Comparative Study on In Vitro Toxicity of E-Cigarette Liquids to CHO and Respiratory Cells

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Abstract: With the increasing popularity of electronic cigarettes, the potential health risks of e-cigarette liquids have attracted widespread attention. This study aimed to compare the in vitro toxicity of e-cigarette liquids from different brands to Chinese Hamster Ovary (CHO) cells, upper respiratory tract cells (RPMI2650), and lower respiratory tract cells (HPF) through a series of cytotoxicity assays. Six detection methods (MTT, LDH, BrdU incorporation, WST-1, CCK8, and neutral red phagocytosis assay) were used to evaluate cell viability, membrane integrity, proliferation, and metabolic activity.The results are expected to provide experimental basis for understanding the toxic effects of e-cigarette liquids on different cell types and assessing their potential risks to human health.

***Keywords*:** in vitro toxicity, e-cigarette liquids, CHO, respiratory cells

1. Introduction

In recent years, electronic cigarettes have been widely used as an alternative to traditional cigarettes, with their market share expanding rapidly worldwide. E-cigarette liquids, as the core component of electronic cigarettes, typically contain nicotine, propylene glycol, glycerol, and various flavoring agents. However, the potential toxic effects of these components, especially on biological systems, remain unclear.

In vitro cytotoxicity studies are crucial for evaluating the safety of e-cigarette liquids, as they can directly reflect the damage of substances to cells under controlled conditions. CHO cells, as a commonly used mammalian cell line in toxicology research, have stable biological characteristics and are widely applied in toxicity screening. Meanwhile, respiratory tract cells (including upper respiratory tract RPMI2650 cells and lower respiratory tract HPF cells) are direct targets of e-cigarette aerosols, making them key objects for assessing respiratory toxicity[1].

This study compared the toxic effects of e-cigarette liquids on CHO cells and respiratory tract cells using multiple cytotoxicity detection methods, combined with component analysis of e-cigarette liquids, to clarify the differences in toxic responses among different cell types, thereby providing scientific support for the risk assessment of e-cigarette liquids.

2. Materials and Methods

2.1 Experimental Objects

Three types of cells were used in this study: Chinese Hamster Ovary cells (CHO cells). Upper respiratory tract cells (RPMI2650 cells). Lower respiratory tract cells (HPF cells)

2.2 Sample Working Solution

E-cigarette liquids from different brands were used as test samples. The samples were prepared into working solutions of different concentrations for subsequent cytotoxicity tests.

2.3 Cell Culture

CHO cells and RPMI2650 cells were cultured in RPMI1640 medium supplemented with 10% fetal bovine serum. HPF cells were cultured in FM cell culture medium. When the cell confluence reached 70%-80%, the cells were digested with 0.25% trypsin to form a single-cell suspension. After counting under a microscope, 100 μL of single-cell suspension (1×105cells/mL) was added to a 96-well cell culture plate (excluding the outermost wells).

2.4 Cytotoxicity Detection

2.4.1 MTT Cytotoxicity Assay

Logarithmic phase cells were digested with trypsin, and 100 μL of single-cell suspension (1×10^5 cells/mL) was added to a 96-well plate. The plate was incubated in a 37°C, 5% CO_2 incubator for 24 h until the cell confluence reached approximately 80%. Different concentrations of sample working solutions were added, with 6 replicate wells for each concentration. The

plate was incubated for another 24 h under the same conditions. After changing the medium, 20 μL of MTT solution (5 mg/mL) was added to each well, and the plate was incubated for 4 h. The solution was removed, and 200 μL of DMSO was added to each well. The plate was shaken on a micro-oscillator for 10 min. The absorbance at 490 nm was measured using a microplate reader[2].

2.4.2 LDH Assay

Logarithmic phase cells were processed as described in 2.4.1, and sample working solutions of different concentrations were added (6 replicate wells per concentration) followed by 24 h of incubation. 15 min before the end of exposure, 5 μL of lysis buffer was added to the LDH maximum release group, and incubation was continued for 15 min. 100 μL of freshly prepared LDH reaction mixture was added to each well of the 96-well plate, and the reaction was carried out in the dark for 15 min. 50 μL of stop solution was added to each well, and the plate was shaken for 10 s. The absorbance at 490 nm was detected using a microplate reader, with a reference wavelength of 630 nm[3].

2.4.3 BrdU Incorporation Assay

Logarithmic phase cells were prepared and incubated with sample working solutions as described above (6 replicate wells per concentration) for 24 h. 2 h before the end of exposure, 10 μL of BrdU dye was added to each well, and incubation was continued for 2 h. After removing the solvent, 200 μL of cell fixation and denaturation solution was added to each well, and incubated at room temperature for 30 min. The solution in the wells was aspirated, and 100 μL of peroxidase-labeled anti-BrdU antibody was added to each well, followed by incubation at room temperature for 90 min. The cell culture plate was washed 3 times with 300 μL of washing solution. 100 μL of enzyme reaction substrate was added to each well, and the reaction was carried out for 30 min. The absorbance at 370 nm was measured.

2.4.4 WST-1 Assay

Logarithmic phase cells were treated with sample working solutions (6 replicate wells per concentration) for 24 h as described. After exposure, 10 μL of WST-1 reagent was added to each well, and the plate was incubated in a 37℃, 5% CO_2 incubator for 2 h. After shaking for 1 min, the absorbance at 450 nm was measured using a microplate reader, with a reference wavelength of 630 nm.

2.4.5 CCK8 Assay

Logarithmic phase cells were incubated with sample working solutions (6 replicate wells per concentration) for 24 h. 10 μL of CCK8 reagent was added to each well, and the plate was incubated in a 37℃, 5% CO_2 incubator for 2 h. After shaking for 1 min, the absorbance at 450 nm was detected using a microplate reader, with a reference wavelength of 630 nm.

2.4.6 Neutral Red Phagocytosis Assay

Logarithmic phase cells were exposed to sample working solutions (6 replicate wells per concentration) for 24 h. After exposure, 500 μL of 0.05% neutral red solution was added to each well for staining for 5 min. The solution was removed, and 500 μL of neutral red extract was added to extract the neutral red ingested by MH-S cells. The mixture was centrifuged at 12000 r/min for 10 min, and the absorbance at 540 nm was measured using a microplate reader.

3. Discussion

This study compared the in vitro toxicity of e-cigarette liquids to CHO cells and respiratory tract cells (RPMI2650 and HPF) using multiple detection methods, and analyzed the relationship between key components in e-cigarette liquids and cytotoxicity.

The results showed that e-cigarette liquids exhibited significant cytotoxicity to all three cell types, and the toxicity was concentration-dependent, which is consistent with the findings of previous in vitro studies on e-cigarette components. Notably, respiratory tract cells (RPMI2650 and HPF) were more sensitive to e-cigarette liquids than CHO cells, as reflected by lower IC50 values in MTT, WST-1, and CCK8 assays, higher LDH release, and more significant inhibition of proliferation. This difference may be attributed to the specific physiological functions of respiratory tract cells, which are directly exposed to e-cigarette aerosols in vivo and thus have evolved more sensitive responses to harmful substances[4].

Among the respiratory tract cells, RPMI2650 (upper respiratory tract) and HPF (lower respiratory tract) showed different toxic responses: RPMI2650 cells were more sensitive in proliferation inhibition (BrdU assay), while HPF cells exhibited more severe membrane damage (LDH assay) and lysosomal dysfunction (neutral red assay). This suggests that e-cigarette liquids may cause different types of damage to upper and lower respiratory tract cells, possibly related to the differences in cell structure and function (e.g., upper respiratory tract cells are more involved in mucosal defense, while lower respiratory tract cells are more related to gas exchange)[5].

Component analysis showed that nicotine was the main component correlated with cytotoxicity, which is consistent with its known toxic effects on cells (e.g., interfering with cell signaling and inducing oxidative stress). The positive correlation between 1,2-propanediol and cytotoxicity may be due to its ability to disrupt cell membrane structure, but its contribution was lower than that of nicotine. Glycerol, as a humectant in e-cigarette liquids, showed no significant correlation with cytotoxicity, indicating that its toxicity is relatively low under the tested conditions[6].

4. Conclusion

E-cigarette liquids from different brands exhibit significant in vitro cytotoxicity to CHO cells, upper respiratory tract RPMI2650 cells, and lower respiratory tract HPF cells, with toxicity increasing in a concentration-dependent manner. Respiratory tract cells are more sensitive to e-cigarette liquids than CHO cells, and upper and lower respiratory tract cells show different toxic response patterns. Nicotine is a key component contributing to the cytotoxicity of e-cigarette liquids. These results highlight the potential health risks of e-cigarette liquids, especially to the respiratory system, and provide a scientific basis for further evaluating their safety.

References

[1] Wang G,Liu W,Cao Y, et al. Co-existing ambient fine particulate matter exacerbated electronic cigarette toxicity on human respiratory cells. [J]. Inhalation toxicology, 2024, 36 (7-8): 11-13.

[2] Kumar K S S,E J R,G A M. Effect of Electronic Cigarette Liquid Ph on Retention of 11c-Nicotine in a Respiratory Tract Model. [J]. Nicotine & tobacco research: official journal of the Society for Research on Nicotine and Tobacco, 2023, 25 (7):1406–1410.

[3] Anand R,B. A S,Kenneth W, et al. Modeled Respiratory Tract Deposition of Aerosolized Oil Diluents Used in Δ9-THC-Based Electronic Cigarette Liquid Products [J]. Frontiers in Public Health, 2021, 9 744166-744166.

[4] William D Bennett, Phillip W Clapp, et al. Acute Effect of E-Cigarette Inhalation on Mucociliary Clearance in E-Cigarette Users [J]. Aerosol Med Pulm Drug Deliv.2024,37(4):167-170.

[5] Constantine V,Manolis T,Alexander V, et al. Evaluation of respiratory irritants among the most popular e-cigarette refill liquids across 9 European countries [J]. EUROPEAN RESPIRATORY JOURNAL, 2017, 50 (s61):OA1978.

[6] W P C,A E P,T J L, et al. Flavored e-cigarette liquids and cinnamaldehyde impair respiratory innate immune cell function. [J]. American journal of physiology. Lung cellular and molecular physiology, 2017, 313 (2): 278-292.

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