The Biological Effects of Toxic Dose Combustion Cigarettes and Heated Tobacco Products in BEAS-2B and A549 Cells

Xiaomin Liu[1], Dawei Yan[1], Xiaonan Li[1], Hong Liu[2], Lehua Lu[1], Ying He[1], Huaquan Sheng[1], Yi Shen[1], Yihan Gao[1,*], Saijing Zheng[1,*]

[1]Shanghai New Tobacco Product Research Institute Co., Ltd., Shanghai 201315, China
[2]Technology Center, Shanghai Tobacco Group Co., Ltd., Shanghai 201315, China

Abstract: The use of novel tobacco products that do not burn tobacco leaves is becoming increasingly popular worldwide. In terms of respiratory system, smoking damage to the epithelial cells of lung is in urgent need of attention and research. In this study, we produced the combustion cigarettes (CC) condensate from conventional cigarettes and the heated tobacco products (HTP) condensate from heat-not-burn (HNB) tobacco, and determine the nicotine and other ingredients content respectively to assessment the CC and HTP cytotoxicity after acute exposure to cigarette smoke. We further select a toxicological dose for comparison of the difference between the CC and HTP, which the impacts on BEAS-2B and A549 cells at a 70% survival rate was evaluated. Then, biological effects of CC and HTP on BEAS-2B and A549 cells, which including cell viability, apoptosis and cell cycle were investigated. Results showed that the BEAS-2B and A549 cells survival rate was decreased with increasing dose of CC and HTP smoke trap for 24 h. Both CC and HTP could suppress the proliferation of BEAS-2B cells, though the degree of inhibition of cell proliferation in CC group was greater than in HTP group. The CC and HTP traps could promote apoptosis in BEAS-2B and A549 cells. The apoptosis rate of HTP group was less than that of CC group. In addition, CC and HTP traps could partly restrain cell cycle progression in G0/G1 phase. These results demonstrate that CC and HTP can cause cytotoxicity, apoptosis and affect cell cycle progression, which thus may be useful to elucidate the underlying biological effects of novel tobacco products and provide new ideas for the preventing and treating diseases.

Keywords: Combustion Cigarettes (CC); Heated Tobacco Products (HTP); Toxicity; Lung Cells

DOI: 10.57237/j.life.2024.02.001

1 Introduction

Cigarette smoking is currently recognized as potentially having adverse effects on the health of humans and is still one of the leading causes of disease worldwide [1, 2]. The composition of smoking are complex, and global tobacco control efforts are increasing as people become more aware of environmental and health issues [3]. Therefore, smoking-induced injury to lung epithelial cells cannot be ignored in the respiratory tract. Studies have shown that smoking may be a risk factor for heart and circulatory disease [4], chronic heart disease [5], stroke and lung cancer [6]. In addition, passive smoking has been found causing respiratory and cardiovascular diseases and prem-

Funding: Shanghai New Tobacco Product Research Institute Technology Project Foundation (K2023-009Z).

*Corresponding author: Yihan Gao, gaoyh@sh.tobacco.com.cn; Saijing Zheng, saijingzh@126.com

Received: 2 February 2024; Accepted: 10 April 2024; Published Online: 13 April 2024 http://www.lifescitech.org

ature deaths among non-smoking adults [7].

At present, there are many evaluation systems for the in vitro toxicological safety assessment of traditional cigarette smoke [8, 9], but the research on the safety assessment system of heated tobacco products (HTP) is not yet comprehensive. At the same time, the HTP of emerging industry, which has no perfect technical regulations for the products in various countries, nor is there any unified standard in the industry. Therefore, the safety assessment of HTP is particularly important and imperative, especially on the effects on the respiratory system, which requires more long-term and in-depth studies. The relationship between smoking and health risks is needed to be clarified.

Regarding the toxic effects of heated cigarettes, studies have shown that combustion cigarettes (CC) can lead to epigenetic destruction and apoptosis by improving the transcription of genes such as EGR1 compared with heated cigarettes [10]. McKarns *et al.* found that TOB-HT smoke condensate had less damaging effects on plasma membrane structure and function in many cell lines (coronary endothelial cells, human bronchial epithelial cells, coronary smooth muscle cells, rat liver endothelial cell, etc.) than CC [11]. Studies have also shown that epithelial differentiation and keratinisation of gingival epithelial cells was influenced by long-term HTP stimulation [12]. Whether it is conventional cigarettes or heated cigarettes, smoking directly exposes the human respiratory system. In this research, human bronchial epithelial cells (BEAS-2B and A549) were selected, which is a cell model widely used in studies related to smoking and lung diseases [13, 14]. A549 cells are squamous cell lung cancer cell, which belongs to nonsmall cell lung cancer (NSCLC). BEAS-2B cells are human lung epithelial cells. The cytotoxicity and apoptosis of BEAS-2B and A549 cells induced by CC and HTP traps were investigated by exposing them to cells, which the different toxic effects of CC and HTP were compared.

2 Materials and Methods

2.1 Preparation Total Particle Phase of CC/ HTP and Analysis Composition

The CC samples were equilibated at (65±5)% relative humidity for 48 h, and the HTP samples were equilibated at (22±2) ℃ for 48 h. Based on the international standard smoking mode of tobacco industry standard method (GB/T 16447-2004), 8 CC were smoked by automatic rotary disk smoking machine, and the total particle phase of the smoke was captured on the 92 mm filter. According to ISO20778 standard suction method, 8 HTPs were smoked by Puffman X500E rotary disk smoking machine, and the total particle phase of the smoke was captured on 44 mm filter. The total particle mass of the captured phase was extracted by DMSO oscillation, and finally the captured substance was fixed to 10 mg/mL, filtered by 0.22 μm microporous filter membrane, and stored at -80 ℃.

Based on the national standards and industry standards, we analysis indexes of nicotine, ammonia, hydrocyanic acid, B[a]P, aldehydes and ketones, phenol, VOCs, TSNAs, aromatic amines and CO in the samples of HTP and CC by means of high-performance liquid chromatography (HPLC) and mass spectroscopy.

2.2 Cell Culture

BEAS-2B cells were sourced from the Cell Bank of Chinese Academy of Sciences (Shanghai, China). The BEAS-2B cell line is an isolation of normal human bronchial epithelium. A549 cells were bought from the American Type Culture Collection (ATCC, Manassas, VA, USA).

The cell lines, BEAS-2B cells were grown in RPMI-1640 medium and A549 cells in Dulbecco's modified Eagle's medium (DMEM, Gibco, Gaithersburg, MD, USA). All media were added with 10% fetal bovine serum (FBS, HyClone Laboratories, Logan, UT, USA), and an antibiotic cocktail of 100 μg/mL streptomycin and 100 U/mL penicillin (Gibco). Cell culture was performed at 37˚C in a humidified environment with 5% CO_2.

2.3 Cell Proliferation Assay

The CCK-8 assay was used to measure cell proliferation. In brief, cells were plated in a 96-well plate at a density of 2×10^3 cells per well and incubated at 37˚C in a humidified environment with 5% CO_2. CCK-8 was added and cells were returned to incubate for 2.5 h. A microplate reader was used daily to measure light absorbance at 450 nm. Triplicate experiments were carried out at least three times independently of each other.

2.4 Colony Formation Assay

Cells were plated at a density of 300 cells per 35 mm tissue culture dish and incubated for 2 weeks at 37˚C in

a humidified environment with 5% CO_2. The colonies were then fixed with methanol. They were stained with crystal violet (0.5% w/v) and counted. Triplicate experiments were carried out at least three times independently of each other.

2.5 Cell Apoptosis Analysis

The analysis of cell apoptosis was carried out as described previously [15]. In order to test the level of cell apoptosis, the Annexin V-fluorescein isothiocyanate (FITC) apoptosis detection kit (BD Pharmingen, San Diego, CA, USA) was used according to instructions of the manufacturer. BEAS-2B and A549 cells were resuspended in 1×binding buffer solution containing propidium iodide (PI) and Annexin V-FITC. Then, the cell samples incubated at room temperature for 15 min with keeping in dark place. Through MoFlo XDP flow cytometer (Beckman Coulter, Inc., Brea, CA, USA) to analysis the cells apoptotic. Triplicate experiments were carried out at least three times independently of each other.

2.6 Cell Cycle Analysis

The analysis of cell cycle was carried out as described previously [16]. A total of 1×10^5 cells were collected. The calls were fixed in 75% ethanol overnight at -20˚C. Then harvested the cells, treated with RNase A (100 ng/mL) for 30 minutes, and PI (50 ng/mL) staining for 15 minutes at normal temperature. After staining, through MoFlo XDP flow cytometer (Beckman Coulter) to analysis the cell cycle distribution. Using Flow Jo software (Treestar Inc., USA), to analysis the data. Triplicate experiments were carried out at least three times independently of each other.

2.7 Statistical Analysis

The results are expressed as the mean of the groups ± SEM. Results were analysed With t-tests for two groups comparisons, using GraphPad Prism 6 software to analysis results data. When $p < 0.05$, differences were considered statistically significant.

3 Results

3.1 Composition of HTP and CC

Analysis a total of 20 indexes of nicotine, ammonia, hydrocyanic acid, B[a]P, aldehydes and ketones, phenol, VOCs, TSNAs, aromatic amines and CO in the samples of HTP and CC, which using high performance liquid chromatography (HPLC) and mass spectrometry. The results of the test are given in Table 1.

The compositional analysis shows that HCN, B[a]P, phenol were not detected in HTP samples, while higher levels were detected in CC. In general, the analysis results showed that except for NNN, the release of components CS was significantly higher than that of HTP, which the difference is even greater than 90%.

Table 1 Chemical composition test results of CC and HTP

Number	Component	1# CC (HCI)	HTP
1	Ethanal (CH_3CHO)	907.49	107.11
2	Acrolein (C_3H_4O)	83.39	2.40
3	Acrylonitrile	8.39	0.15
4	4-aminobiphenyl	1.92	ND
5	1-naphthylamine	14.21	ND
6	2-naphthylamine	13.23	ND
7	ammonia	17.44	1.32
8	Benzene	44.17	0.26
9	B[a]P	7.86	ND
10	1,3-butadiene	62.32	0.28
11	CO	16.1	0.35
12	Crotonaldehyde	29.31	5.61
13	Methanal	98.44	21.21
14	Isoprene	449.59	0.41
15	Nicotine	2.00	0.82
16	NNK	16.56	3.31
17	NNN	9.031	30.66
18	Methylbenzene	60.47	2.21
19	Phenol	20.63	ND
20	HCN	179.5	ND

(ND: No detection)

3.2 Effects of HTP and CC on the Viability of Cells and Morphology

Since lung is the primary target organ of cigarettes, BEAS-2B and A549 cells were selected to study the function of HTP and CC. Through cytotoxicity assay CCK-8, we screened HTP and CC poisonous dose to BEAS-2B and A549 cells. We found that with increasing CC and HTP concentrations, the relative of BEAS-2B and A549 cells survival rate were decreased, and there was a dose-response relationship between the dose of CC and the dose of HTP and the relative survival rate of cells after a period of 24 h exposure to different concentrations of HTP or CC (Figure 1 A, B). The IC_{50} of CC (47.64 μg/mL) was smaller than that of HTP (110.2 μg/mL) in BEAS-2B (Figure 1 A). In A549 cells, the IC_{50} of CC (56.69 μg/mL) was also smaller than that of HTP (116.03 μg/mL) (Figure

1 B). This indicates that the cytotoxicity of CC is more sensitive than that of HTP. Subsequently, in order to research the biological functions of CC and HTP on cells, we selected the dose with the relative survival rate of cells above 70% as the dose for the follow-up experimental index detection. For BEAS-2B cells, the selected doses of CC and HTP were as 25 μg/mL and 45 μg/mL, respectively. For A549 cells, the dose of CC and HTP were selected as 20 μg/mL and 40 μg/mL.

Then, we investigated the function of CC and HTP on cellular morphology. Results shown that the control group (NC and DMSO) cells were in well adhesion condition, transparent cells and good refraction with CC and HTP exposure for 24 h, whereas the treatment group (CC group and HTP group) shown the cell density reduced, the cell wrinkled, the cell adhesion ability was weakened, and the apoptotic morphology was obvious. Moreover, it can be seen from the cell morphology that the state of the cells in the CC is worse than in the HTP group (Figure 1C).

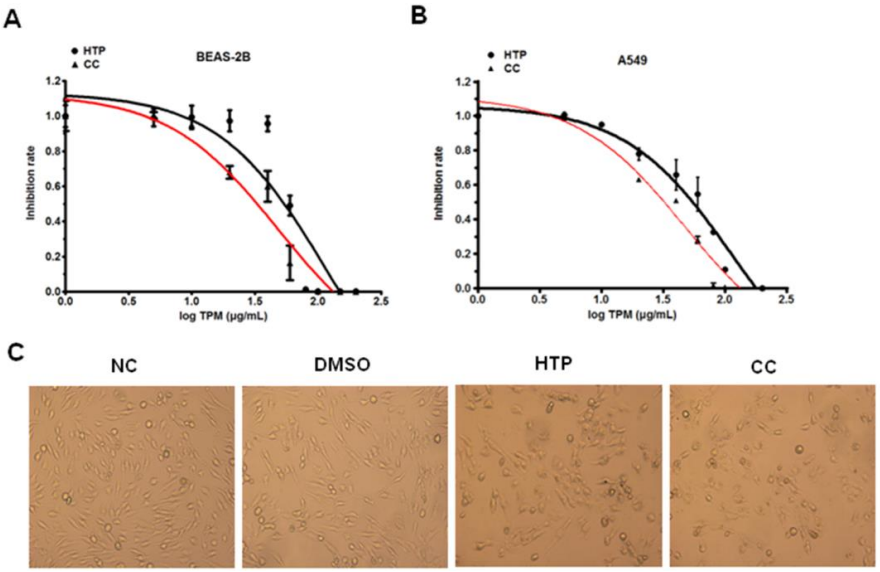

Figure 1 The cell viability and morphological changes of lung cells after treatment HTP and CC. (A-B) Survival of BEAS-2B and A549 cells from HTP and CC (dose-response relationship). (C) Effects of HTP and CC on the growth status of BEAS-2B cells

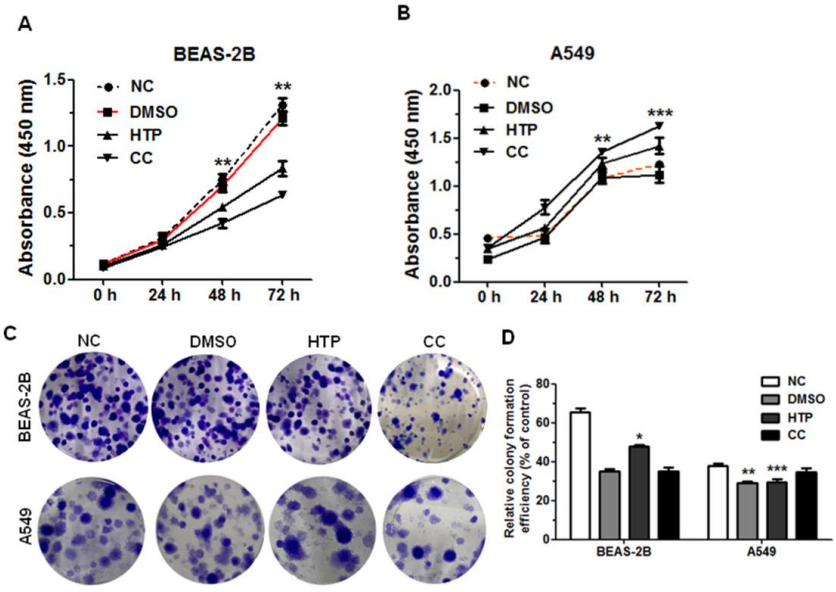

Figure 2 HTP and CC could inhibit the proliferation of BEAS-2B and A549 cells. (A-B) Cell proliferation ability of BEAS-2B and A549 cells which treatment with HTP and CC measured by CCK-8 assay. (C-D) Colony formation assay in BEAS-2B and A549 cells which treatment with HTP and CC respectively. Representative images and quantitative data are shown. **P*<0.05, ***P*<0.01, ****P*<0.001

3.3 HTP and CC Suppress the Cell Proliferation

After selecting doses of CC and HTP, using cell counting kit-8 (CCK-8) assay, we examined the function of cell proliferation in BEAS-2B and in A549 cells. Results showed that poisoning 24 h of CC and HTP significantly inhibited the BEAS-2B proliferation, and the degree of proliferation inhibition in CC group was greater than that in HTP group (Figure 2A). Whereas, in A549 cells, the CC and HTP groups promoted the proliferation of lung cancer cells after poisoned 24 h, the degree of cell proliferation inhibition in the HTP group was more than in the CC group, implying that CC promoted the proliferation of lung cancer cells (Figure 2B).

To further illustrate the cell proliferation effects of CC and HTP, we carried out the colony formation assay. The results indicated that CC and HTP could significantly suppress colony formation in BEAS-2B and A549 cells with poisoned 24 h, when compared with the control (NC and DMSO) (Figure 2C–2D). This corresponds to the previous experiment.

3.4 CC and HTP Promotes Cell Apoptosis and Arrests Cell Cycle

To research the underlying mechanism of action of CC and HTP in inhibiting cell proliferation, we carried out flow cytometry after infecting BEAS-2B and A549 cells with CC and HTP for a period of 24 h. We analyzed the impact of cell apoptosis and cell cycle progression. The results showed that CC and HTP promoted apoptosis in BEAS-2B and A549 cells after poisoned 24 h, which compared with the control (NC and DMSO) (Figure 3A–3B). From the apoptosis results, the rate apoptosis CC group was higher than that of HTP group. With regard to cell cycle, treatment with CC and HTP for 24 h in BEAS-2B and A549 cells, could partly restrain cell cycle progression in G0/G1 phase (Figure 3C–3D). This indicates CC and HTP have also effect on cell cycle progression.

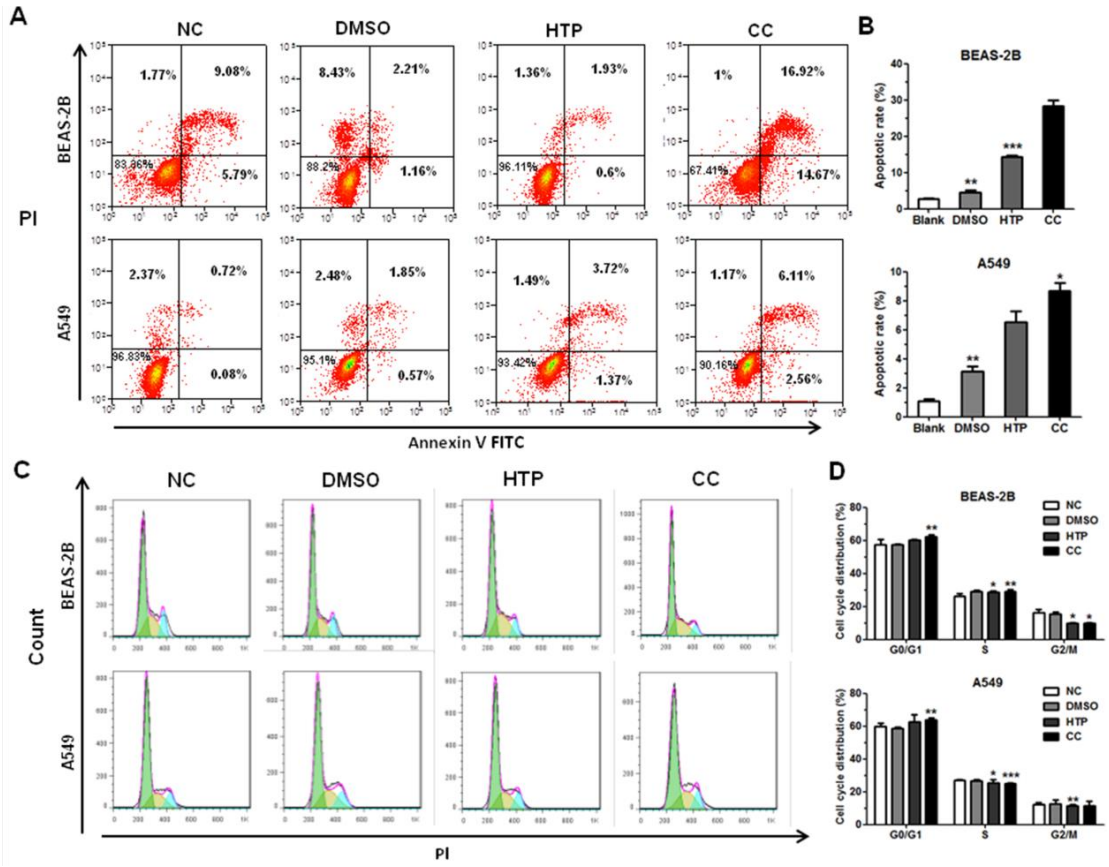

Figure 3 CC and HTP could promote cell apoptosis and arrests cell cycle. (A-B) The rate of apoptosis was analyzed by flow cytometry following treatment with HTP and CC in BEAS-2B and A549 cells. (C-D) Cell cycle distributions of BEAS-2B and A549 cells treatment with HTP and CC were detected by flow cytometry. *P<0.05, **P<0.01, ***P<0.001

4 Discussion

Tobacco smoke exposure can cause cytotoxicity [17, 18], inflammation [19, 20], oxidative stress [21] and apoptosis [22]. In this study, CC and HTP smoke traps were applied to human bronchial epithelial cells, that the cell survival rate gradually decreased and even cell death occurred with the increase of dose. The cytotoxicity test demonstrated that both CC and HTP could suppress BEAS-2B proliferation, but the degree of inhibition of cell proliferation in CC was greater than in HTP group, implying that the toxicity of HTP was less.

Apoptosis, also known as programmed cell death (PCD), is a genetically controlled process that causes active cell die, triggered by changes in the internal and external environment of the body or death signals [23, 24]. The unrestricted proliferation of tumor caused by apoptosis disorder and abnormal cell cycle is one of the important reasons for tumorigenesis [25, 26]. The regulation between tumor and other diseases and apoptosis is a hot topic at home and abroad [27, 28]. In this study, it was found that CC and HTP traps could promote apoptosis of A549 and BEAS-2B cells, and the HTP apoptosis rate group was less than in CC group. Whether it is CC or HTP, apoptosis is considered to be the key pathophysiological mechanism in various human pathologies, and is an important manifestation of the early formation of various diseases [29, 30]. In addition, the cell cycle process also plays an important role in cell growth [31, 32]. In this study, we found that CC and HTP traps could partly restrain progression of cell cycle in the G0/G1 phase. In summary, strengthening the research on apoptosis, cell cycle induced by tobacco smoke and its mechanism may be the source of new ideas and new ways of preventing and treating of diseases.

Overall, these finding indicate that both CC and HTP can cause cytotoxicity, apoptosis and affect cell cycle progression. When the cell survival rate was above 70%, the cytotoxicity of HTP was less than that of traditional cigarettes. However, whether the harm of HTP to the human body is lower than that of CC can't be determined. Further studies are needed to evaluate the safety of exposure to tobacco products, such as how induced apoptosis mechanism changes and whether there are changes in lung related inflammatory factors. Therefore, systematic studies are still needed for in vitro testing of the mechanism of toxicity of tobacco smoke and for assessment of the safety of tobacco products, so as to provide basic support for assessing the health risks posed by tobacco products.

5 Conclusion

In conclusion, this study showed that both CC and HTP could cause cytotoxicity, apoptosis and affect the cell cycle process, with HTP causing less cytotoxicity than CC. This study provides a new experimental basis for in vitro toxicity mechanism testing and safety evaluation of tobacco product smoke.

Acknowledgments

We thank Wei Pan for her technical assistance in this study. Thanks for the stimulating discussions to all authors.

Author Contributions

LXM conducted the experiments and wrote the primary manuscript. LXM, YDW, LXN, LH, LLH, HY and SHQ performed experiments and analysised the data. SY, ZSJ and GYH designed the experiment supervision and project administration. LXM and ZSJ revised the manuscript. The published version of the manuscript has been read and approved by all authors.

Conflict of Interest

The authors have declared that no competing interest exists.

References

[1] Chang JT, Anic GM, Rostron BL, Tanwar M and Chang CM. Cigarette Smoking Reduction and Health Risks: A Systematic Review and Meta-analysis. Nicotine & tobacco research: official journal of the Society for Research on Nicotine and Tobacco. 2021; 23(4): 635-642.

[2] Sliwinska-Mosson M and Milnerowicz H. The impact of smoking on the development of diabetes and its complications. Diabetes & vascular disease research. 2017; 14(4): 265-276.

[3] Zhang P, Chen PL, Li ZH, Zhang A, Zhang XR, Zhang YJ, Liu D and Mao C. Association of smoking and polygenic risk with the incidence of lung cancer: a prospective cohort study. British journal of cancer. 2022; 126(11): 1637-1646.

[4] Bullen C. Impact of tobacco smoking and smoking cessation on cardiovascular risk and disease. Expert review of cardiovascular therapy. 2008; 6(6): 883-895.

[5] Kreuz J, Skowasch D, Kamrath P, Lorenzen H, Tiyerili V, Linhart M, Nickenig G and Schwab JO. Influence of smoking dosage and chronic obstructive lung disease on the incidence of appropriate therapies and mortality in patients with structural heart disease and an implantable cardioverter defibrillator. Pacing and clinical electrophysiology: PACE. 2015; 38(1): 71-76.

[6] Warren GW and Cummings KM. Tobacco and lung cancer: risks, trends, and outcomes in patients with cancer. American Society of Clinical Oncology educational book American Society of Clinical Oncology Annual Meeting. 2013: 359-364.

[7] Hart CL, Hole DJ and Smith GD. Comparison of risk factors for stroke incidence and stroke mortality in 20 years of follow-up in men and women in the Renfrew/Paisley Study in Scotland. Stroke. 2000; 31(8): 1893-1896.

[8] Dempsey R, Coggins CR and Roemer E. Toxicological assessment of cigarette ingredients. Regulatory toxicology and pharmacology: RTP. 2011; 61(1): 119-128.

[9] Mallock-Ohnesorg N, Rinaldi S, Malke S, Dreiack N, Pieper E, Laux P, Schulz T, Zimmermann R and Luch A. Oral nicotine pouches with an aftertaste? Part 1: screening and initial toxicological assessment of flavorings and other ingredients. Archives of toxicology. 2023; 97(9): 2357-2369.

[10] Hattori N, Nakagawa T, Yoneda M, Nakagawa K, Hayashida H and Ito T. Cigarette smoke, but not novel tobacco vapor products, causes epigenetic disruption and cell apoptosis. Biochemistry and biophysics reports. 2020; 24: 100865.

[11] McKarns SC, Bombick DW, Morton MJ and Doolittle DJ. Gap junction intercellular communication and cytotoxicity in normal human cells after exposure to smoke condensates from cigarettes that burn or primarily heat tobacco. Toxicology in vitro: an international journal published in association with BIBRA. 2000; 14(1): 41-51.

[12] Uehara O, Nakamoto N, Hiraki D, Paudel D, Sugiyama N, Morikawa T, Yoshida K, Kawano Y, Shimo T, Furuichi Y, Miura H and Abiko Y. Effects of prolonged stimulation with heated tobacco products (Ploom TECH(+)) on gingival epithelial cells. Journal of periodontal research. 2023; 58(3): 553-563.

[13] Wang L, Wang Y, Chen J, Yang XM, Jiang XT, Liu P and Li M. Comparison of biological and transcriptomic effects of conventional cigarette and electronic cigarette smoke exposure at toxicological dose in BEAS-2B cells. Ecotoxicology and environmental safety. 2021; 222: 112472.

[14] Wang M, Zhang Y, Xu M, Zhang H, Chen Y, Chung KF, Adcock IM and Li F. Roles of TRPA1 and TRPV1 in cigarette smoke -induced airway epithelial cell injury model. Free radical biology & medicine. 2019; 134: 229-238.

[15] Ma ZL, Hou PP, Li YL, Wang DT, Yuan TW, Wei JL, Zhao BT, Lou JT, Zhao XT, Jin Y and Jin YX. MicroRNA-34a inhibits the proliferation and promotes the apoptosis of non-small cell lung cancer H1299 cell line by targeting TGF-betaR2. Tumour biology: the journal of the International Society for Oncodevelopmental Biology and Medicine. 2015; 36(4): 2481-2490.

[16] Ma ZL, Zhang BJ, Wang DT, Li X, Wei JL, Zhao BT, Jin Y, Li YL and Jin YX. Tanshinones suppress AURKA through up-regulation of miR-32 expression in non-small cell lung cancer. Oncotarget. 2015; 6(24): 20111-20120.

[17] Czekala L, Simms L, Stevenson M, Tschierske N, Maione AG and Walele T. Toxicological comparison of cigarette smoke and e-cigarette aerosol using a 3D in vitro human respiratory model. Regulatory toxicology and pharmacology: RTP. 2019; 103: 314-324.

[18] Nakanishi T, Sakiyama S, Takashima H, Honda R, Shumba MN, Nakamura Y, Kasahara K and Tamai I. Toxicological implication of prostaglandin transporter SLCO2A1 inhibition by cigarette smoke in exacerbation of lung inflammation. Toxicology and applied pharmacology. 2020; 405: 115201.

[19] Kopa PN and Pawliczak R. IQOS - a heat-not-burn (HnB) tobacco product - chemical composition and possible impact on oxidative stress and inflammatory response. A systematic review. Toxicology mechanisms and methods. 2020; 30(2): 81-87.

[20] Han H, Peng G, Meister M, Yao H, Yang JJ, Zou MH, Liu ZR and Ji X. Electronic Cigarette Exposure Enhances Lung Inflammatory and Fibrotic Responses in COPD Mice. Frontiers in pharmacology. 2021; 12: 726586.

[21] Szoka P, Lachowicz J, Cwiklinska M, Lukaszewicz A, Rybak A, Baranowska U and Holownia A. Cigarette Smoke-Induced Oxidative Stress and Autophagy in Human Alveolar Epithelial Cell Line (A549 Cells). Advances in experimental medicine and biology. 2019; 1176: 63-69.

[22] Liu Q, Zhao M, Chen W, Xu K, Huang F, Qu J, Xu Z, Wang X, Wang Y, Zhu Y and Wang W. Mainstream cigarette smoke induces autophagy and promotes apoptosis in oral mucosal epithelial cells. Archives of oral biology. 2020; 111: 104646.

[23] D'Arcy MS. Cell death: a review of the major forms of apoptosis, necrosis and autophagy. Cell biology international. 2019; 43(6): 582-592.

[24] Kopeina GS and Zhivotovsky B. Programmed cell death: Past, present and future. Biochemical and biophysical research communications. 2022; 633: 55-58.

[25] Goldar S, Khaniani MS, Derakhshan SM and Baradaran B. Molecular mechanisms of apoptosis and roles in cancer development and treatment. Asian Pacific journal of cancer prevention: APJCP. 2015; 16(6): 2129-2144.

[26] Evan GI and Vousden KH. Proliferation, cell cycle and apoptosis in cancer. Nature. 2001; 411(6835): 342-348.

[27] Huang H, Fan X, Qiao Y, Yang M and Ji Z. Knockdown of KNTC1 Inhibits the Proliferation, Migration and Tumorigenesis of Human Bladder Cancer Cells and Induces Apoptosis. Critical reviews in eukaryotic gene expression. 2021; 31(1): 49-60.

[28] Nagler R, Weizman A and Gavish A. Cigarette smoke, saliva, the translocator protein 18 kDa (TSPO), and oral cancer. Oral diseases. 2019; 25(8): 1843-1849.

[29] Gal K, Cseh A, Szalay B, Rusai K, Vannay A, Lukacsovits J, Heemann U, Szabo AJ, Losonczy G, Tamasi L and Muller V. Effect of cigarette smoke and dexamethasone on Hsp72 system of alveolar epithelial cells. Cell stress & chaperones. 2011; 16(4): 369-378.

[30] Sakhatskyy P, Gabino Miranda GA, Newton J, Lee CG, Choudhary G, Vang A, Rounds S and Lu Q. Cigarette smoke-induced lung endothelial apoptosis and emphysema are associated with impairment of FAK and eIF2alpha. Microvascular research. 2014; 94: 80-89.

[31] Pezzuto A, Citarella F, Croghan I and Tonini G. The effects of cigarette smoking extracts on cell cycle and tumor spread: novel evidence. Future science OA. 2019; 5(5): FSO394.

[32] Sobus SL and Warren GW. The biologic effects of cigarette smoke on cancer cells. Cancer. 2014; 120(23): 3617-3626.