Early View

Research letter

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Heated Tobacco Product IQOS Induces Unique Metabolic Signatures in Human Bronchial Epithelial Cells

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To the editor,
The tobacco epidemic is one of the most significant health threats, causing 8 million deaths annually [1]. Currently, major tobacco companies have shifted their strategies, effectively enticing the public including youth, through aggressive and misleading advertisements for new electronic nicotine and flavor delivery systems (ENDS) mainly e-cigarettes (E-Cigs). More recently, a novel category of e-devices that heat tobacco in specialized cigarettes known as heated tobacco products (HTPs) has been introduced which aims to replace the dwindling combustible cigarette (CC) market. Unlike traditional CCs, HTPs such as IQOS are a hybrid between a traditional CC and an E-Cigs. They work by heating a metal filament to heat a solid rolled tobacco sheet to 350°C to generate aerosol without combustion [2]. Differentiating biological effects of IQOS from E-Cigs or CCs is a critical step to understanding mechanisms of disease, and to facilitate the development of a clear long-term strategy to prevent an E-Cig or vaping use-associated lung injury (EVALI)-like epidemic in the future. At present, there is no published information available regarding the metabolomics of disease pathology associated with IQOS in comparison to traditional CC and E-Cigs. Why is this an important question? Understanding the biochemical and metabolic changes induced by such inhaled products will lead to critical clues as to underlying disease mechanisms of action. Hence, there is an immediate need to investigate the impact of IQOS and other emerging HTPs on airway structural cells (such as smooth muscle cells or epithelial cells) to identify unique and novel damaging biosignatures (metabolites) that can help predict lung pathology in individuals who consume these products, and direct future research efforts.

IQOS induces greater cytotoxicity in bronchial epithelial cells compared to E-Cig [3], but it is equally toxic when compared to CC [2]. In here, we aimed to investigate whether IQOS exposure can induce distinct metabolites that are either up- or down-regulated in human bronchial epithelial cells (HBE-1 cell line as described in [4, 5]) vs traditional CC and E-Cig. We sought to identify novel metabolites and pathways associated with IQOS that may be
different than CC smoke and/or E-Cig vapor. E-Cig vapor was generated using e-liquid (18 mg/ml nicotine, 1:1 PG and VG, VaporEmpire, USA); IQOS aerosol was generated using IQOS HeatStick (Marlboro, USA); and CC smoke extract was generated using 1R6F Kentucky Research Cigarette (University of Kentucky, USA) using an automated exposure system (CH Technologies, USA). Confluent submerged HBE-1 cells were exposed to either room air (un-exposed), CC smoke, E-Cig vapor, or IQOS aerosol for a total of 6 minutes. We chose a 6-minute exposure time to align with the average duration it takes for an individual to smoke a combustible cigarette. It's noteworthy that IQOS allows only 14 puffs before extinguishing, a process also taking approximately 6 minutes. This exposure duration was selected to closely mimic or approximate the physiological real-life exposures observed in human subjects. Cells were then subsequently incubated (37°C, 5%CO₂) for a total of 48 hours with the exposed media, and then cell lysates were collected using liquid nitrogen with RIPA buffer (CST, USA) with protease and phosphatase inhibitors (Selleck Chemicals, USA). Cell lysates were stored at −80°C until they were ready to be processed and analyzed at the UC Davis West Coast Metabolomics Center for the abundance of primary metabolites, lipids, and biogenic amines. MetaboAnalyst 5.0 (Canada) was used for the statistical analysis [one factor]. MetaboAnalyst combines the fold change (FC) analysis (FC threshold:2; FDR: 0.05) and T-test (p-value < 0.05) based on either biological or statistical significance to create the significant metabolites followed by Qiagen’s Ingenuity Pathway Analysis (IPA) to rank the output pathways by relative abundance from the list and express their enrichment based on p-value < 0.05.

Using the MetaboAnalyst, we were able to determine the up- and down-regulated metabolites after being exposed to IQOS, E-Cig, and CC. There were 34 primary metabolites, 39 biogenic amines, and 10 lipids that were statistically different from unexposed (UE) cells vs IQOS, E-Cig, and CC exposed cells (Fig. 1A-C). Also, we found common primary metabolites, biogenic amines, and lipids that were statistically significant in the IQOS, E-Cig,
and CC exposed groups vs UE (Fig. 1D-F). When IQOS-exposed cells were compared to UE cells, there were unique metabolites that were not common with E-Cig or CC exposed cells (Fig. 1G). In addition, when compared to UE cells, there were common metabolites between IQOS and E-Cig (Fig. 1H), as well as IQOS and CC (Fig. 1I). Even though IQOS has metabolites similar to E-Cig and CC aerosol exposed cells, our data suggest that IQOS has a unique biochemical fingerprint that is not observed with the other exposure devices.

E-Cigs and CC are known to cause carbonyl exposure, and carbonyls are the most abundant toxic species in aerosols generated from smoking devices [6]. Each exposure group when compared to UE cells were enriched with a few metabolites that were linked to carbonyls (Fig. 1J). The common carbonyl-grouped metabolite in all three comparisons is 1-methylnicotinamide (a metabolite of nicotinamide, and product of nicotinamide N-methyltransferase) [7]. It was significantly increased by 10- to 18-fold in each group and recent studies have shown that nicotinamide N-methyltransferase is highly expressed in various cancers such as oral squamous cell carcinoma and gastric cancer [8, 9]. While there was no common carbonyl metabolite between all groups; 1,3-cyclohexanedione was abundant in both IQOS and CC vs UE. This finding is significant because the high levels of carbonyls in these products indicate that despite people using E-Cigs and IQOS as a "safe alternative" to traditional CC smoking, these products may still be harmful.

Using the IPA, we were able to do a core analysis that identified canonical pathways and diseases and disorders associated with these metabolites. All exposures induced the canonical pathway of 4-hydroxyphenylpyruvate biosynthesis, which is synthesized by 4-hydroxyphenylpyruvate dioxygenase which is found to be highly expressed in lung cancer [10]. IQOS-exposure induced unique canonical pathways that were not observed with E-Cig and CC-exposure (Fig. 1J). Furthermore, the pathways induced by IQOS are associated with numerous human diseases and disorders, including developmental and hereditary disorders,
metabolomic diseases, organismal injury, and dermatological diseases. The most common pathology associated with IQOS exposure is organismal injury and abnormalities (Fig. 1J).

Smoking is still a leading cause of chronic obstructive pulmonary disease (COPD), adversely impacts other airway diseases such as asthma and it negatively affects other organs in the body (e.g., aerodigestive tract, cardiovascular, gut) [11]. Despite the declining use of traditional CC smoking, this trend could reverse with the introduction of emerging smoking products acting as a novel gateway to new generations of ENDS users. In our study, we utilized immortalized human bronchial epithelial cells (HBE-1) that are well-established in the field [4, 12]. Airway epithelial cells serve as the initial defense barrier in the human lungs to environmental contaminants, particularly in the context of smoke exposure. Additionally, epithelial cells preserve the integrity of the mucosal lining, regulate local immune responses, mucus release, balance inflammatory responses, and produce biologically active substances to maintain innate immunity function against infections and other noxious agents [13].

This study is the first to reveal the substantial increase in metabolites induced by IQOS, some of which are distinct compared to those induced by E-Cig and CC. These metabolites activate pathways linked to various diseases including inflammation and cancer. Furthermore, the identification of unique metabolites associated with each type of exposure enables the creation of a distinct product-specific signature. Such signature profiles have the potential to aid in predicting the development of disease pathology in the future. In summary, our initial yet significant findings here will lay the groundwork for more extensive human and basic mechanistic studies focused on validating and further characterizing the causal role of these metabolites.

Conflict of interest: The authors have no conflict of interest to declare.

REFERENCES

Figure Legend

**Figure 1. The Effects of IQOS on the Airway Epithelial Cellular Metabolome.** Comparison of the effects of IQOS, E-Cig and CC on HBE-1 cells on the abundance of primary metabolites (A), biogenic amines (B) and lipids (C). Heat maps showing statistically significant up- or down-regulation (p-value < 0.1) for IQOS, E-Cig, and CC were compared to unexposed cells using MetaboAnalyst 5.0. Primary metabolites (D), biogenic amines (E) and lipids (F) that are common between IQOS, E-Cig, and CC when compared to unexposed cells. Unique metabolites that are seen only in IQOS when compared to unexposed cells (G). Common
metabolites that are seen in IQOS and E-Cig (H); IQOS and CC (I) when compared to unexposed cells. (J) Table showing canonical pathways and associated diseases and disorders and carbonyl related metabolites for IQOS, E-Cig, and CC when compared to unexposed cells. Data presented are the mean of three independent experiments carried out using human bronchial epithelial (HBE-1) cells while significance is shown for 2-fold or greater change when compared to unexposed cells using MetabAanalyst 5.0 (IQOS represents heated tobacco product; E-Cig: electronic cigarette; CC: combustible cigarette).