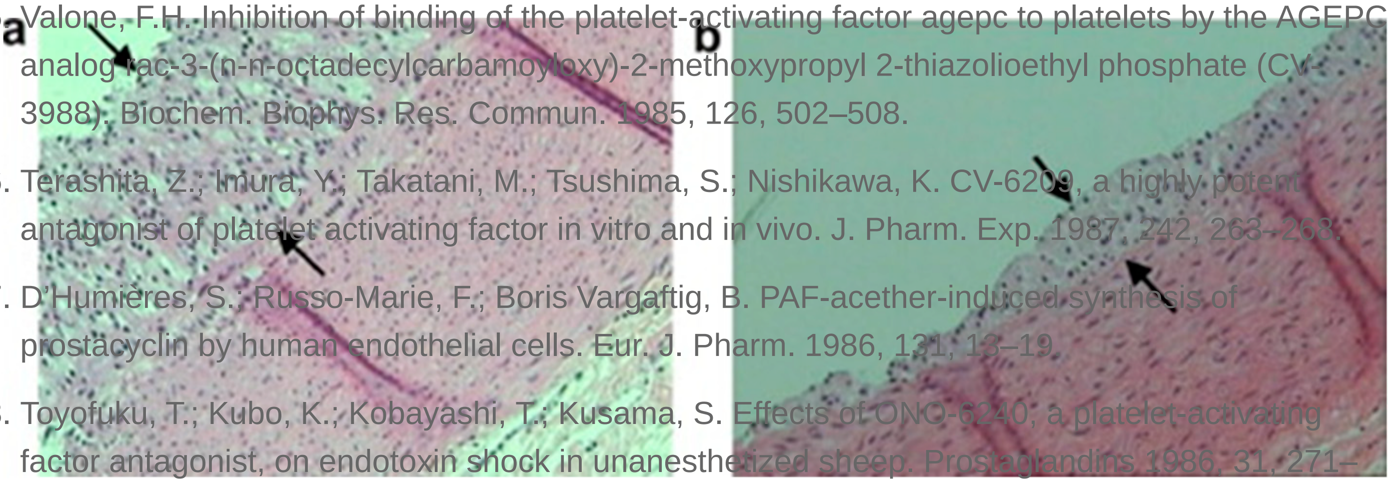

Figure 2. Representative optic micrographs ×100 of aortic wall cross-sections stained with hematoxylin and eosin obtained from the two rabbit experimental groups. Atherosclerotic lesions appear as foam cells between the arrows. Each tissue sample was approximately 5 μm thick. (**a**) Group A (atherogenic diet) and (**b**) group B (atherogenic diet enriched with seabream polar lipids). Reproduced with permission from Nasopoulou et al. [139].

However, after discovering that polar lipids could inhibit PAF in vitro and in vivo, the question remained whether these compounds of natural origin could affect human health? It is now known that there have been some promising nutritional trials that indicate that PAF antagonists in wine may affect platelet aggregation and metabolism postprandially in humans [43][140]. In people with metabolic syndrome, consumption of meals including

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wild plants of the Mediterranean diet rich in PAF inhibitors postprandially reduced PAF-induced platelet aggregation [141]. Other results from dietary intervention studies have shown that the administration of traditional Mediterranean diet meals [142][143] to either normal volunteers or individual's with type II diabetes mellitus (who have a predisposition to CVD) resulted in the characteristic lower PAF activity in blood (measured as PAF-induced platelet aggregability), which correlates with inhibition of atherogenesis according to experiments [136].

Likewise, dietary supplements can reduce PAF-induced platelet aggregation and increase PAF catabolism in healthy humans [144]. These studies collectively indicate that consumption of PAF antagonists from foods and nutraceuticals may benefit the consumer by reducing the pro-inflammatory effects of PAF either through inhibition of PAF/PAF-R signaling or by influencing the metabolic enzymes of PAF.

Considering, the potential use of dietary polar lipids for the prevention of CVD, several recent studies have discovered PAF antagonists in various fish species and by-products of the fishing industry including salmon fillet and head, minced boarfish, and sardine [145][146][147], and other foods such as sheep and goat meat [148], milk and fermented dairy products [149][150][151][152], and beer and brewing by-products [153][154]. Future research in this area aims to develop novel functional foods and nutraceuticals that incorporate these bioactive polar lipid extracts for the prevention of CVD and other inflammation-related diseases. For more extensive reviews of the anti-inflammatory and antithrombotic properties of various food polar lipids the authors suggest the following literature [155][58].

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