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Effect of electronic cigarettes on human middle ear

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ABSTRACT

Objective: Electronic cigarettes (e-cigarettes) are the most commonly used electronic nicotine delivery systems and are a relatively new product designed for smoking cessation. The market scale of electronic cigarettes is growing rapidly, but the potential impact of e-cigarettes on public health has not yet been verified. In this study, we examined the effect of e-liquids on a human middle ear epithelial cell (HMEEC) line.

Material and methods: The main components of e-liquids are propylene glycol, vegetable glycerin and flavoring agents with or without nicotine. We analyzed 73 bottles of e-liquids from 12 different manufacturers, evaluated the trace elements in e-liquids, and identified the cytotoxicity of e-liquids on HMEECs in the presence or absence of nicotine.

Results: In the trace elements analysis, nickel, arsenic, cadmium, and lead were detected in the e-liquids. E-liquids without nicotine decreased cell viability, and the average IC 50 value of total e-liquids (n = 73) was 2.48 ± 0.93%. Among the different flavors, menthol-flavored e-liquids significantly reduced cell viability, and their average IC 50 value (n = 28) was 1.85 ± 0.80%. The average IC 50 values were distinct among manufacturers and the proportion of the solvents.

Conclusion: The present study provides evidence that e-cigarettes influence and reduce human middle ear cell viability even without the application of nicotine. Additionally, the cytotoxicity of e-liquids was affected by the flavoring agents.

1. Introduction

Electronic cigarettes (e-cigarettes) are the most commonly used electronic nicotine delivery systems (ENDS), and they are a relatively new product designed to simulate and help quit smoking. E-cigarettes officially entered the market in 2007 [1,2], and the market has been growing rapidly, reaching $3 billion in 2013 globally [3]. There are little data on ENDS use at the global level, but ENDS use has been reported to be at least doubled among both adults and adolescents from 2008 to 2012 [4]. Other recent data report that e-cigarette use by adolescents has increased, and over a quarter of a million never-smoking youths have tried e-cigarettes by 2013 [5–7]. Although e-cigarettes are used as cessation tools and are a safer alternative to traditional cigarettes [8], they paradoxically create demand among youth [5,7], and evidence of e-cigarette efficacy in reducing traditional cigarette smoking remains to be determined [9,10].

An e-cigarette is composed of a battery, a vaporizing chamber and an electronic liquid (e-liquid); e-cigarettes heat a solution to deliver an aerosol to users when they inhale. The main components of e-liquid are propylene glycol, vegetable glycerin, and flavoring agents with or without nicotine. While all the components of e-liquid, except nicotine, are used as food ingredients and are generally recognized as safe, these concepts apply only to ingestion, not to inhalation [11]. Additionally, since there are no regulations or manufacturing standards for the ingredients of e-liquids, an increasing number of new flavor chemicals are introduced into the market annually.

Although e-cigarettes are considered to be less toxic than conventional tobacco, relatively little is known about the content and toxicity of e-liquids and e-cigarette aerosols. Toxic chemicals, including carcinogens and heavy metals, that are not typically found in e-liquids have been identified at low levels in diverse e-cigarette aerosols [12,13]. Studies have reported that e-liquids are cytotoxic to human pulmonary fibroblasts, human embryonic stem cells and mouse neural stem cells and that the cytotoxicity is related to the flavor chemicals [14,15].

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Other studies have shown that both e-liquids and aerosols induce toxicity, oxidative stress and an inflammatory response in lung epithelial cells and in the mouse lung [16].

The potential effect of e-cigarettes on the upper airway or middle ear has not yet been identified. The middle ear is connected to the nasopharynx through the eustachian tube and is vulnerable to bacterial infection and environmental pollutants. Additionally, since conventional smoking of a family member is a well-known cause of otitis media, the use of e-cigarettes may influence middle ear mucosa and increase the occurrence of otitis media. In this study, we examined the effect of e-liquids on human middle ear.

2. Material and methods

We selected the most popular brands of e-liquids that dominate the domestic market after reviewing various rapidly changing products. We analyzed 73 bottles of e-liquids from 12 different brands purchased from local retailers. The bottles were kept at room temperature and protected from light until they were used for analysis. The solutions were classified into five flavor groups: tobacco (n = 13), coffee (n = 6), fruit (n = 21), mint/menthol (n = 28) and other flavor varieties (e.g., nuts, caramel, coke, honey, bubble gum) (n = 5) (Table 1).

HMEECs (kindly provided by Dr. David J. Lim, House Ear Institutes, Los Angeles, CA, USA [17] immortalized with the *E6/E7* genes of human papilloma virus type 16 [18] were maintained in a mixture of Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA, USA), bronchial epithelial basal medium (BEBM) (Lonza, Walkersville, MD, USA) (1:1) and other growth medium supplements, including bovine pituitary extract (52 μg/mL), hydrocortisone (0.5 μg/mL), human epidermal growth factor (hEGF; 0.5 ng/mL), epinephrine (0.5 mg/mL), transferrin (10 μg/mL), insulin (5 μg/mL), triiodothyronine (6.5 ng/mL), retinoic acid (0.1 ng/mL), gentamycin (50 μg/mL), and amphotericin B (50 ng/mL). To study the effects of e-liquids, the cells were grown to 60% confluence in 96-well culture plates at 37 ˚C in a carbon dioxide-enriched (95% air, 5% CO2) humidified atmosphere. These cells were starved for 2 h, exposed to e-liquids and subsequently incubated for 24 h. The experimental group was exposed to various concentrations of e-liquids (0, 0.01, 0.1, 1, 2, 3, 4 or 5%), whereas the control group was not exposed to e-liquids.

Three different tobacco flavors from diverse manufacturers (A, B, C) were examined to identify the presence and the concentration of heavy metals in the e-liquids. Approximately 0.5 g of each sample was accurately weighed and placed in a PFA (Perfluoroalkoxy) digestion vessel, and then, 5 mL of 70% HNO3 (Electronic grade, Dongwoo Fine Chem. Korea) was added. The closed vessel was heated on a hot plate at 150 ˚C for about 5 h. Samples were cooled to room temperature with the vessels open. The vessels were heated on a hot plate until white fumes were released, and then, the residues were redissolved in a polypropylene bottle with 10 mL of 1% HNO_3. The concentrations of Cu, Pb, Zn, Cr, and other elements were determined using inductively coupled plasma mass spectrometry (ICP-MS 7700, Agilent Technologies).

Cell viability was measured using cell counting kit-8 (CCK-8, Dojindo Laboratories, Kumamoto, Japan). HMEECs were seeded onto 96-well plates, with 1×10^4 cells in each well. The following day, the cells were treated with 0, 0.01, 0.1, 1, 2, 3, 4, or 5% e-liquid. CCK-8 solution was added to each well after 24 h, and the plates were incubated for 150 min at 37 ˚C. The contents of the plates were mixed using a shaker (at room temperature for 5 min), and the optical density was measured at 450 nm using a microplate reader (Spectra Max plus 384; Molecular devices, Sunnyvale, CA, USA). ED50plus v 1.0 software was used to calculate the IC 50 (half maximal inhibitory concentration). First, three different tobacco-flavored e-liquids from diverse manufacturers (A, B, C) were applied to test cell viability in the presence or absence of nicotine. Then, the effects of all 73 e-liquids without nicotine on cell viability were evaluated.

The main components of e-liquids are propylene glycol (PG), vegetable glycerin (VG), and flavoring agents (with or without nicotine); the former two components are considered solvents. Since there are no known manufacturing standards for the ingredients and the compositions of the solution varied among the brands, we selected 5 different ratios of PG and VG for the positive control (PG:VG; PG only, 3:7, 5:5, 7:3 and VG only). HMEECs were treated with 0, 0.01, 0.1, 1, 2, 3, 4, and 5% of five different ratios of solvents. Then, cell viability was measured using cell counting kit-8 as described above.

All values are represented as the mean ± SD. For data analysis, we used the SPSS 24.0 statistical program. Kruskal-Wallis test was used to compare between two groups and ANOVA was used to compare multiple groups in the cell viability assay. A p value of < 0.05 was considered statistically significant. For multiple comparisons, Bonferroni correction was performed.

Table 1
Flavors of e-liquids from 12 brands.

Flavor	Number of e-liquids
Tobacco	13
Coffee	6
Fruits (different sub-flavors as below)	21
aloe, apple(2), banana, blackberry, cherry(2)	
citron, coconut, grape, green grape, hanrabong (JeJu orange)	
lemonade, mango, melon, peach, pear	
plum, pomegranate, raspberry, strawberry	
Menthol (different sub-flavors as below)	28
menthol only (6), apple, blueberry (2), bubbleberry, chocolate	
green tea, lemon, litch, melon, peach	
pineapple, pomegranate, raspberry, tobacco, tropical fruit	
tundraberry (2), other mixed fruit (4)	
Etc	5
bubble gum, caramel, cola, honey, nut	
Total	73

3. Results

Three different tobacco-flavored e-liquids (A, B and C) were examined to identify the presence and the concentration of heavy metals. All three samples contained Ni, As, Cd and Pb (Table 2). Sample A contained 1.32 ppb of nickel, 2.66 ppb of arsenic, 0.95 ppb of cadmium, and 13.10 ppb of lead. Sample B contained 5.11 ppb of nickel, 3.04 ppb of arsenic, 1.28 ppb of cadmium, and 24.39 ppb of lead. Sample C contained 3.87 ppb of nickel, 0.83 ppb of arsenic, < 0.25 ppb of cadmium, and < 0.25 ppb of lead.

The effect of three different tobacco flavors (A, B and C) on cell viability was examined to identify the effect of nicotine. When cultured cells were exposed to the three different e-liquids without nicotine, cell viability was (Fig. 1) inversely proportional to the concentration of e-liquids. However, when the cells were exposed to e-liquids with nicotine, cell viability was more significantly reduced ($p < 0.05$).

We analyzed 73 bottles of e-liquids from 12 different brands. Nicotine was not added, and all the solutions did not contain nicotine. The effect of each e-liquid on cell viability was measured using CCK-8 analysis, and the IC 50 was obtained using ED50plus v1.0 software (Fig. 2). The average IC 50 value of the total e-liquids (n = 73) was

Table 2
The concentration of trace elements in samples (ppb).

Elements	A	B	C
Ni	1.32	5.11	3.87
As	2.66	3.04	0.83
Cd	0.95	1.28	< 0.25
Pb	13.10	23.49	< 0.25