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Vitamin E acetate as linactant in the pathophysiology of EVALI

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A B S T R A C T

The recent identification of Vitamin E acetate as one of the causal agents for the e-cigarette, or vaping, product use associated lung injury (EVALI) is a major milestone. In membrane biophysics, Vitamin E is a linactant and a potent modulator of lateral phase separation that effectively reduces the line tension at the two-dimensional phase boundaries and thereby exponentially increases the surface viscosity of the pulmonary surfactant. Disrupted dynamics of respiratory compression-expansion cycling may result in an extensive hypoxemia, leading to an acute respiratory distress entailing the formation of intraalveolar lipid-laden macrophages. Supplementation of pulmonary surfactants which retain moderate level of cholesterol and controlled hypothermia for patients are recommended when the hypothesis that the line-active property of the vitamin derivative drives the pathogenesis of EVALI holds.

Introduction

As of January 14, 2020, there were 2,668 reported hospitalized cases of e-cigarette, or vaping, product use associated lung injury (EVALI) in the United States [1]. Patients with EVALI typically exhibit respiratory symptoms, such as dyspnea and tachypnea, and often require the receipt of supplemental oxygen due to progressive hypoxemia [2,3]. Computed tomography (CT) scans of the chest of these patients mostly display diffuse ground-glass opacity, a non-specific sign characteristic of lung injury that is often indistinguishable from those induced by viral infections [4]. Extensive investigation into EVALI has revealed an emerging link between the disease and tetrahydrocannabinol (THC). Reportedly, as many as 82% of patients with EVALI used THC-containing e-cigarettes, while those who solely used nicotine-containing products accounted for a mere 14% of all cases [1]. Correspondingly, on October 4, 2019, the Food and Drug Administration issued a public warning against THC-containing vaping products, and the rate of emergency department (ED) visits due to EVALI has significantly declined since then [5].

The pathophysiology and the definitive cause of EVALI are yet to be established. Initial efforts to elucidate the pathophysiology of the disease have mainly focused on its association with THC. However, repeated inhalation of THC via propylene glycol vapor in murine models (0.32 M solution, 30 min inhalation session, twice daily, two weeks of exposure) did not induce respiratory symptoms, but rather resulted in a partially tolerated THC-induced hypothermia, hypolocomotion, and antinociception, raising suspicions against the pathological role of THC in EVALI [6,7]. What is exacerbating the situation is the lack of characterization of the injury. Other than the observation of type II alveolar pneumocyte hyperplasia [8], vacuolization [9], multinucleation [10], and intraalveolar lipid-laden macrophages [11], we still do not have a comprehensive understanding on what the injury exactly is. As a consequence, health care providers still mostly rely on a diagnosis of exclusion when a patient with a history of THC-containing vaping product usage exhibits severe respiratory symptoms indicative of EVALI [3]. Since the epidemic of EVALI has already reached its post-peak period and is expected to enter a post-epidemic one in a near future [5], thanks to global efforts to strengthen regulations against e-cigarettes, it is likely that we might not be able to fully characterize the disease before the epidemic is finally over. Nonetheless, for thousands of hospitalized patients who still suffer from the disorder and for the deceased who died without knowing what they were exactly dying from, the pathophysiological characterization of the disease should be continued and recent breakthroughs in the field are tremendously supporting us.

Recently, investigators at the Centers for Disease Control and Prevention (CDC) identified Vitamin E acetate, a chemical widely utilized as a diluent for THC-containing vaping fluids, as one of the potential causal agents of EVALI [12]. Among 51 submitted samples of bronchoalveolar lavage (BAL) fluid from patients, 48 samples contained high content of Vitamin E acetate exceeding 2.32 nM, while none was observed in those of healthy e-cigarette users without EVALI. Primary toxicants other than Vitamin E acetate were nearly absent in BAL fluid samples from patients, indicating a strong association of Vitamin E acetate in the pathology. In a field predominated by confusion, this discovery sets an important milestone.

Hypothesis.

Vitamin E has long been thought of as an antioxidant dwelling in the lipid bilayer. In fact, the discovery of the vitamin itself has come from reports of fetal resorption in murine models which were depleted of the vitamin [13]. The lethality of Vitamin E deficiency was later attributed

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to the lack of its antioxidative capacities, and it is now well-established that the vitamin is required for the protection of polyunsaturated fatty acids from being unwantedly oxidized [14]. However, Vitamin E acetate, given the additional ester moiety, is deprived of the antioxidative property and is markedly more thermostable. Furthermore, its affinity to α-tocopherol transfer protein (αTTP) is ~ 50-fold lower than that of the natural stereoisomer of Vitamin E [15], indicating the significance of an extensive water bridge between the hydroxyl group and residues Tyr117, Ser136, and Ser140 in the binding of the vitamin to αTTP [16]. Despite αTTP often being recognized as a hepatocyte-specific protein which presents Vitamin E from endosomal compartments to the plasma membrane for secretion [17], it is also expressed in the lung where it non-canonically increases the level of Vitamin E upon hypercapnia [18]. Due to these significant differences between Vitamin E and Vitamin E acetate, it is important to clarify whether the lung injury observed in patients with EVALI is induced solely by the esterified derivative of the vitamin. Unfortunately, the differences between the two compounds has largely been neglected in the field of EVALI, and the scarcity of information on the esterified derivative of the vitamin has forced many investigators to rely on reported biochemical properties of unmodified Vitamin E in elucidating the pathophysiology of EVALI.

Unlike those administered via dietary uptake [19], wherein the compound is readily hydrolyzed by cellular esterases, including pancreatic carboxyl ester hydrolase and cholesteryl ester hydrolase [20,21], inhaled Vitamin E acetate does not undergo esterase-mediated hydrolysis in the time scale of several hours [22,23]. This indicates that most of the inhaled Vitamin E acetate would remain unhydrolyzed in the lung of e-cigarette users even until the next vaping session in a typical setting. This is analogous to those observations made in skin, wherein the hydrolysis rate of the esterified derivative was measured as mere 5% [24]. The mechanistic detail on why inhaled Vitamin E acetate is resistant to esterase-mediated hydrolysis, however, is poorly understood.

Despite the remaining controversy on which of these compounds is responsible for the pathogenesis of EVALI, there is ample amount of evidence to rule out the antioxidative property of Vitamin E as its driving force. Antioxidative activities have long been recognized as a protective mechanism against injury in respiratory disorders. Indeed, pulmonary surfactants contain significant amounts of superoxide dismutase and catalase activities, which act to scavenge extracellular reactive oxygen species, such as hydrogen peroxide, within the pulmonary system [25]. Similarly, type II alveolar pneumocytes secrete Vitamin E alongside pulmonary surfactants to protect the respiratory system against inhaled oxidants [26,27]. However, it is worth to note that the protective effect of Vitamin E in the pulmonary system is not solely due to its antioxidative property. Indeed, the transcriptional activation of *α*TTP, which increases the level of Vitamin E in the pulmonary system, protects against ventilator-induced lung injury in murine models without affecting antioxidant response signaling, such as those dependent on nuclear factor erythroid 2-related factor 2 (NRF2) [18].

Recent investigations on the biological action of the vitamin have raised an emerging appreciation of its non-antioxidative properties [28,29]. Although it remains a matter of controversy whether non-antioxidative properties of the vitamin can be strictly discriminated from its antioxidative property [30], thorough examination of the effects of Vitamin E other than antioxidation in this poorly characterized respiratory disorder remains valuable. In this regard, herein I discuss several possible mechanisms by which Vitamin E acetate in vaping fluids may drive the pathogenesis of EVALI. As the biochemical properties of the vitamin derivative have been insufficiently characterized, I focus on those of an unmodified vitamin. Currently, there are five established non-antioxidative properties of Vitamin E in the biological system: i) its ability to induce gel-liquid crystalline phase transition, ii) its active deposition in the lipid droplet of macrophages, iii) its modulation of diacylglycerol kinase (DGK) and protein kinase C (PKC) signaling pathway with an antidiabetic regulatory role, iv) its activation of the xenobiotic-sensing pregnane X receptor (PXR) signaling, and v) its ability to modulate lateral phase separation. In each section, I discuss possible ways by which these properties may contribute to the pathophysiology of EVALI and whether these properties are expected to be shared by Vitamin E acetate. At last, I argue that the line-active property of the vitamin deserves academic attention. Although toxic byproducts of heating Vitamin E acetate have recently garnered considerable interest [31], I instead focus on the biological action of the vitamin derivative itself, as there is yet lacking amount of evidence that pinpoints toxic byproducts as the pathological driving force for EVALI. Indeed, Lanzarotta and colleagues have recently shown that the majority of vaporized Vitamin E acetate exist either as an equimolar complex with THC or as a dimer [32].

Vitamin E acetate as an inducer of gel-liquid crystalline phase transition

Among the five non-antioxidative properties of Vitamin E, investigators from the Lung Injury Response Laboratory Working Group have focused on its peculiar ability to induce gel (L_{β}) to liquid crystalline (L_c) phase transition in model saturated phosphatidylcholine bilayers [12] (L in the notation stands for *lamellar*). This property of the vitamin is believed to be the consequence of its structural deviance from phospholipids. As the structure of Vitamin E greatly differs from a typical phospholipid, especially in its rigid chromane double ring, the vitamin greatly perturbs the packing of phospholipids within the L_{β} phase, resulting in a facilitated gel-liquid crystalline phase transition (Fig. 1) [33]. For instance, the addition of either Vitamin E or Vitamin E acetate in molar ratios as high as 16 mol% in cholesterol-free model dimyristoylphosphatidylcholine (DMPC) membranes lowers the critical temperature required for gel-liquid crystalline phase transition

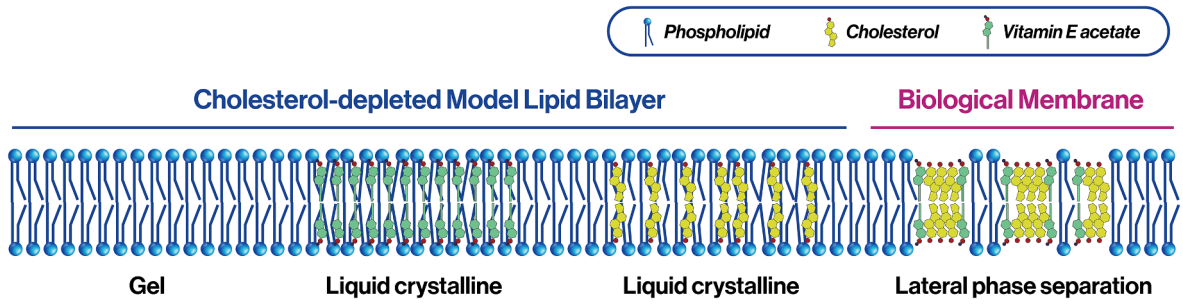

Fig. 1. Gel-liquid crystalline phase transition in lipid bilayers. It has been hypothesized that Vitamin E acetate may facilitate gel-liquid crystalline phase transition to confer acute respiratory distress [12]. In this picture, Vitamin E acetate perturbs the packing of phospholipids within the lipid bilayer such that the system thermodynamically prefers the liquid crystalline phase over the gel phase. However, cholesterol, which exists in large excess to Vitamin E acetate in the biological membrane, not only exerts similar effect in model saturated phospholipid bilayers, but also reportedly abolishes gel-liquid crystalline phase transition [38]. Therefore, gel-liquid crystalline phase transition may be irrelevant in the pathogenesis of EVALI.

by ~ 1.2 K [33,34]. However, since cholesterol, a major constituent of biological membranes, also exhibits profound structural deviance from a typical phospholipid, it also facilitates gel-liquid crystalline phase transition in a similar manner. Compared to free cholesterol, Vitamin E acetate is 1.5 times more potent in modulating the phase behavior of the lipid bilayer. As a consequence, one possible mechanism by which Vitamin E acetate intoxication may lead to a fatal lung injury is its induction of liquid crystalline phase in pulmonary surfactants, which would likely influence the respiratory compression-expansion cycling.

A major downfall of Blount and his colleagues' hypothesis is the abundance of free cholesterol in human pulmonary surfactants. Indeed, free cholesterol level in BAL fluids were measured as ~2.6 mM in murine models of pulmonary alveolar proteinosis (PAP) [35], while the level of Vitamin E acetate observed in patients with EVALI, who occasionally feature signs of PAP [36], was in nanomolar range [12]. In contrast, the decrease in the critical temperature induced by the addition of Vitamin E acetate was of roughly the same orders of magnitude compared to that of free cholesterol. This indicates that the overall contribution of Vitamin E acetate in the modulation of phase behavior is at least three orders of magnitude smaller compared to that of endogenously available free cholesterol. Furthermore, the existence of cholesterol in human pulmonary surfactants is known to completely abrogate gel-liquid crystalline phase transition [37]. In explanation, the driving force for gel-liquid crystalline phase transition induced by Vitamin E acetate in model saturated phosphatidylcholine bilayers has been its perturbation of phospholipid packing by structural deviance. The abundance of free cholesterol, of which structure is as rigid as the head portion of the vitamin, in biological membranes offsets the structural deviance of Vitamin E in lipid bilayers, resulting in an inability to induce gel-liquid crystalline phase transition (Fig. 1). Indeed, the observation of gel-liquid crystalline phase transition has been limited to the cholesterol-free model saturated phosphatidylcholine bilayers [33,34] and the membrane of *Mycoplasma laidlawii*, which is depleted of cholesterol and is enriched with saturated fatty acids [38]. Accounting for the rationale behind the selection of *Mycoplasma laidlawii* as the model system for investigating gel-liquid crystalline phase transition, Steim and colleagues concisely stated, "because cholesterol interferes, to detect a phase change above the ice point in a membrane, an organism containing rather saturated fatty acids but little or no cholesterol must be chosen" [38].

Vitamin E acetate as an inducer of exogenous lipoid pneumonia

Another important characteristic of Vitamin E acetate in the biological system is its deposition in lipid droplets [39,40], and many investigators have especially focused on the observation of intraalveolar lipid-laden macrophages as it has been proven to be one of the most prominent features of lung biopsies from patients with EVALI [41]. Indeed, intraalveolar lipid-laden macrophages were also evident in a murine model of Vitamin E acetate inhalation (daily inhalation of 77.3–167.5 μg Vitamin E acetate per gram of body weight, two weeks of exposure), of which effect was largely absent in mice inhaled a mixture of propylene glycol and vegetable glycerin [42]. Intriguingly, when a single dose of Vitamin E acetate (inhalation of 0.1 μg Vitamin E acetate per gram of body weight) was inhaled into the lipopolysaccharide-treated lungs of murine models, the substance greatly attenuated the inflammatory response, despite being 3-fold less potent than Vitamin E [23]. This indicates that it is the excessive accumulation of Vitamin E acetate, rather than its transient exposure, which is responsible for the pathogenesis of EVALI.

Although it is tempting to speculate that intraalveolar lipid-laden macrophages observed are merely the consequence of excessive deposition of Vitamin E acetate within the pulmonary system, increasing amount of reports suggest that such assumptions are erroneous. Importantly, there is a lacking histological evidence of exogenous lipoid pneumonia (ELP) in patients suffering from EVALI, despite the prevalence of intraalveolar lipid-laden macrophages in BAL fluids. For instance, when a 63-year-old woman had regularly smoked cod-liver oil, she acquired a non-oncologic perihilar mass in the right middle lobe of her lung, indicative of ELP [43], but these ELP-like histological signs were mostly absent in patients with EVALI [11]. As Guerrini and colleagues importantly mention, intraalveolar lipid-laden macrophages do not always originate from an excessive uptake of exogenous lipids, but may often emerge as a common immunopathological response following sustained inflammation [44]. However, unmodified Vitamin E is indicated in the downregulation of CD36 [45], which functions to promote the formation of lipid-laden macrophages (foam cells) [46]. Furthermore, Vitamin E also ameliorates nuclear factor-κB (NF-κB) signaling to prevent the formation of lipid-laden macrophages [47]. Although there is yet lacking amount of literature on the esterified derivative of the vitamin, it is somewhat unlikely that a single addition of an acetyl moiety onto the structure of the vitamin would completely reverse its effects on the formation of lipid-laden macrophages.

This hypothesis also does not provide us with an exact mechanism by which Vitamin E acetate may initiate inflammation. Since Vitamin E acetate is a viscous liquid at physiological temperature, activation of the NLRP3 inflammasome through crystal-induced lysosomal rupture as in models of silicosis [48] seems unlikely, despite robust activation of the inflammasome in e-cigarette users [49]. Vitamin E is also indicated in an active inhibition of 5-lipoxygenase (5-LO), which converts fatty acids to leukotrienes, promoting inflammation [50,51]. Importantly, Vitamin E acetate is also reported to inhibit 5-LO activity [50], despite being twice less potent than the unmodified vitamin, supporting the view that Vitamin E acetate does not induce ELP in patients with EVALI via the inflammatory response.

Vitamin E acetate as a modulator of DGK-PKC pathway

In the field of diabetes mellitus, Vitamin E is a potent agonist of DGKα, which catalyzes the conversion of diacylglycerol (DAG) to phosphatidic acid (PA) and thereby inhibits the catalytic activity of PKCα [52–54]. Agonism of DGKα by Vitamin E derivatives are known to be dependent on the chromane double ring of the molecule, as both Vitamin E analogs containing the moiety, such as troglitazone and trolox, and non-antioxidative Vitamin E derivatives, such as Vitamin E succinate, exhibited robust activation and subsequent plasmalemmal translocation of the kinase [55]. Vitamin E acetate may also antagonize the catalytic activity of PKCα in a DGK-independent manner, as there are studies indicating that the substance is capable of competing with DAG for its binding site in PKCα [56]. Intriguingly, *d*-α-tocopherol, but not *d*-β-tocopherol nor *d*-γ-tocopherol exhibited robust inactivation of PKCα, indicating a pivotal role of methylation at C5 and C7 of the chromanol structure [57]. Supplementation of Vitamin E acetate in murine models of diabetic cardiomyopathy showed largely protective role for the chemical [58], but it is worth to note that most of the substances supplemented via dietary uptake undergo hydrolysis to yield Vitamin E. The protective effect of Vitamin E derivatives in diabetes mellitus was completely ameliorated in DGKα knockout mice, indicating a pivotal role of the kinase in this antidiabetic signaling cascade [54].

Since the observed level of Vitamin E acetate in BAL fluids is of same orders of magnitude with that of DAG, a potent second messenger with which Vitamin E acetate competes for binding [59], the activity of the substance as a modulator of DGK-PKC pathway is physiologically relevant. However, the reported anti-inflammatory effects of *d*-α-tocopherol in the pulmonary system through post-translational inactivation of PKCα significantly undermines this hypothesis [60,61]. Since many observations suggest that the antagonism of PKCα by *d*-α-tocopherol is independent of its antioxidative property [57], it is anticipated that Vitamin E acetate might play an analogous anti-inflammatory role in the pulmonary system [62,63]. However, whether the esterified derivative antagonizes PKCα still remains to be investigated. Overall, the

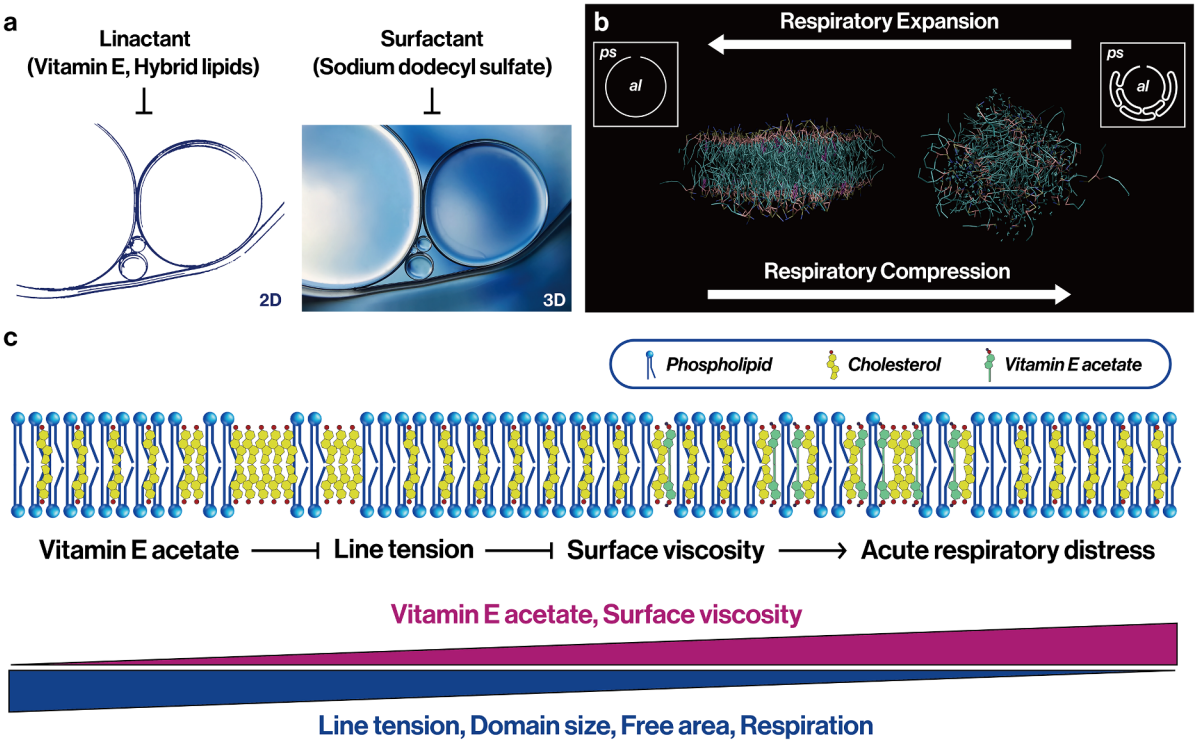

Fig. 2. Vitamin E acetate as linactant in the pathophysiology of EVALI. (a) Comparison between linactants and surfactants. While surfactants reduce the surface tension at the three-dimensional phase boundary, linactants, such as Vitamin E and hybrid lipids, act to decrease the line tension at the two-dimensional phase boundary. (b) Dynamics of respiratory compression-expansion cycling within pulmonary surfactants. The upper insets depict the structural change in the alveolar surface during the respiratory compression-expansion cycling, while the main illustrations show the detailed structure of the lipid bilayer comprising the pulmonary surfactant in each state. Upon breathing, pulmonary surfactants rapidly cycle between respiratory expansion and compression to confer dramatic changes in the alveolar surface area required for efficient air exchange. Increased surface viscosity may disrupt this dynamic process, causing extensive hypoxemia. al, alveolus. ps, pulmonary surfactants. (c) Consequences of the linactivity of Vitamin E acetate on pulmonary surfactants. From left to right, Vitamin E acetate is gradually added onto a biological membrane exhibiting an L_α/L_o lateral phase separation. Vitamin E acetate, if line-active as unmodified Vitamin E, may reduce the tensile force along the two-dimensional phase boundary and thereby increase the surface viscosity of pulmonary surfactants. Reduced line tension within the two-dimensional phase boundary confers smaller microdomains and decreases the free area of lipids [87]. As a result, the surface viscosity of pulmonary surfactants exponentially increases to perturb the dynamics of respiratory compression-expansion cycling, causing acute respiratory distress analogous to those seen in patients with EVALI.

modulation of DGK-PKC pathway by Vitamin E acetate seems yet incapable of accounting for the observation of pro-inflammatory intraalveolar lipid-laden macrophages in patients with EVALI, although there is yet lacking amount of evidence to completely rule out this hypothesis.

Vitamin E acetate as a pro-agonist of PXR

When Vitamin E is administered into the biological system, it transforms into a plethora of bioactive metabolites [64]. This includes several of the PXR agonists, such as *α*-tocopherol-13′-COOH, γ-tocotrienol, and garcinoic acid [65,66]. Since PXR forms a heterodimer with 9-*cis* retinoic acid receptor (NR2B) to activate the transcription of cytochrome P450 monooxygenase genes indicated in the clearance and detoxification of xenobiotic substances [67], pro-agonist role of Vitamin E acetate may be relevant in the pathophysiology of EVALI. Indeed, polymorphisms in the cytochrome P450 genes have been associated with drug-induced interstitial lung diseases (DIILD), characterized by coarse reticular opacity and intraalveolar lipid-laden macrophages [68]. This pathology is consistent with that of EVALI, wherein patients often reported patterns of giant-cell interstitial pneumonia [4]. In this picture, EVALI can be seen as a variant of DIILD caused by an excessive accumulation of Vitamin E acetate in the pulmonary system. Despite its pathophysiology being not fully characterized, DIILD often involves the generation of reactive oxygen species [69,70]. Vitamin E acetate, however, is known to protect the biological system against reactive oxygen species even in the absence of apparent hydrolysis [24], possibly through its inhibition of 5-LO [15] or through infinitesimal hydrolysis to Vitamin E as previously speculated [24]. Furthermore, DIILD is a highly heterogenous disorder, that is often dependent on genetic polymorphisms rare enough to avert federal regulations during drug approval [71,72]. For instance, the demonstration of respiratory symptom after oral administration of fenfluramine or dexfenfluramine was on a patient with a rare CYP2D6*1/*3 haplodeficient variant of the cytochrome P450 2D6 (CYP2D6) gene [68]. How haplodeficiency of the CYP2D6 gene contributes to the pathogenesis of DIILD remains unclear.

If Vitamin E acetate behaves as a pro-agonist of PXR, a transcriptional activator of cytochrome P450 genes, and thereby contributes to the pathogenesis of EVALI, the symptoms of EVALI should be replicated by inhalation of other well-established PXR agonists, such as corticosteroids [73–75]. Nebulized budesonide or fluticasone, however, did not induce respiratory distress when inhaled repeatedly (inhalation of 125–200 μg corticosteroids each session, 2–4 times daily, 8 days) in young children with a severe onset of asthma [76]. Furthermore, even in the absence of Vitamin E acetate, vaping induced significant increase in the activity of cytochrome P450 enzymes, including those hydroxylates Vitamin E, such as CYP2B1, CYP2C11, and CYP3A1, but without apparent respiratory distress [77]. However, it remains to be studied whether an excessive accumulation of Vitamin E acetate produces PXR-agonizing metabolites above a certain threshold such that it may induce an acute respiratory distress. Overall, the pro-agonist role of Vitamin E acetate on PXR seems relevant in the pathophysiology of EVALI, but there is yet lacking amount of evidence which indicates that the PXR

agonism drives the pathogenesis of EVALI.

Vitamin E acetate as a linactant

Recent developments in membrane biophysics indicate that an unmodified Vitamin E has an active role in lateral phase separation. Due to the rigid chromane double ring, which significantly reduces the entropic cost required for its association with cholesterol [78], unmodified Vitamin E efficiently releases the line tension (i.e. two-dimensional counterpart of a surface tension) between the liquid-disordered L_α and the liquid-ordered L_o phase of lipid polymorphs [79]. Indeed, oral supplementation of Vitamin E acetate in HIV/AIDS patients, which is readily converted to Vitamin E by cellular esterases, has been successful in reducing the viral load [80], as the molecule disrupts the tensile two-dimensional L_α/L_o phase boundary required for viral entry [81,82]. In membrane biophysics, this vitamin is classified as a linactant [83], which is a two-dimensional counterpart of a surfactant, as it reduces the line tension at the two-dimensional phase boundaries (Fig. 2a).

In explanation, when one puts a drop of oil into the water, gravity and surface tension compete to generate the lobular shape of the droplet. The addition of sodium dodecyl sulfate (SDS), a well-known surfactant, reduces the surface tension such that the oil droplet loses its ability to maintain its structure against gravitational force. As a consequence, oil emulsifies into the water, if not become completely miscible [84]. Similarly, when one adds Vitamin E onto the lipid bilayer exhibiting L_α/L_o phase separation, it reduces the line tension, which competes with entropy to form an enlarged cholesterol-enriched microdomain. As it has been shown that the molar ratio of Vitamin E within a typical lipid bilayer is insufficient to completely abolish lateral phase separation [85], Vitamin E acts to reduce the size of the microdomain and thereby emulsifies cholesterol-enriched L_o into cholesterol-depleted L_α (Fig. 2c). Despite the fact that the line-active property of Vitamin E acetate has not yet been explicitly tested, the small size and the abundance of ideally localized nonbonding electron donors of the ester moiety provide ample reason to hypothesize that the substance is a linactant. The content of Vitamin E within the lipid mixture that is sufficient to function as a potent linactant is typically below 10 mol% [79,85], which is comparable to that of Vitamin E acetate, estimated based on its concentration in BAL fluid samples from patients with EVALI.

An important consequence of the dissemination of the cholesterol-enriched microdomain is a profound increment in the surface viscosity of pulmonary surfactants [86]. Lateral phase separation is an important source of free area, defined as the difference between observed and minimum area per molecule [87]. This is analogous to the observation of reduction in the total volume after mixing ethanol with water. In such a case, the phenomenon can be interpreted as if the free volume of each molecule has decreased following mixing. Similarly, mixing of L_o with L_α results in a substantial decrease in the free area, while demixing induces its subsequent increase. According to the free area theory of surface viscosity [88], surface viscosity exponentially increases following the reduction of free area, driven by reduced tensile force along the two-dimensional phase boundaries. Indeed, a mere 3% decrease in the free area resulted in a striking 12-fold increase in the surface viscosity of pulmonary surfactants [87]. In accordance, upon hyperpnea, the biological system increases free cholesterol level in pulmonary surfactants, to reduce surface viscosity, which is required for facilitated respiration [89]. Similarly, the reduced free area following the inhalation of Vitamin E acetate may considerably increase the surface viscosity of the pulmonary surfactant, resulting in a disrupted respiratory compression-expansion cycling and a subsequent hypoxemia (Fig. 2b).

In this picture, intraalveolar lipid-laden macrophages are consequences of hypoxemia-induced excessive intracellular accumulation of triglycerides [90], and extensive vacuolization, multinucleation, and reactive hyperplasia observed in type II alveolar pneumocytes are products of an extensive tissue hypoxia [91,92]. Taking into account that the pulmonary-associated surfactant protein B (SP-B) often cooperates with cholesterol in reducing the surface viscosity of pulmonary surfactants during respiratory compression-expansion cycling [86], other than its known role in the reduction of surface tension in the steady state, it is worth to note that the partial deficiency of the protein results in a respiratory distress analogous to those observed in patients with EVALI [93]. In sum, vaporized Vitamin E acetate, a thermostable derivative of the well-established linactant, may substantially increase the surface viscosity of pulmonary surfactants and thereby confer the pathogenesis of EVALI.

Evaluating the hypothesis

The above-described biological actions of Vitamin E derivatives are closely intertwined to each other [83,94], and the effect of Vitamin E acetate on the pulmonary system in patients with EVALI is likely to be the sum of all these actions, rather than originating from a single action of the vitamin derivative. For instance, in the plasma membrane of cells, cholesterol-enriched microdomains, or lipid rafts [95], are involved in a plethora of cellular activities, including PKCα [96] and 5-LO signaling [97]. As a consequence, it is worth to note that the evaluation of these hypotheses should focus on identifying the major driving force for the pathogenesis of EVALI, rather than a sole cause, which most likely does not exist.

To evaluate whether the line-active property of Vitamin E acetate is driving the pathogenesis of EVALI, we must answer the following questions. First, is Vitamin E acetate, like *d-α*-tocopherol, a potent linactant? If it is, to what extent does the substance reduce the line tension at the two-dimensional phase boundaries and increase the surface viscosity? Molecular dynamics simulations, such as those previously introduced by Rosetti and colleagues [98], would particularly be useful, as they enable us to roughly estimate the fold change in the surface viscosity of pulmonary surfactants and thereby to evaluate the validity of the hypothesis. Second, are linactants, in general, capable of inducing respiratory distress when inhaled through e-cigarettes? One obvious experiment that must be done is the repeated inhalation of hybrid lipids in murine models. Hybrid lipids, which are phospholipids that contain both saturated and unsaturated acyl chains, are one of the most widely accepted class of linactants [98–101]. As a consequence, repeated inhalation of hybrid lipids, analogous to those attempted by Bhat and colleagues, should induce similar histological signs, for the hypothesis to hold. To rule out the potential role of Vitamin E acetate as an inducer of gel-liquid crystalline phase transition, rescue experiments using an inhalation setup for dimethyl sulfoxide (DMSO), which abrogates gel-liquid crystalline phase transition [102], can be attempted. To rule out the possibility that Vitamin E acetate drives the pathogenesis of EVALI through modulating DGK-PKC or PXR signaling, Vitamin E acetate inhalation experiment using either PKC*α* [103] or PXR knockout mice [104] can be pursued. Characterization of the lipid composition of the intraalveolar lipid-laden macrophages is also crucial, as it may assist us to rule out the hypothesis that an acute respiratory distress associated with EVALI is indeed a form of ELP. Lastly, as we still suffer from the lack of characterization of the type of injury in EVALI, I propose an RNA sequencing (RNA-seq) experiment for lung tissues obtained from murine models of EVALI [42]. Analyses on the level of transcripts associated with the biological action of the vitamin, such as cytochrome P450 monooxygenase genes, hypoxia-inducible factor 1 (HIF-1), and NRF2, as well as gene ontology analyses would greatly assist us to better define the pathology of the new respiratory disorder. RNA-seq experiment would also allow us to develop reliable biomarkers for EVALI to better assist health care providers in the differential diagnosis of the vaping-induced respiratory disorder against viral infections. Living in the era of the pandemic outbreak of the coronavirus disease 2019 (COVID-19), wherein tens of millions of

Table 1
Summary of possible mechanisms by which Vitamin E acetate may drive the pathogenesis of EVALI.

Role of Vitamin E acetate	Findings	Counterarguments
Inducer of gel-liquid crystalline phase transition	Vitamin E acetate lowers the critical temperature required for gel-liquid crystalline phase transition in model saturated phosphatidylcholine bilayers	Cholesterol, which exists in large excess to Vitamin E acetate in human pulmonary surfactants, reportedly abolishes gel-liquid crystalline phase transition in biological membranes
Inducer of exogenous lipoid pneumonia	Vitamin E acetate is stored in lipid droplets, and BAL fluids of patients with EVALI typically feature lipid-laden macrophages	ELP-like histological signs are absent in patients with EVALI, and the hypothesis does not explicitly explain how Vitamin E acetate initiates inflammation to induce ELP
Modulator of DGK-PKC pathway	Observed level of Vitamin E acetate in BAL fluids is of same orders of magnitude with that of DAG	Vitamin E derivatives inhibit inflammation via the DGK-PKC pathway
Pro-agonist of PXR	PXR target genes are associated with drug-induced interstitial lung diseases, of which pathology is consistent with that of EVALI	Repeated inhalation of well-established PXR agonists does not induce respiratory distress in human subjects
Linactant	Vitamin E is a potent linactant. Linactants exponentially increase the surface viscosity of pulmonary surfactants, and thereby cause extensive hypoxemia. This accounts for all major pathological observations in EVALI	It is yet to be proved whether Vitamin E acetate is line-active as unmodified Vitamin E, and whether the extent of linactivity of Vitamin E acetate is sufficient to confer an acute respiratory distress

people worldwide are being hospitalized with symptoms often indistinguishable from those of EVALI [105,106], establishing diagnostic biomarkers for EVALI would particularly be valuable.

Conclusion

In summary, I discussed several possible mechanisms by which Vitamin E acetate may drive the pathogenesis of EVALI and specifically advocated the role of Vitamin E acetate as a linactant (Table 1). Based on recent discoveries from the field of membrane biophysics, I proposed that the alleged linactivity of Vitamin E acetate, a thermostable derivative of a well-established linactant, significantly increases the surface viscosity of pulmonary surfactants in the alveoli to confer acute respiratory distress associated with EVALI. This hypothesis leads to new possible medication approaches for the new pulmonary disease. If it is true that the line-active property of the vitamin derivative is what is causing the disease, supplementation of pulmonary surfactants as well as corticosteroid treatments should be valid. To reduce the elevated viscosity of pulmonary surfactants, pulmonary surfactants which retain a moderate level of cholesterol, such as Infasurf [107], may be recommended for use. In addition, in a biophysical perspective, since the entropy component of the free energy, which is proportional to the temperature of the system, works against lateral phase separation in regular solutions [108,109], one can reverse the effects of Vitamin E acetate by slightly lowering the temperature of the system. Controlled hypothermia to rescue lateral phase separation and thereby reduce surface viscosity, as introduced by Autilio and colleagues [110], might be a useful way to manage acute respiratory distress syndrome associated with EVALI.

The current outbreak of EVALI is an emerging crisis affecting a great portion of the global population. As pathophysiologists, we have a duty to decipher the pathophysiology of EVALI for the good of all patients suffering from the disease, especially after the recent identification of the widely utilized derivative of the vitamin as one of the causal agents for the new catastrophic pulmonary disease.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mehy.2020.110182.

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