E-Cig Flavors May Damage Cardiovascular Cells

-Study shows that flavored e-liquids had varying effects on cell survival

by Zeena Nackerdien , CME Writer, MedPage Today
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CME Author: Zeena Nackerdien

Study Authors: Won Hee Lee, Sang-Ging Ong, et al.; Jane Freedman, Chinmay Trivedi

Target Audience and Goal Statement:

Pulmonologists, cardiologists, addiction specialists, toxicologists, pediatricians, family medicine physicians

The goal of the study was to examine whether electronic cigarette (e-cigarette) use and flavorings increased the risk for heart disease, based on research using human induced pluripotent stem cell–derived endothelial cells (iPSC-ECs).

Question Addressed:

- How did e-liquid, the flavored fluid that is used inside electronic cigarettes, affect human endothelial cells (cells that line blood vessels from the aorta to microvessels) generated in the laboratory from iPSC-ECs?
- What molecular insights could be gained from the impact of e-cigarette use on endothelial cells?
- Were the nicotine levels in the blood serum of e-cigarette and traditional cigarette users similar after smoking at a constant rate for a defined time period?

Synopsis and Perspective:

One of the risk factors for cardiovascular (CV) diseases is smoking-induced endothelial dysfunction. Joseph C. Wu, MD, PhD, of the Stanford School of Medicine in California, and colleagues used iPSC-ECs to assess the health impact of e-cigarettes. By definition, these cells can become many different cell types, and therefore provided investigators with a means to study cells that would be hard to isolate from any given patient.

Smoking cigarettes harms nearly every organ in the human body. According to the CDC, for every person who dies because of smoking, at least 30 people live with a serious smoking-related illness.

While the health consequences of smoking are clear, there is no one "best" way to quit. Many people who try to stop smoking continue to crave cigarettes, and may relapse before quitting permanently. E-cigarettes are battery-operated devices that typically contain a nicotine solution, flavorings, and other chemicals. Vaping, an alternate term for e-cigarette use, has been described as "a step in the right direction towards managing peoples' addictions and creating a pleasant experience out of it." However, limited research supports that claim.

While e-cigarettes do not contain the tar and smoke found in traditional cigarettes, the devices do contain nicotine. An addictive substance, nicotine is known to constrict blood vessels, temporarily raise blood pressure and heart rate, and elevate glucose levels. However, the potential harmful CV effects of inhaled e-cigarette flavoring chemicals were not known.

Wu's group exposed iPSC-ECs derived from three healthy individuals to six different, flavored e-liquids with varying nicotine concentrations (0, 6, and 18 mg/mL) and to serum from e-cigarette users. The popular flavors were fruit, tobacco, sweet tobacco with caramel and vanilla, sweet butterscotch, cinnamon, and menthol.

Participants from whom sera were drawn were five healthy non-smokers, five active cigarette smokers (cigarette), and two dual users of e-cigarettes and cigarettes as well as two sole users of e-cigarettes (e-cigarette). The latter two groups were combined into one category, because the participants were cigarette smokers who had started the use of e-cigarettes as an alternative to quitting the use of cigarettes, the authors noted in the *Journal of the American College of Cardiology*.

Vapers reported using their e-cigarettes an average of 27.5 days/month, with 9.8 e-cigarette sessions (more than two puffs) per day. Traditional smokers used, on average, 10.1 cigarettes per day and reported 29.8 days of smoking in the past 30 days. Dual users smoked less than two packs of cigarettes/month. All the participants were free from other CV risk factors. They were asked not to use combustible cigarettes for 7 days prior to all study visits and to abstain from the use of cigarettes, e-cigarettes, food, and caffeinated drinks for 12 hours prior to the study day.

The authors reported that flavored e-liquids had varying effects on cell survival, with cinnamon and menthol exerting the most potent cytotoxic effects. Interestingly, some of the damage caused by e-liquids occurred in the absence of nicotine, and the severity depended on the flavor.

Exposure of iPSC-ECs to the e-liquids or blood collected from vapers shortly after e-cigarette use led to less cell viability and an increase in molecules implicated in DNA damage and cell death. Inflammatory cytokines were also observed in the sera of e-cigarette users. Treated cells were also less able to participate in angiogenesis.

Comparable nicotine levels were found in the blood of e-cigarette and traditional cigarette users after 10 minutes of smoking at a constant rate.

Wu and colleagues emphasized that the study was conducted in a laboratory, and that in real life, cells other than endothelial cells could be affected by e-cigarettes and cigarettes. Because the effects of aerosols were assessed indirectly by looking at the addition of sera from vapers to iPSC-ECs, the results need to be interpreted within the context of a lack of heat or combustion.

The investigators suggested further studies to understand the potential risk from toxic compounds generated by e-cigarette products. They also cautioned that the set puffing protocol used in their experimental setting might not reflect actual user puff practice.

Study Highlights: Explanation of Findings:

Endothelial cells support vascular and heart health. Injured endothelial cells could impede the ability to form new blood vessels from existing vasculature and to heal wounds.

Selected e-liquid flavorings had detrimental effects on the viability and function of endothelial cells. Some of the molecular changes included increased production of reactive oxygen species and caspase 3/7. The latter enzymes serve as accelerants of cell death. Investigators also found that e-cigarettes provided effective and measurable nicotine delivery, as using the devices increased plasma nicotine concentrations comparable to levels achieved via traditional cigarettes.

"Increased ROS [reactive oxygen species] generation and inflammatory cytokines present in serum was observed in concert with acute e-cigarette use-induced endothelial dysfunction, as indicated by impaired tube formation of iPSC-ECs," Wu and colleagues wrote.

Statistics about e-cigarette use among U.S. youth has shown a 1-year increase of 78% among high-school students. A similarly high percentage of current youth e-cigarette users (81% in a 2013-2014 survey) cited the availability of appealing flavors as the main reason for use.

"One in five high school students have tried e-cigarettes, perhaps because they feel they are relatively safe," noted co-author Won Hee Lee, PhD, of the University of Arizona in Phoenix, in a Stanford news release. "But we found the e-liquids caused changes in the endothelial cells that are closely related to those seen during the development of cardiovascular disease."

In an accompanying editorial, Jane Freedman, MD, and Chinmay Trivedi, MD, PhD, both of the University of Massachusetts Medical School at Worcester, wrote that a "clear limitation" of the study was the reliance on a culture system.

"Due to organ specific molecular heterogeneity of the vascular endothelium, it would be critical to examine effects of the various liquids in animal models. Most importantly, these in vivo models with hard endpoints after longer-term use, or after multiple uses, would allow us to determine a causal role

of these liquids in cardiovascular disease," they wrote.

Despite this limitation, Freedman and Trivedi said the findings clearly demonstrate "that e-liquid flavorings had stronger effects on cytotoxicity, vascular dysfunction, and angiogenesis than nicotine. Thus, in addition to harm from the nicotine, the additives are a potential source of adverse vascular health and one that is being disproportionately placed on the young."

They noted that mounting data continue to show health harms for e-cigarette use, and the uptake of e-cigarettes among youth highlights the need for regulation of the products.

"These observations suggest that, even without the smoke of combustible cigarette products, there may be a smoldering fire of adverse health effects," Freedman and Trivedi stated.

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