CORRESPONDENCE

An Animal Model of Inhaled Vitamin E Acetate and EVALI-like Lung Injury

TO THE EDITOR: As of February 4, 2020, electronic-cigarette, or vaping, product use–associated lung injury (EVALI) has resulted in the hospitalization of 2758 people across the United States and has been linked to at least 64 deaths (www.cdc.gov/tobacco/basic_information/e-cigarettes/severe-lung-disease.html). Although recent testing by the Centers for Disease Control and Prevention showed the presence of vitamin E acetate in bronchoalveolar-lavage (BAL) fluid samples obtained from patients with EVALI,[1,2] additional studies are necessary to determine whether a causal link exists between inhalation of vitamin E acetate and EVALI.

We exposed 30 mice (in three groups of 10) to aerosols generated from vitamin E acetate, a mixture of propylene glycol and vegetable glycerin (PG–VG), or air (controls). We estimated that the mice exposed to vitamin E acetate in our experiments would have inhaled 77.3 to 167.5 μg of vitamin E acetate per gram of body weight per day, a dose equivalent to the amount that an adult e-cigarette user would inhale by daily use of 0.52 to 1.13 ml of vaping product containing 88% vitamin E acetate. The levels of vitamin E acetate measured in mouse BAL fluid (Fig. 1A) suggest that this chemical was effectively delivered to the lungs of the exposed mice and match the findings of vitamin E acetate in BAL fluid from patients with EVALI.[1,2] At the end of the 2-week exposure period, the mice were euthanized, BAL fluid was harvested, and lung sections were examined by a pathologist. The details of study procedures are provided in the Supplementary Appendix, available with the full text of this letter at NEJM.org.

Numerous markers measured in our study indicated that pulmonary injury was present after inhalation of vitamin E acetate aerosols. Exposure to vitamin E acetate increased the levels of albumin in BAL fluid (a surrogate marker of lung epithelial damage) to a greater extent than exposure to PG–VG or to air (Fig. 1B). The total numbers of leukocytes in the lungs of mice exposed to vitamin E acetate were significantly higher than those in mice exposed to PG–VG or to air (Fig. 1C). Cells isolated from the BAL fluid of vitamin E acetate–exposed mice contained numerous lipid-laden macrophages (Fig. 1D), a finding consistent with clinical observations in patients with EVALI.[3,4] BAL cells recovered from mice exposed to PG–VG contained fewer identifiable macrophages, and there was no specific staining by oil red O (Fig. 1E). Examination of lung-tissue sections from mice exposed to vitamin E acetate revealed alveolar macrophages containing abundant oil red O–stained lipid, often in clusters, residing with pneumocytes lining the alveoli (Fig. 1F). In contrast, tiny oil red O–stained granules in the cytoplasm of cells lining the alveoli were observed in mice exposed to PG–VG (Fig. 1G).

This study contributes to our understanding of EVALI through the development of an animal model that can be used to evaluate the potential role of the suspected toxicant vitamin E acetate. A limitation of the study is that the aerosols generated by an e-cigarette may contain by-products of the thermal degradation of vitamin E acetate after heating. A chemical evaluation of the generated aerosols would be required to identify such by-products. Another limitation is that we did not expose animals to aerosols that contained tetrahydrocannabinol (THC) or nicotine in a dose-dependent manner. Finally, it is possible that aerosols generated from other lipophilic solvents may produce outcomes similar to the outcome seen with vitamin E acetate in this study.

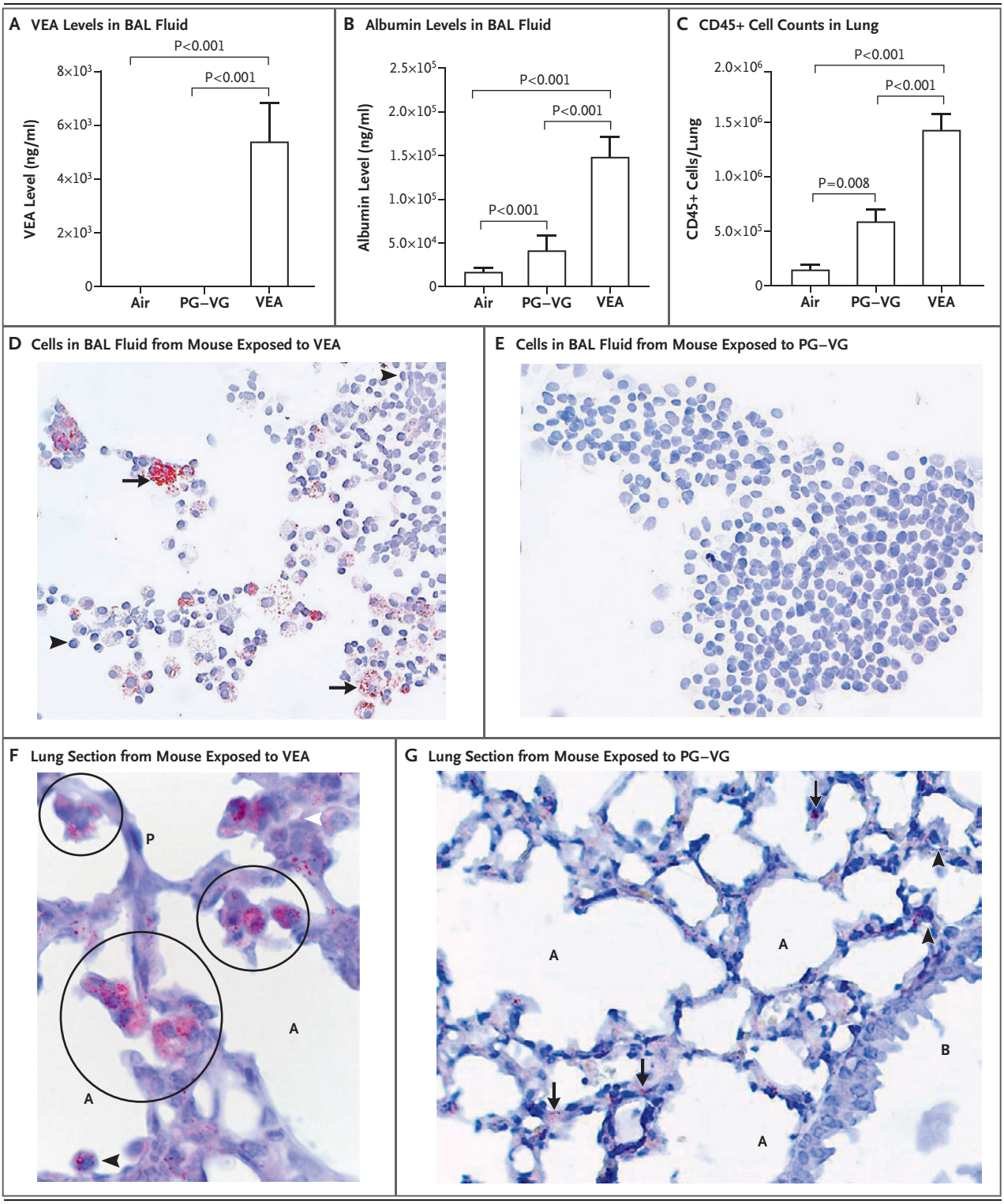
A VEA Levels in BAL Fluid
P<0.001
P<0.001
VEA Level (ng/ml)
8×10³
6×10³
4×10³
2×10³
0
Air
PG–VG
VEA
B Albumin Levels in BAL Fluid
P<0.001
P<0.001
P<0.001
Albumin Level (ng/ml)
2.5×10⁵
2.0×10⁵
1.5×10⁵
1.0×10⁵
5.0×10⁴
0
Air
PG–VG
VEA
C CD45+ Cell Counts in Lung
P<0.001
P<0.001
P=0.008
CD45+ Cells/Lung
2.0×10⁶
1.5×10⁶
1.0×10⁶
5.0×10⁵
0
Air
PG–VG
VEA
D Cells in BAL Fluid from Mouse Exposed to VEA
E Cells in BAL Fluid from Mouse Exposed to PG–VG
F Lung Section from Mouse Exposed to VEA
P
A
A
G Lung Section from Mouse Exposed to PG–VG
A
A
B
A

Figure 1 (facing page). Findings from a Mouse Model of Electronic-Cigarette, or Vaping, Product Use–Associated Lung Injury (EVALI).

Panel A shows levels of vitamin E acetate (VEA) quantified by isotope-dilution mass spectrometry in bronchoalveolar-lavage (BAL) fluid harvested from mice. Values are means and standard deviations for 10 mice. Panel B shows albumin levels measured in BAL fluid from mice exposed to air, a mixture of propylene glycol and vegetable glycerin (PG–VG), or VEA. Values are means and standard deviations for 10 mice. Panel C shows the total number of CD45+ cells infiltrating the lung in mice exposed to air, PG–VG, or VEA. Values are means and standard deviations for 10 mice. The P values in Panels A, B, and C were calculated by two-way analysis of variance in Tukey's post-test comparisons among the exposure groups. Panel D shows BAL fluid from a mouse exposed to VEA, containing lipid-laden macrophages (representative examples are indicated with arrows) with cytoplasmic staining by oil red O in a vesicular pattern. The macrophages are numerous and contain variable amounts of lipid. Background pneumocytes (arrowheads) show comparatively scant cytoplasm and are present as single cells or loose sheets. Panel E shows BAL fluid from a mouse exposed to PG–VG, which contained fewer identifiable macrophages and had minimal to no specific staining by oil red O. Without lipid staining, it is more difficult to distinguish between small alveolar macrophages and pneumocytes in these preparations. Panels F and G show findings in lung sections. In mice exposed to VEA (Panel F), alveolar macrophages (arrowheads and circles) in residence among pneumocytes (P) lining the alveoli (A) contained abundant oil red O–stained lipid. In mice exposed to PG–VG, tiny oil red O–stained granules in the cytoplasm of cells lining the alveoli, including pneumocytes (arrows) and alveolar macrophages (arrowheads), were observed. B denotes bronchiole.

Future studies are needed to address these issues. Our findings, coupled with previous research identifying vitamin E acetate in BAL fluid from patients with EVALI[1,2] and in samples of case-associated product liquids,[5] provide additional evidence for vitamin E acetate as a possible cause of EVALI.

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Disclosure forms provided by the authors are available with the full text of this letter at NEJM.org.

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