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Article

Comparing the effects of electronic cigarette smoke and conventional cigarette smoke on lung cancer viability

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SUMMARY

The popularity of electronic cigarettes (e-cigarettes) has been noted to be increasing in recent years — for multiple reasons, including the fact that e-cigarettes are often cheaper, available in a variety of "flavors," and easily accessible, to both adolescents and young adults find e-cigarettes to be a more appealing option than traditional cigarettes. Even though levels of carcinogens are reduced in e-cigarettes in comparison with tobacco cigarettes, there is increasing concern that vaping e-cigarettes may also increase the risk of lung cancer. Instead of burning tobacco, e-cigarettes contain liquid solutions of three main components: solvents, flavors, and, mainly, nicotine. We hypothesized that the chemicals from e-cigarettes and cigarette smoke might affect lung cancer cell viability. To test this hypothesis, we collected smoke extracts from different types of cigarettes: regular tobacco, cigar-like e-cigarette, and vape-type e-cigarette. By exposing A549 cells, human lung cancer cells, to the different types of smoke extracts, we wanted to see how cell viability would be affected. Among the three different cigarette extracts, vape-type e-cigarette smoke extract significantly increased the A549 cell viability. Since vape-type e-cigarettes contain the highest concentration of nicotine compared to the other types of cigarettes, we hypothesized that nicotine might be the cause of increased lung cancer viability. When different concentrations of nicotine were tested on the A549 cell line, the results showed that up to 2% nicotine concentration increased the A549 cell viability significantly, but more than 5% nicotine concentration induced cell death due to its high toxicity. In conclusion, contrary to conventional thought, e-cigarettes, or vapes, may be more dangerous than tobacco cigarettes in terms of lung cancer viability.

INTRODUCTION

E-cigarettes, otherwise known as vapes, are often used as a substitute for regular cigarettes. To date, e-cigarettes are not approved by the United States Food and Drug Administration (FDA) federal agency for smoking cessation. Since e-cigarettes are generally thought to contain fewer toxic chemicals than regular cigarettes, some researchers claim that they are a safer alternative to other smoked tobacco products (1).

The first e-cigarette device was developed in 2003 by Chinese pharmacist Hon Lik, who was looking for a healthier alternative to smoking. Since then, e-cigarettes have been rising rapidly, with reports showing an increase in the adolescent population's interest (2). Many people believe that e-cigarettes are better alternatives to cigarettes for different reasons: e-cigarettes are thought to be less harmful in terms of health effects; they do not leave behind the pungent odor from smoke, and they are considered to be "sleek" and thus contribute to social bonding and other social interactions (3). Therefore, many people often look for e-cigarettes, unaware of the possible health effects they might cause on human lungs.

Various types of vaping e-cigarettes are currently released on the market. Among them, cigar-like e-cigarettes are composed of similar content as conventional tobacco cigarettes, containing both nicotine and tobacco. However, the device is electronic and thus utilizes a heating method, not combustion, to produce the smoke (4). Another form of e-cigarettes contains liquid instead of the traditional solid components. Naturally, instead of "smoking," the users of e-cigarettes inhale vapor (thus the alternative term for the use of an e-cigarette, "vaping") (1). Vapor is produced by heating the liquid cartridges within the e-cigarette, which are comprised of propylene glycol or glycerin, flavorings, and nicotine (5). After the heating process, the liquid becomes an aerosol, which can be inhaled from the device.

Inhaling the aerosol vapor produced by the e-cigarette device allows the particles of these ingredients to permeate into the lung tissue (6). Even though most of the ingredients present in the liquid are known to be generally recognized as safe by FDA, that is only relevant when consuming these ingredients individually. The claim does not consider the action of breathing such components after combustion or heating (7). In fact, many of the ingredients commonly found in vaping devices break down to form dangerous compounds when heated, with some ingredients being found to have cancer-causing properties (8). There are even instances where vapes have been found to include traces of toxic heavy metals leached from the heating elements themselves (9).

Even now, little is known about the effects these ingredients have on the human body when inhaled, especially for a long duration, with research in this area still developing (10). A previous study, for instance, indicated that while short-term exposure to e-cigarettes is suggested to induce harmful

effects, such as cardiovascular diseases, the effects of long-term exposures are still unknown (11). As such, the effect of e-cigarettes on lung cancer has not been fully established.

The chemicals from tobacco cigarette smoke extract (CSE), cigar-like e-cigarette smoke extract (ECSE), and vape extract (VE) may affect lung cancer cell viability. Since a previous study indicated that vaping contains a significantly higher percentage of nicotine (maximum of 15.4 mg in comparison to only 1.1~1.8 mg intake in a traditional cigarette when inhaled), we hypothesized that VE might enhance lung cancer cell viability more than the smoke extract from the other two types of smoke (12). In this research, we observed the increased cell viability in the VE-treated lung cancer cell line. We concluded that, contrary to conventional belief, vape-type e-cigarettes might have more significant effects than tobacco cigarettes regarding lung cancer progression.

RESULTS

The purpose of this experiment was to compare the lung cancer cell viability when the cell cultures were treated with either CSE, ECSE, or VE. In doing so, we hoped to find the effect of smoke extracts on lung cancer viability. We prepared the soluble smoke extracts with phosphate-buffered saline (PBS) buffer solution in a 50 ml syringe **(Figure 1)**. Using these, we analyzed the effect of smoke extracts on lung cancer cell viability. After the extraction of the soluble chemicals from the smoke, we exposed the A549 cell line to six different concentrations of CSE, ECSE, and VE for 24 h: 0, 1, 2, 5, 7, and 10% weight/volume (w/v). The extract obtained from one cigarette type was defined as 100% concentration. They are diluted to the indicated w/v percentage in RPMI1640 cell medium. As CSE, ECSE, or VE concentrations increased up to 10% (w/v) in the RPMI1640 cell medium, lung cancer cell viability increased as well **(Figure 2)**. Overall, treatment of all CSE, ESCE, and VE extract significantly increased the lung cancer cell viability in comparison to the control condition with 0% treatment **(Figure 2)**.

In the next experiment, we further investigated the effect of a 10% (w/v) concentration of cigarette extracts on lung cancer cell viability. In doing so, we increased the incubation time to 48 hr. Compared to the control sample, all cell cultures treated with various smoke extracts displayed significantly greater cell viability **(Figure 3)**. Between CSE-treated and ECSE-treated samples, the difference in the cell viability was insignificant **(Figure 3)**. Interestingly, however, the VE-treated cell line displayed significantly greater cell viability in comparison to the other two **(Figure 3)**. Overall, all three different cigarette extracts enhanced lung cancer cell viability at 10% concentration, but the VE-treated sample showed the most increase in viability among other extracts.

We speculated that the notable difference between VE-treated samples and other types of samples has to do with the substantial nicotine content within Vape-type cigarettes. Previous research indicated that vape cigarettes have more nicotine than traditional cigarettes (12). Therefore, we hypothesized that nicotine would be one of the substances inside the smoke extract that might enhance lung cancer viability. Therefore, we tested lung cancer cell viability in the presence of an increasing concentration of nicotine. Six different concentrations of nicotine were tested: 0, 1, 2, 5, 7, and 10% (w/v). No significant difference was observed between 0% and 1% nicotine-treated lung cancer cell viability **(Figure 4)**. However, compared to the untreated samples, a significant increase in cell viability was detected with 2% nicotine **(Figure 4)**. For concentrations of 5% and greater, however, the cell viability decreased significantly **(Figure 4)**. We concluded that nicotine seems to increase cell viability until it reaches concentrations that appear toxic, in which the cells are no longer able to survive.

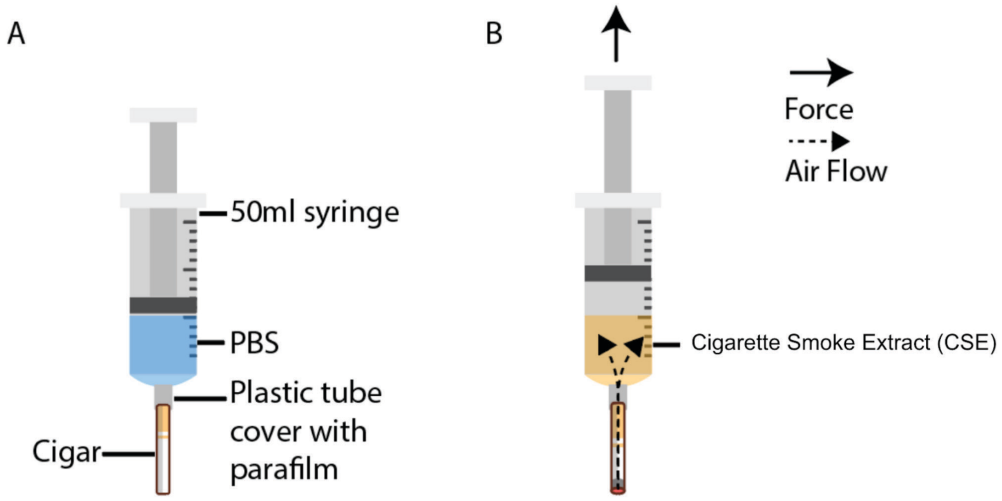

Figure 1: A simple syringe method used to collect the smoke extract. (A) The basic setup of the method, and (B) the process by which the smoke extract was collected.

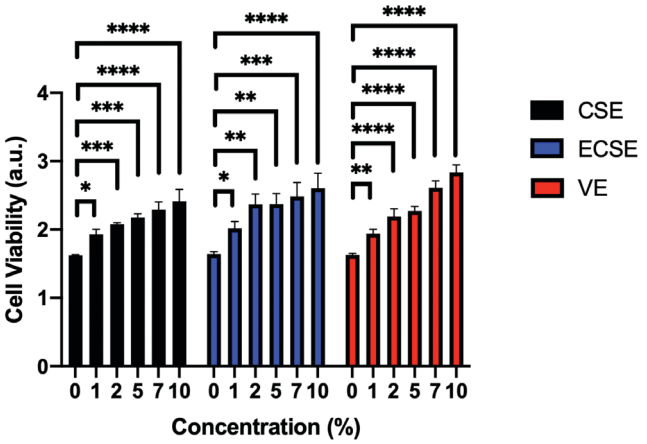

Figure 2: Effect of different concentrations of CSE, ECSE, and VE on A549 cell viability. Bar graph showing mean ± SD of cell viability using arbitrary unit (a.u.) of 570 nm absorbance (n=3). A549 cancer cells were grown under either 0%, 1%, 2%, 5%, 7%, 10% (w/v) concentration of CSE (black), ECSE (blue), or VE (red) for 24 h. One way ANOVA with Tukey's post hoc test, $^{ns}p > 0.05$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

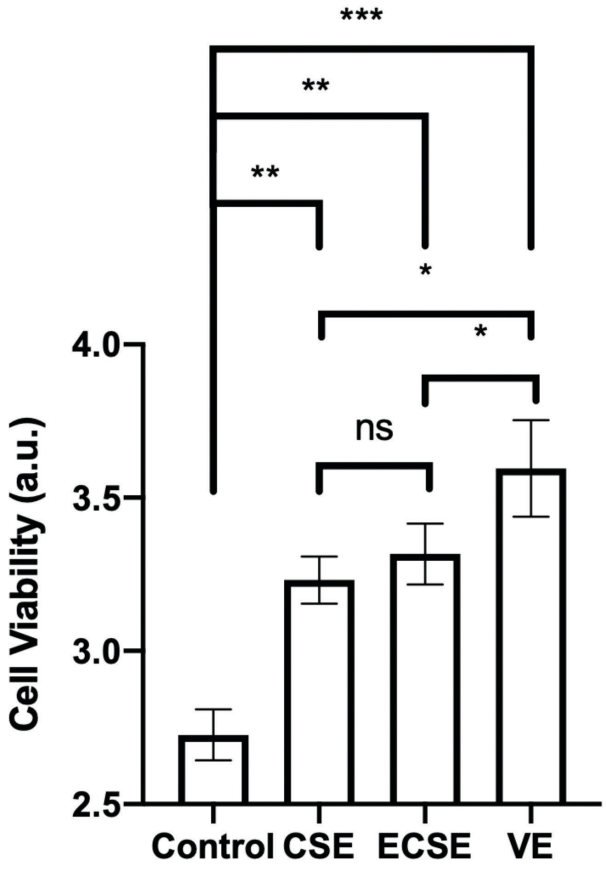

Figure 3: Effect of different types of the smoke extract on A549 lung cancer cell viability. Bar graph showing mean ± SD of cell proliferation using arbitrary unit (a.u.) of 570 nm absorbance (n=4). A549 cancer cells were grown under either control conditions without smoke extract, with 10% w/v CSE, with 10% w/v ECSE, or with 10% w/v VE for 48 h. One way ANOVA with Tukey's post hoc test, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{****}p < 0.0001$.

DISCUSSION

In treating the cell lines with different types of smoke extract, we observed that cell viability shows an overall positive trend that correlates with the increased concentration of smoking extract. While CSE- and ECSE- treated lung cancer cells did not show a significant difference in comparison to each other, we found VE enhances lung cancer viability most effectively compared to both CSE and ECSE **(Figure 3)**.

Due to the high concentration of nicotine in VE, we speculated that nicotine might be a potential promotor of lung cancer cell viability. Previous research has indicated nicotine may induce lung cancer development (13). Consistent with previous research, treatment of lung cancer cells with 2% nicotine resulted in an overall increase in cell viability. Concentrations of nicotine 5% or greater, however, caused a drastic decrease in cell viability. This result is consistent with the previous study indicating that high nicotine concentrations (>1.0 μM) had cytotoxic effects and induced cell death (14). Overall, we concluded that VE leads to a significant increase in cancer cell proliferation, perhaps due to its high nicotine concentration. Upon additional experiments, we identified nicotine as a potential cause of this phenomenon, but further investigation is necessary.

There are some limitations to this research. When we extracted chemicals from the smoke for each type of cigarette, we used one standardized cigarette or e-cigarette pod per cigarette extract. Thus, there may have been slight variation stemming from the different brands and models. Also, the experiments were conducted with only one type of lung cancer cell line, which may have led to a restrictive understanding of how different types of cigarette smoke extracts affect lung cancer progression. Therefore, more types of lung cancer cell lines should be used to obtain a more comprehensive understanding of the influence of cigarette exposure. Also, in vivo experiments such as using a mouse model may further verify our results. In vivo studies would allow the investigators to address many of the shortcomings of in vitro studies, for scientists can better evaluate the safety, toxicity, and efficacy of a drug candidate in a complex animal model.

Further, the method we used to obtain the smoke extract was not fully optimized. There was leakage of the PBS solution while extracting the smoke from the cigarette, causing the cigarette to dampen and be extinguished. Moreover, some amount of smoke escaped the syringe, and cigarettes were not fully combusted due to safety and logistical reasons. Such limitations of the experimental design may have led to inaccurate results.

Additionally, we treated the cells only with the soluble substances from the extract. We speculate that it is most likely that soluble substances in the smoke will have a greater effect on the lung cells than the insoluble ones as they can cause intracellular effects more effectively. At the same time, however, it is possible that the insoluble substance would also affect lung cancer cell viability through interactions with the cell surface and the extracellular matrix. The way that this

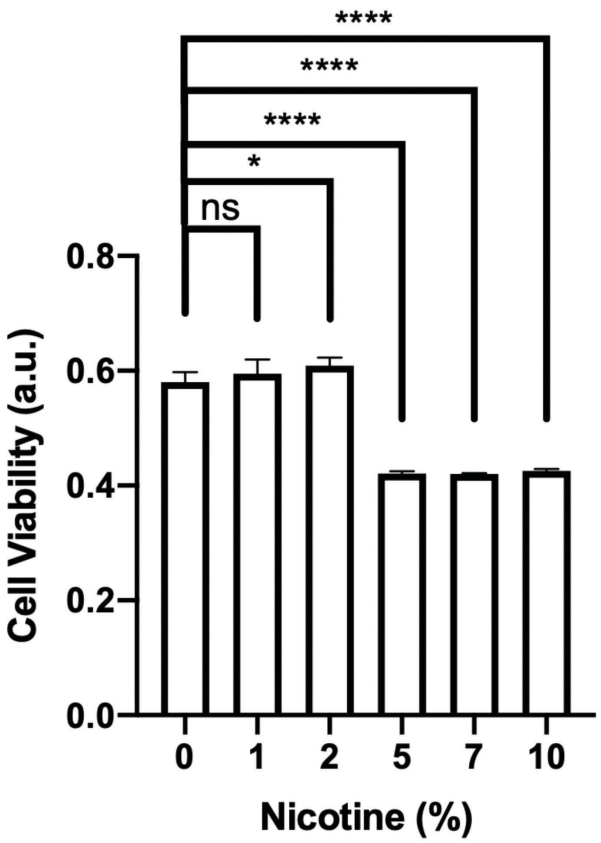

Figure 4: Effect of different concentrations of nicotine on cell viability of A549 lung cancer cell line. Bar graph showing mean ± SD of cell proliferation using arbitrary unit (a.u.) of 570 nm absorbance (n=4). A549 cancer cells were grown under either 0%, 1%, 2%, 5%, 7%, or 10% nicotine for 24 h. One way ANOVA with Tukey's post hoc test, $^{ns}p > 0.05$, $^{*}p < 0.05$, $^{****}p < 0.0001$.

experiment was designed does not allow us to test for the effect of the insoluble substances on lung cancer viability.

We only investigated the effect of the smoke extract on the degree of cancer cell viability. Further research analyzing different functional assays related to cancer progressions such as cancer cell migration, invasion, and metastasis could be implemented. Overall, we found increased cell viability in the VE-treated lung cancer cell line. Therefore, our study indicates that a high concentration of nicotine in vape-type e-cigarettes may facilitate lung cancer progression.

MATERIALS AND METHODS

Cigarette Smoke Extraction from Cigarette, Cigarlike e-Cigarette, and Vape Pen

A syringe was used to collect CSE, ECSE, and VE. All three types of cigarettes (RASON BLUE, MIXX, and Classita Juice) were purchased at Seven Eleven in South Korea. We placed the cigarette, cigarlike e-cigar, and vape pen on the tip of the 50 mL syringe, which was filled with 30 mL of PBS buffer solution **(Figure 1A)**. Smoke was extracted by pulling the syringe, and the air was pulled inside through the cigarette to produce the smoke **(Figure 1B)**. After the smoke was generated, the smoke was injected through the PBS solution. In this process, soluble substance from the smoke was solubilized in PBS buffer solution.

Cell Culture and Maintenance

Using one vial of A549 human lung cancer cells, purchased (Korea Cell Line Bank, Cat# 10185) for research purposes, we cultured A549 cells in a 37°C, 5% CO_2 incubator. A549 cells were maintained in RPMI1640 medium (Gibco, Cat# R8758) supplemented with 10% fetal bovine serum (Gibco, Cat# f4135) and 1% penicillin and streptomycin (Gibco, Cat# 15140122). The cell medium was changed every three days.

Cell Viability Assay

After cells were seeded with 100,000 cells in each well of a 96-well cell culture plate, CSE, ECSE, and VE with or without nicotine (Sigma) was diluted into percent weight per volume (% w/v) in RPMI1640 cell media. When the cells were incubated with CSE, ECSE, VE, and nicotine for 24 or 48 h, PrestoBlue cell viability reagent (Invitrogen, Cat# A13261) was added to each sample according to the protocol provided by the manufacturer. After 1 h incubation, 570 nm absorbance was quantified by a microspectrometer (Biotek).

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