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Anaphylaxis (P Lieberman, Section Editor)

Potential Therapeutic Strategies for Severe Anaphylaxis Targeting Platelet-Activating Factor and PAF Acetylhydrolase

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Opinion statement

Characterization of mediators and mechanisms of anaphylaxis will allow for more specific and effective treatment with fewer side effects. Recent studies have shown that platelet-activating factor (PAF) is a pivotal mediator of the life-threatening manifestations of anaphylaxis. The putative role of PAF in human anaphylaxis is based on a large body of evidence in experimental and human anaphylaxis. In animal models of anaphylaxis, intravenous administration of PAF reproduces the severe physiologic derangements of anaphylaxis. PAF receptor knock-out mice are resistant to experimental anaphylaxis. Data from human anaphylaxis show that levels of PAF increase proportionately with the severity of anaphylaxis, whereas a deficiency in the enzyme that inactivates PAF predisposes to severe or fatal anaphylaxis. Many of the biologic effects of PAF appear to be transduced by nitric oxide production. PAF receptor antagonists protect against the lethal effects of exogenous PAF, but, importantly, also protect against experimental anaphylaxis following allergen challenge. Mice treated with an enzyme that inactivates PAF are similarly resistant to anaphylaxis. Some clinically available medications for anaphylaxis act at different points of the PAF pathway. Epinephrine, the first-line treatment for anaphylaxis, appears to act in part by phosphorylation and inactivation of the PAF receptor, whereas methylene blue, which reduces

the actions of nitric oxide, can reverse severe anaphylaxis that is refractory to conventional treatment. Taken together, these studies have shown that therapies targeting the PAF pathway might hold the potential for more specific and effective treatments for this potentially life-threatening condition.

Introduction

An anaphylactic reaction is an acute life-threatening event generally involving mast cell and basophil activation. The major triggers of anaphylaxis in the North American population are age dependent and generally include foods, drugs, and insect stings [1]. Most epidemiologic studies suggest that the incidence of anaphylaxis is increasing in both young and old individuals [2].

The severity of anaphylaxis can be stratified using several validated scoring systems [3–5]. These scoring systems differ in their specifics, but all reflect that severe anaphylaxis is an event with marked cardiovascular and/or respiratory compromise. Clinical factors that appear to predispose to severe anaphylaxis include the presence of asthma and other co-morbid conditions, such as hypertension and coronary artery disease; use of medications, such as ASA, NSAIDs, and certain antihypertensive medications; and underlying mast cell disorders [6]. However, the mechanistic basis for severe and potentially fatal anaphylaxis is only now being studied, and relevant biomarkers are now being identified.

The pathophysiologic understanding of anaphylaxis is advancing. Treatments based on specific mediators of anaphylaxis will allow for targeted interventions in anaphylaxis. Several candidate mediators have recently been identified, including preformed and newly synthesized mediators, that could be potential therapeutic targets. In recent years, the role of platelet-activating factor (PAF), long known to be a key substance in murine anaphylaxis [7], has been shown to have a central role in severe and fatal human anaphylaxis [8, 9•].

Platelet-activating factor

Background and characteristics of platelet-activating factor

PAF is a newly synthesized phospholipid mediator secreted by many cell types and possessing pleiotropic effects [10]. PAF is able to act on the vascular endothelium and bronchial smooth muscle, as well as multiple cell types. PAF directly induces histamine release from basophils, activates mast cells, induces mast cell chemotaxis and eosinophil chemotaxis, activates IL-4 production from T cells, is a mitogen for vascular smooth muscle cells, recruits neutrophils, and aggregates platelets, among many other functions [11].

PAF acts through the PAF receptor. The PAF receptor has been demonstrated on many cells, including mast cells, eosinophils, basophils, macrophages, monocytes, endothelial cells, and others. Expression of the PAF receptor is regulated at many levels [12, 13], including at the level of transcription, as well as by both phosphorylation and by intracellular cAMP levels. Residues in the carboxy-terminal cytoplasmic tail serve as a phosphorylation target. Phosphorylation of these sites may modulate binding of G-proteins to the receptors, accounting for the rapid desensitization of the PAF receptor. The PAF receptor undergoes homologous desensitization, a control mechanism that potentially limits its signaling actions. Internalization or sequestration of the receptor from the cell surface into the intracellular compartment leads to downregulation of receptor numbers on the cell surface. Phosphorylation by second messenger kinases and G-protein-coupled kinases helps to maintain receptors uncoupled from G-protein, but the role of phosphorylation in receptor sequestration is not as well defined; agonist-induced phosphorylation of the cytoplasmic tail of the PAF receptor facilitates, but is not essential for, receptor sequestration. The PAF receptor has been shown to undergo a ligand-specific, temperature-dependent internalization in transfected cells. The binding of PAF to its receptor results in the initiation of downstream signaling events, including the release of nitric oxide (NO) [14], causing vascular smooth muscle relaxation, leading to circulatory collapse and uterine, airway, and coronary smooth muscle contraction.

PAF is a highly potent vasoactive mediator. The typical serum concentration of PAF in healthy individuals is generally less than 400 pg/ml, whereas it can exert its biologic effects at concentrations as low as 10^{-12} mol/l [15]. It is inactivated by the enzyme platelet-activating factor acetylhydrolase (PAF-AH). The half-life of PAF is

determined by the serum concentration of PAF-AH and is typically in the order of minutes [8].

Data supporting a role for platelet-activating factor in anaphylaxis in experimental animals

Data from experimental animals support the important role of PAF in anaphylaxis [16–18]. PAF can cause severe cardiac and respiratory symptoms and is involved in the biphasic allergic response. In numerous studies of animal models of anaphylaxis, PAF was elevated in the systemic circulation in proportion to the severity of anaphylaxis. The direct injection of exogenous PAF in mice causes decreased peripheral vascular tone, decreased myocardial contractility, and increased end-diastolic and pulmonary artery pressures, as well as smooth muscle contraction in the coronary arteries, gut, and uterus [19]. PAF receptor knock-out mice were resistant to experimental anaphylaxis, as compared with wild-type mice [20]. In mice without active IgE or IgG receptors, and therefore resistant to anaphylaxis, reconstitution with wild-type neutrophils restored allergen-induced anaphylaxis. This effect was shown to be due to the release of PAF from neutrophils via IgG receptors. In addition, transfer of human neutrophils was also able to restore anaphylaxis [21•].

Clinical data supporting a role for the platelet-activating factor pathway in severe and fatal anaphylaxis

There are compelling clinical data in support of a pivotal role for PAF in severe and fatal anaphylaxis in humans. Serum concentrations of PAF are significantly higher in severe anaphylaxis [8] than in milder allergic reactions and controls. The level of the enzyme that hydrolyzes PAF to a biologically inactive form, PAF-AH, correlates with the severity of anaphylaxis in patients with acute anaphylaxis [8, 9•]. In addition, the PAF-AH level is lower in fatal anaphylaxis than in controls with nonfatal anaphylaxis, controls with life-threatening and non-life-threatening asthma, and patients who die from nonallergic causes [8]. Intracutaneous PAF injection causes both an early- and a late-phase reaction in humans [22], suggesting that PAF contributes to the biphasic response to allergen. Very recently, a low basal PAF-AH level was reported to be a risk factor for severe reactions to Hymenoptera stings [23•]. Inhaled PAF has been documented to cause bronchoconstriction [24, 25].

The current treatment for severe anaphylaxis is prompt intramuscular administration of epinephrine [26] with supportive measures, including postural maneuvers to support blood flow to the brain and administration of intravenous fluids. Additional supportive medications include antihistamines and bronchodilators. The use of epinephrine for anaphylaxis was first reported in the 1960s and is based on empiric observation and expert opinion. Epinephrine acts through multiple pathways and mechanisms, although the exact action in anaphylaxis is acknowledged to be nonspecific and unknown. In addition, epinephrine has many side effects and a narrow therapeutic window [27]. More directed treatments with a wide safety margin are needed for anaphylaxis.

Current treatment options directly related to PAF are limited. The purpose of this review is to summarize the recent advances in the understanding of the role of PAF in severe anaphylaxis, with particular attention to clinically available and preclinical therapeutic interventions related to the actions of this chemical mediator.

Treatment

- This section discusses medications with respect to their relation to the PAF pathway, with the express purpose of illustrating the role of blocking the effects of PAF in anaphylaxis. The clinically available medications—epinephrine, methylene blue, and rupatidine—are not specific to the PAF pathway, but aspects of their mechanisms of action support the role of PAF in anaphylaxis. Epinephrine has been shown to reduce the effects of PAF in vitro. The successful use of

methylene blue, which reduces the manifestations of one of the key downstream effectors of PAF, NO, has been reported in refractory anaphylaxis. The antihistamine rupatidine has both anti-PAF and antihistamine effects.

- The preclinical agents reviewed below are targeted to the effect of PAF in severe anaphylaxis but are not available for human use at this time. Nevertheless, they provide the most compelling evidence to support the role of targeting PAF to treat or prevent severe anaphylaxis.

Pharmacologic treatment

- The aims of drug therapy are to rapidly reverse the cardiovascular and respiratory complications of anaphylaxis to reduce morbidity and mortality. In the future, medications may have the goal of preventing anaphylaxis in selected patient populations.

β-adrenergic agonist

Epinephrine

- Epinephrine is the first-line agent for the treatment of anaphylaxis [26]. This recommendation is based on expert opinion and observation because there are no high-quality studies to support its use [28].
- Epinephrine has multiple pharmacologic actions, including β-adrenergic activity and β1-adrenergic vasoconstrictor activity [29]. This section will concentrate on the actions of epinephrine that are relevant to the PAF pathway.
- Epinephrine has been shown to reduce PAF-induced prostaglandin release [30•]. In cultured human vascular smooth muscle cells (HVSMCs) stimulated with PAF, prostaglandin E_2 (PGE_2) was released in a time- and dose-dependent manner. Incubation of HVSMCs with epinephrine reduced PAF-mediated prostaglandin release, with the greatest effect being seen if epinephrine was added before PAF stimulation of HVSMCs. The effect on PGE_2 release was reduced as the time of the addition of epinephrine after PAF stimulation was increased. The ability of epinephrine to reduce prostaglandin release in this model was prevented by β-blockade with propranolol. In this model, epinephrine acts through the β-adrenergic receptor to modify PAF-induced prostaglandin release in HVSMCs.
- The function and expression of the PAF receptor is known to be controlled by multiple factors, including intracellular cAMP levels [12]. To further elucidate how signaling through the β-adrenergic

	receptor could alter PAF action, the effect of increasing intracellular cAMP levels was examined. In this model [30•], agents that increased intracellular cAMP also reduced PAF-mediated prostaglandin release. Taken together, these findings suggest that epinephrine likely exerts its effect on PAF signaling by phosphorylation of the PAF receptor via increased intracellular cAMP.
Standard dosage	0.01 mg/kg IM to a body weight of 30 kg. For a body weight of >30 kg, 0.1–0.5 mg of a 1:1000 solution IM every 5–15 minutes as needed.
Contraindications	There are no absolute contraindications to use of epinephrine in the setting of acute anaphylaxis. Caution is advised in patients with heart disease, diabetes, psychiatric disease, closed angle glaucoma, or pregnancy.
Main drug interactions	The efficacy of epinephrine in the treatment of anaphylaxis is reduced in persons taking β-blockers.
Main side effects	A detailed discussion of epinephrine can be found in other reviews. Epinephrine has a narrow therapeutic window and can cause severe side effects, including arrhythmias, pulmonary edema, hypertension [31], and cerebral hemorrhage [32]. More commonly, epinephrine leads to side effects of pallor, tremor, and anxiety [29].
Special points	Up to one third of patients will require more than one injection of epinephrine, so access to at least two doses is recommended [33]. At very low doses, epinephrine may cause vasodilatation and increased release of mediators through its action on β2 adrenergic receptors [29].
Cost/cost effectiveness	Epinephrine is inexpensive when purchased in unit dose vials. When supplied in autoinjectors, the cost is approximately $50–100 per autoinjector.

Guanylate cyclase inhibitor

Methylene blue

- Methylene blue is indicated for the treatment of drug-induced methemoglobinemia, as well as in imaging applications such as sentinel node biopsies [34], but it is also included in anaphylaxis guidelines as an agent to consider in the treatment of anaphylaxis [35]. No formal clinical trial of methylene blue in the treatment of anaphylaxis has been undertaken.
- Methylene blue was postulated to be effective in treating anaphylaxis with hypotension in 1997 [36]. Methylene blue competes with NO to inhibit guanylate cyclase, thereby preventing synthesis of cyclic guanosine monophosphate (cGMP) and resultant smooth muscle relaxation and vasodilation.
- PAF induces NO synthesis. Direct injection of PAF had a reduced physiologic effect in mice treated with a nonselective NO synthase inhibitor as well as in endothelial NO synthase (eNOS) knock-out mice. Together, these observations show that PAF exerts its effects through NO and that the NO is generated through eNOS [37, 38].

The effect of methylene blue is illustrated in Fig. 1.

- Clinical case reports have shown that methylene blue is effective in reversal of refractory anaphylaxis with hypotension [36]. Recently, methylene blue was used successfully in treating a patient with refractory anaphylaxis with upper airway obstruction but without hypotension [39•].

Standard dosage	In anaphylaxis, methylene blue 1 % has been administered at a dose of 1.5–2 mg/kg IV in a solution of 5 % dextrose [36].
Contraindications	Caution is advised in renal failure and G6PD deficiency. It should not be administered subcutaneously, because of the risk of necrotic abscess. It is a category C drug with respect to pregnancy. No safety information is available for lactation [34].
Main drug interactions	Methylene blue is a monoamine oxidase inhibitor and can produce serotonin syndrome if used with serotonergic medications [34].
Main side effects	Methylene blue may cause anaphylaxis, even in patients who have not been exposed to this dye before. A well-documented case of anaphylaxis occurred on first exposure for fallopian tube imaging [40]. Positive skin prick tests and in vitro leukocyte histamine release assay supported the diagnosis. However, anaphylaxis to methylene blue is very rare [41]. Methylene blue can cause fever, hypertension, chest pain, diaphoresis, headache, dizziness, confusion, nausea, vomiting, and abdominal pain [34].
Special points	Because of the blue color, methylene blue interferes with pulse oximetry and can cause blue urine and stools [34].
Cost/cost effectiveness	Methylene blue is generic and is inexpensive.

Emerging therapies

Platelet-activating factor receptor antagonists

- The PAF receptor is a G-protein-coupled transmembrane receptor, which triggers multiple intracellular signaling pathways and is responsible for both immediate actions as well as new gene transcription [42]. There are two alternatively spliced transcripts of the PAF receptor, and PAF receptor expression can be modified via multiple mechanisms [12].
- The PAF receptor is able to recognize PAF, as well as oxidized PAF-like compounds [43].
- PAF receptor antagonists (PAF-RAs) block the action of PAF by competing with binding at the level of the receptor. Numerous naturally occurring compounds with PAF-RA activity have been discovered, and a number of potent PAF-RAs have been synthesized [44].
- The PAF-RAs have been studied extensively in experimental models of anaphylaxis but have not yet been studied in human anaphylaxis. Experience in experimental models suggests that these compounds show great promise in the treatment and prevention of anaphylaxis.

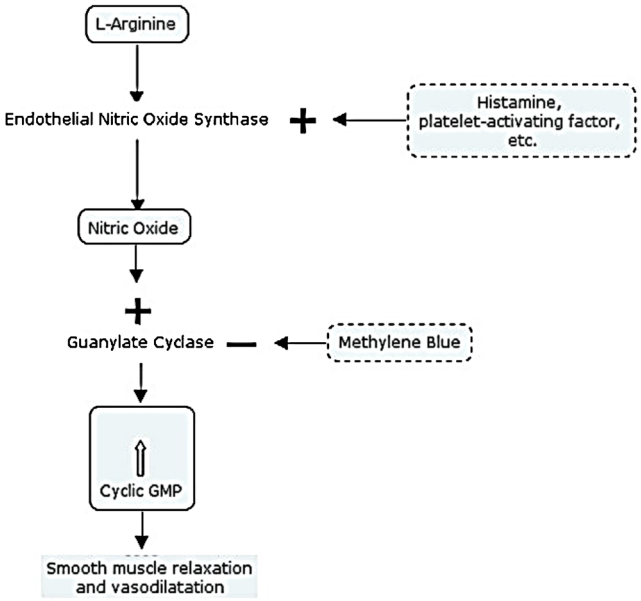

Fig. 1. Endothelial nitric oxide synthase converts L-arginine to nitric oxide, a reaction that is upregulated in the presence of histamine and platelet-activating factor. Nitric oxide increases the activation of guanylate cyclase, which then increases cyclic guanosine monophosphate (cyclic GMP). These events result in smooth muscle relaxation and vasodilation. + indicates upregulates/activates, – indicates downregulates/inhibits. Reprinted from [39•] with permission from Elsevier. http://www.sciencedirect.com/science/journal/07356757.

Synthetic platelet-activating factor receptor antagonists

- There are multiple synthetic PAF-RA compounds. In this section, CV3988, CV6209, WEB2086, WEB2170, ABT-491, UK74505, and SR27417A will be discussed.

In experimental models

CV3988 protected mice from death due to PAF injection and due to anaphylaxis from bovine serum albumin (BSA) injection [45]. Importantly, CV3988 was effective if given prior to or after allergen challenge, suggesting that PAF-RA may have potential as a rescue treatment for anaphylaxis.

WEB2086 attenuated the late cardiac manifestations of anaphylaxis in ovalbumin-sensitized guinea pigs when used in combination with the H1 and H2 receptor antagonists mepyramine and cimetidine but had little effect on its own [46]. The antihistamines alone prevented early cardiac effects but did not prevent death. In this model, blocking PAF and histamine was more effective in preventing severe anaphylaxis than blocking either mediator alone.

Additional studies have shown that the protective effect on anaphylaxis of combining a PAF-RA with an antihistamine is even more profound. In ovalbumin-sensitized BALB/c mice, CV6209 reduced anaphylactic hypotension, whereas the H1 antihistamine diphenhydramine worsened hypotension. In addition, a synergistic effect was seen in which the two compounds almost completely prevented hypotension [47].

In a different murine model in which mice were orally sensitized with peanut protein, antagonism of PAF with the oral PAF-RA ABT-491, given 1 hour prior to peanut challenge, reduced the symptoms of anaphylaxis [48]. Greater protection was achieved by blocking both histamine and PAF, although blocking histamine alone did not have an effect.

The synergistic effect of combined PAF and histamine receptor blockade can be partially explained by the observations that histamine can induce PAF release [49] and PAF can induce histamine release [50].

Animal studies with PAF-RAs have shown that they have variable effects on bronchoconstriction and edema, depending on different pathophysiologic states and animal models. For example, PAF-RAs, including WEB2170 and CV6209, failed to prevent PAF-induced bronchoconstriction in guinea pigs sensitized to ovalbumin but did prevent lung edema [51]. Paw edema caused by PAF injection was reduced by WEB2170 in sensitized mice far more than in sensitized mice that had received an additional (booster) injection of antigen [52].

Conversely, in anaphylaxis in mice, WEB2170 was shown to protect against death from PAF-induced and allergen-induced anaphylaxis in the mice that had received a booster injection of antigen and in those that did not [53]. Additionally, the dose of WEB2170 needed to prevent PAF-induced death was 16 times lower than the dose needed to protect against PAF-induced paw edema in unsensitized mice. These studies demonstrate that different mechanisms account for bronchoconstriction, edema, and anaphylaxis.

In clinical studies

There have been no clinical studies of the efficacy of PAF-RA in anaphylaxis. However, they have been studied in allergic asthma. The experience with PAF-RA in asthma has been reviewed [54].

The PAF-AH UK74505 looked promising in a trial of 12 healthy males [55]. In this study, UK74505 was administered orally at 25 or 100 mg. Three and 24 hours after ingesting UK74505, subjects inhaled PAF. The lower dose of UK74505 was able to prevent bronchoconstriction from PAF inhalation 3 hours after UK74505 inhalation and the higher dose of UK74505 also prevented PAF-induced bronchoconstriction at 24 hours. In addition, UK74505 was able to reduce neutrophil changes and levels of other mediators such as LTE4. However, when studied in a randomized, double-blind, placebo-controlled crossover study in asthmatic patients, a 100 mg dose had no effect on the early or late response, despite showing evidence of bioavailability via potent effects on platelet aggregation [56].

Similarly, WEB2086 abolished the bronchoconstriction caused by inhaled PAF in humans [57], but clinical trials of this PAF-RA in asthma were disappointing. Administered at higher doses orally 3 times daily for a week prior to challenges, it had no significant effect on either histamine- or allergen-induced bronchoconstriction [58]. In a 12-week placebo-controlled study of WEB2086 in subjects with chronic asthma on inhaled corticosteroids, there was no effect on the ability to taper inhaled corticosteroids, peak expiratory flow, time to relapse, or symptom score [59].

SR27417A is considered to be a very potent PAF-RA. At doses of 10 mg per day taken for a week prior to allergen challenge, this compound attenuated the late but not the early allergen-induced asthmatic response [60].

From a pharmacologic standpoint, some synthetic PAF-RA compounds are very appealing as a prophylactic treatment. For example, a single 2.5 mg oral dose of SR27417A can inhibit platelet aggregation for more than 24 hours in humans [61]. As discussed above, in animal models, intravenous PAF-RA preparations have been shown to be effective in treating anaphylaxis when given before or after antigen exposure [45]. Therefore, PAF-RA compounds have potential both as rescue medications for acute anaphylaxis and as treatments for long-term prevention.

In all of these clinical trials for asthma, the PAF-RAs were well tolerated. In the safety and tolerability trial for WEB2086, healthy volunteers were administered the drug three times a day at doses of up to 100 mg for 7–12 days, and there were no drug-related adverse effects or laboratory changes [62]. In the study by Spence et al. [59], 63 subjects completed 6 or more weeks of oral treatment dosed at 40 mg TID, and no side effects were reported.

Special points

The clinical trials of synthetic PAF-RA in allergic asthma used monotherapy, and the observed effects on bronchoconstriction were insignificant or minor. However, there was evidence of an ability to lessen the late effect of allergen exposure, which is in keeping with animal models in which PAF-RAs protected against late cardiovascular death.

It is possible that the PAF-RA compounds may be more efficacious if used synergistically with antihistamines, given that a synergistic effect with PAF-RAs and antihistamines has been well demonstrated in animal models.

Animal models have shown that different pathophysiologic states respond differently to PAF-RAs, and so the clinical results in asthma may not apply to anaphylaxis.

Naturally occurring platelet-activating factor receptor antagonists

- The naturally occurring PAF-RAs are ginkgolides, kadsurenones, and glitoxins [63]. They have been studied in experimental models and in humans. In this section, the ginkgolides BN52021 and BN52063 will be briefly discussed.

In experimental models

BN52021 is the best-studied PAF-RA. This compound, derived from the *Ginkgo biloba* tree, has been shown to antagonize bronchoconstriction due to PAF, passive sensitization, and active sensitization to antigen in guinea pigs [64, 65].

In a guinea pig model in which the animals were sensitized to ovalbumin, BN52021 effectively blocked bronchoconstriction with first intratracheal exposure after sensitization, but if the animals received a booster injection of allergen, BN52021 was much less effective [66]. As discussed in the previous section, a booster injection did not reduce the efficacy of the synthetic PAF-RA WEB2170 [53] in preventing death from anaphylaxis in mice. Therefore, the effects of PAF-RA compounds on anaphylaxis need to be studied in the setting of anaphylaxis, as extrapolation between models may not be possible.

In clinical studies

Oral administration of a mixture of ginkgolides (BN52063) inhibited PAF-induced wheal and flare responses in nonatopic subjects [67] and late-onset wheal and flare responses in atopic subjects [68]. In patients with asthma, ginkgolides reduced the immediate response to inhaled allergen challenge [69].

Inhaled BN52021 (also known as ginkgolide B) completely prevented PAF-related bronchoconstriction in asthmatic children. Importantly, BN52021 also inhibited bronchoconstriction caused by inhaled allergen challenge in a subset of asthmatic children. It was able to prevent the PAF-related reduction in eosinophils and neutrophils in nonasthmatics but not in asthmatics [70].

Pharmacokinetic studies on ginkgolides have been performed. As one example, the oral absorption of ginkgolide B from a standardized *Gingko biloba* extract is slowed by food and the half-life of ginkgolide B ranges from about 4 hours under fasting conditions to over 10 hours after a meal [71]. Inves-

	tigation into the pharmokinetics of various ginkgolide compounds is ongoing [72].
Contraindications	*Ginkgo biloba* is contraindicated when there is hypersensitivity to the compound. There is no specific contraindication for breast feeding or pregnancy, but the safety of *Gingko*-derived compounds has not been proven in these settings [73].
Complications	In all of these clinical trials for asthma, the PAF-RAs were well tolerated. No serious side effects of ginkgolide B have been reported from trials for any of its uses [73]. Ginkgo compounds may interfere with platelet aggregation. It is often recommended to withhold ginkgolides for 3 days prior to surgical procedures. Rare cases of spontaneous bleeding have been associated with use of *Ginkgo biloba*.
Special points	Human trials are in progress, investigating use of *Ginkgo biloba* extracts for several indications [http://www.clinicaltrials.gov/ct2/results?term=gingko&pg=2]. These compounds require further study for use in anaphylaxis.

Platelet-activating factor acetylhydrolase

- PAF-AH is the enzyme that hydrolyzes PAF, to form a biologically inactive compound, lysoPAF. It is a circulating enzyme present in picogram amounts in serum. The lower limit of normal serum activity is 20 nmol/ml/min [8]. It is secreted from the liver and cells of the myeloid lineage, predominantly macrophages [74].
- There are two intracellular isoforms and one extracellular isoform of PAF-AH [12]. In human plasma, PAF-AH is bound to high- and low-density lipoproteins (HDL and LDL). Plasma PAF-AH activity can be influenced by altering the lipid composition and structure of the lipoproteins [74].
- The activity of PAF-AH is specific for choline phosphoglycerides with a short-chain fatty acid at the sn-2 position [12]. This enzyme is therefore able to hydrolyze PAF, as well as structurally similar compounds, including PAF-like lipids [75, 76].
- PAF-AH may exert both pro-inflammatory and anti-inflammatory actions, depending on the available substrates [77].
- In experimental models, the secretion of PAF-AH was altered by PAF-RA compounds in rat hepatocytes [78]. Some PAF-RA compounds reduced the secretion of PAF-AH and also reduced its activity. However, other PAF-RA compounds increased PAF-AH secretion in these cells. This adds another layer of complexity to the therapeutic potential of modifying the PAF pathway.

Recombinant platelet-activating factor acetylhydrolase

- PAF-AH is available as an IV preparation.
- Pretreatment of mice with 1 mg/kg intravenous recombinant human PAF-AH (rPAF-AH) 15 minutes before challenge reduced mortality in

mice caused by exogenous PAF injection from 100 % to 0 %. In antigen-induced anaphylaxis, treatment with rPAF-AH reduced mortality from 100 % to 20 % [79].

- In humans, a relative deficiency of PAH-AH has been linked to death from anaphylaxis [8]. A strong inverse correlation of PAF-AH activity with the severity of anaphylaxis has been observed in patients with acute anaphylaxis [8, 9•]. A low basal PAF-AH is a risk factor for severe reactions to Hymenoptera stings [23•].
- A recombinant human PAF-AH has been used in a phase IIa clinical trial for asthma but with disappointing results [80]. In this placebo-controlled trial, a 1 mg/kg dose of rPAF-AH was given in a single intravenous dose. The target dose in this trial was a plasma level of rPAF-AH of 10 mcg/ml. A non-statistically-significant trend toward a reduction in the sputum neutrophil count was found, but the allergen-induced dual-phase asthmatic response was not significantly altered. The authors suggested that future studies of rPAF-AH in asthma should target those patients with neutrophil-predominant disease.
- Transient drowsiness has been reported at 3 mg/kg IV dosing, and 1 of 12 subjects experienced fatigue during the trial conducted by Henig et al. [80].

Special points

Considerations: PAF-AH is a protein. As such, it might be suitable for IV administration in the treatment of acute anaphylaxis, but, without an oral or subcutaneous route, it would be unlikely to be a candidate for chronic therapy. It was well tolerated in trials, but, as with other biologic agents, there would be a potential risk of developing anaphylaxis with repeated administration.

Asthma is a very complicated, heterogeneous disease, in which there are many phenotypes and variable cell populations. The results of rPAF-AH in human asthma may not apply to human anaphylaxis.

Dual histamine and platelet-activating factor receptor antagonists

Rupatadine

- One second-generation antihistamine with both selective peripheral H1 receptor antagonist and PAF receptor antagonist activities approved for human use is rupatadine [81]. Rupatadine is not FDA approved at the time of writing but is available in the UK, Europe, South America, and India.
- There are, as yet, no data supporting use of rupatadine in anaphylaxis, although such studies would be of interest.

Pediatric considerations

- The lowest available dose of epinephrine in autoinjectors is 0.15 mg, which does not provide adequate flexibility for use in children with a

body weight of less than 15 kg.

- Neonates are at risk of serious side effects from methylene blue [34], such as hemolytic anemia, pulmonary edema, and others.

Compliance with Ethics Guidelines

Conflict of Interest

Julia Upton declares that she has no conflict of interest.

Peter Vadas has provided expert testimony in medical legal cases of injury due to anaphylaxis and owns US patent no. 8257697 ("Use of platelet activating factor acetylhydrolase as a biomarker for anaphylaxis"); however, no money was issued to him or his institution.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

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