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Oxidative stress induced by electronic nicotine delivery systems (ENDS): Focus on respiratory system

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Abstract

In recent years, a huge and still growing number of electronic nicotine delivery systems (ENDS, or electronic cigarettes) has been introduced to the market. Despite the claims of the producer companies and the consumers' perception, ENDS safety is hotly debated, especially in comparison with combustible tobacco products. In this review, we focus on oxidative stress and alterations in antioxidant activity caused by ENDS' aerosols or liquids in oral cavity and lung tissues, which are the primary targets of cigarette smoke. Although detectable levels of oxidative stress are induced by ENDS, it should be emphasized that lower levels of oxidative stress markers and of related gene and protein alterations are observed compared to conventional cigarettes. More scientific studies need to be performed related to ENDS' toxicity and induction of oxidative stress in a pathophysiological context in order to allow evidence based conclusions.

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Keywords

Electronic nicotine delivery systems (ENDS), Oxidative stress, Electronic cigarettes, Heat not burn, Lung tissue, Cigarette smoke, Electronic cigarette aerosol.

Abbreviations

BER, Base excision repair; ecigs, Electronic cigarettes; ENDS, Electronic nicotine delivery systems; HnB, Heat not burn; NER, Nucleotide excision repair; NHBE, Primary normal human bronchial epithelial; ROS, Reactive oxygen species; TPM, Total particulate matter.

Oxidative stress is a disequilibrium state between the systemic exposure to reactive oxygen species (ROS) and a biological system's ability to detoxify these reactive intermediates, through neutralization by antioxidants or by repairing the resulting damage.

Disturbance of normal redox balance of cells by excess of ROS, generated by endogenous and exogenous sources, can cause toxicity to major cell compartments, to homeostatic mechanisms of cellular signaling and to macromolecules including protein, lipids, and DNA [1,2]. To repair or to tolerate DNA damage, mammalian cells have evolved several different defense mechanisms, such as free radical scavengers [*e.g.*, glutathione (GSH), vitamin C and E], antioxidant enzymes [*e.g.*, catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx)] and sophisticated DNA repair mechanisms [3]. Despite these mechanisms, there are numerous disorders which are thought to be caused or exacerbated by oxidative stress such as adult attention deficit disorder (ADHD), cancer, Alzheimer's disease, heart failure, Parkinson's disease, atherosclerosis, myocardial infarction, sickle cell disease, lichen planus, vitiligo, autism, chronic fatigue syndrome, depression and schizophrenia [4–18].

1. Electronic nicotine delivery devices (ENDS) as an alternative to tobacco combustion products (conventional cigarettes)

Tobacco smoke, the most common tobacco consumption form in most societies, contains more than 7000 chemicals of which at least 69 are known to damage DNA and cause cancer [19]. It includes a high number of oxidants (about 10^{14} free radicals per puff) [20,21], causing a significant increase in oxidative DNA damage and a reduction of the antioxidant capacity of tissues, correlating with overall low plasma antioxidant activity [22,23].

Owing to the acknowledged harmful effects of tobacco combustion, new nicotine delivery products have been appearing in the market to attract nicotine addicted consumers concerned about the health effects of

tobacco smoke. In the last decade, electronic nicotine delivery systems (ENDS), such as electronic cigarettes (ecigs) and heat not burn devices (HnB), have been continuously evolving and become increasingly popular, especially among younger individuals, pregnant smokers and as tools for smoking cessation.

ENDS' vapor delivers stimulant nicotine to the users in aerosol state, the process commonly referred as vaping [24], without the tobacco burning process. Similar to tobacco smoke, ENDS aerosol contains ultrafine particles that transport the nicotine deep into the lung tissue, where it is absorbed and transferred to the brain within a few seconds. Ecigs' ultrafine particles vary in particle size, which is similar to that of conventional cigarettes (range: ≈120–165 nm) but differs in composition [25–28], still however containing some components known to be toxic [29]. Higher nicotine level in the eliquid is associated with higher number of particles in the aerosol without changing particle size distribution. Longer puffing duration results in more particles, while addition of flavor does not affect particle number or size [28].

Many consumers believe that ENDS are a safer alternative to conventional cigarettes; however, the potential long term effects of inhaled aerosols are not well-known. In early studies comparing ecigs aerosol to tobacco smoke, lower levels (9–450 fold lower) of potentially toxic compounds (*e.g.* formaldehyde, acetaldehyde, acrolein, and toluene) were reported [30–34].

Ecig aerosol is formed in lower heating temperatures (roughly around 100–250 °C) compared to tobacco (combustion temperature: 800 °C), thus it is largely devoid of combustion byproducts such as tobacco nitrosamines that are potent carcinogens [35,36]. Nevertheless, the majority of ENDS still contain the addictive drug nicotine [37,38], which is known to contribute to cardiopulmonary diseases, neurodegenerative disorders and cancer [35,39,40]. The aerosol produced by ENDS through thermal decomposition of the solvents contains numerous toxicants and organic compounds such as propylene oxide (from propylene glycol) that is not present in traditional tobacco cigarettes. Considerable levels of potential carcinogens including toxic metals (aluminum, cadmium, chromium, copper, lead, magnesium, manganese, nickel and zinc), a number of organic compounds including carbonyls (*e.g.*, acrolein from glycerol/glycerine), and potentially harmful compounds such as silicate beads, tin, and flavorings are present in ecig aerosols [27,38,48,39,41–47]. These particles may reach the alveolar epithelium and mediate oxidative stress and inflammation [49,50].

1.1. Electronic nicotine delivery devices (ENDS)

1.1.1. Electronic cigarettes (ecigs)

Ecigs or electronic vapor products contain no tobacco and do not burn tobacco; they produce an inhalable vapor by electronically heating an eliquid (with or without nicotine). Ecigs are devices comprised of three components: a battery powered heating system, a replaceable cartridge (or a tank) to store the solution (eliquid) and an atomizer that vaporizes the solution by heating and produces the aerosol inhaled by the consumer.

Companies offer their customers countless variety of eliquid options based on nicotine content, flavor, and propylene glycol/vegetable glycerin (PG/VG) composition, multiplied by the available features of the electronic devices such as color, led indicator, voltage adjustment, battery life etc.

1.1.2. Heat not burn (HnB) technology products

In contrast to ecigs, HnB products contain tobacco that is directly or indirectly heated (but not burnt), to create an inhalable tobacco vapor. The majority of the products are electrically heated, using a variety of heat sources. Below, we introduce some representative examples of the HnBs with proprietary market technologies.

IQOS™ (from Philip Morris International) is a tobacco heating system with three main components: a heated tobacco unit, a holder, and a charger. The consumer inserts the replaceable tobacco unit into the holder and controls the device electronically by pushing the button while inhaling the aerosol. Its electronic system ensures that only the tobacco unit is heated (up to 350 °C) and the nicotine containing vapor is released without burning the tobacco (i.e. temperatures of >600 °C), thus avoiding combustion and diminishing the release of harmful chemicals in the nicotine containing vapor compared to tobacco cigarette smoke, such as carbonyls [51]. TEEPS™ also releases flavors and nicotine without combustion, using a different carbon heat source to control the temperature transfer.

iFuse™, from British American Tobacco, is a hybrid device that combines standard ecigs features with some characteristics of HnB technology, aiming to create a more genuine tobacco taste. Principally, the eliquid is heated and the formed vapor passes through a compartment that contains tobacco. The tobacco is heated at a low temperature of ∼50 °C), to release sufficient tobacco flavor.

Ploom TECH™ (Japan Tobacco Inc.) also uses a hybrid technology to deliver the tobacco flavored vapor by heating a non-nicotine liquid, which passes through a capsule containing granulated tobacco leaves. The tobacco is indirectly heated around 30 °C, thus no combustion occurs.

2. Electronic nicotine delivery systems (ENDS) and oxidative stress

The association between oxidative stress, the inflammation caused by tobacco use and the resulting lung

disease are already well established [52]. ROS and tars found in cigarette smoke are the main mediators of an inflammatory state, which have been implicated in the pathogenesis of diseases, such as chronic obstructive pulmonary disease (COPD) and lung cancer [53].

Byproducts from the devices and oxidative stress induced by ENDS may vary and depend on a set of parameters, such as different volume of vapor, adjustable voltage settings, type and state of the heating system and type of atomizers, as well as on the composition of eliquids (humectant mixture, nicotine quantity and addition of flavors etc.). Aldehydes and free radicals found in ecig aerosols and tobacco smoke are reported to trigger various types of DNA damage which are repaired mainly by nucleotide excision repair (NER) and base excision repair (BER) mechanisms [35,54]. Byproduct acrolein has been reported to induce oxidative stress and inflammation that result in loss of endothelial cell barrier integrity in the lung [55]. Propylene glycol, glycerine, and methanol have all been shown to increase generation of H_2O_2 [56].

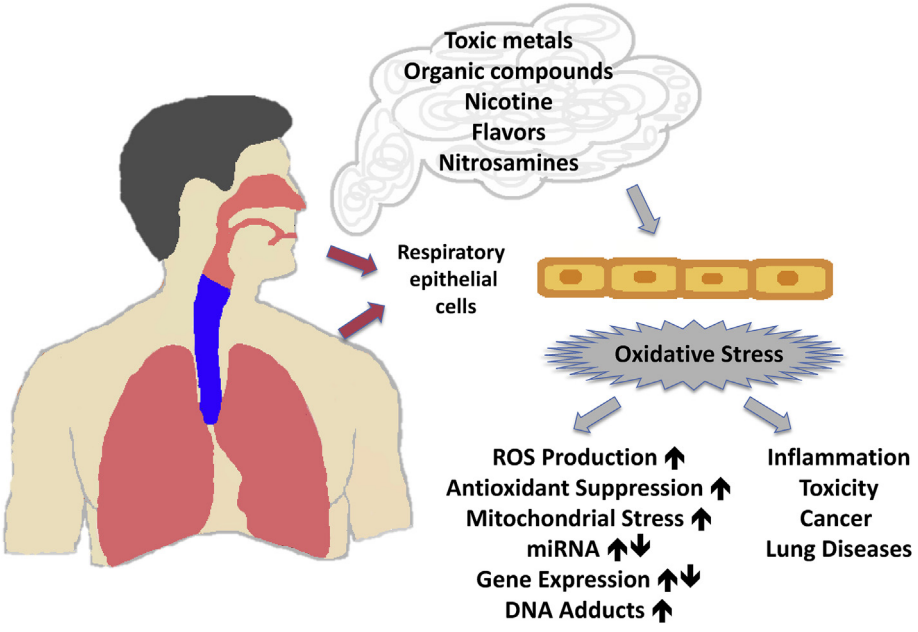
Similar to tobacco smoke, ecigs aerosols contain several potential toxicants and have been reported to share various adverse effects with tobacco smoke, comprising oxidative stress [54,57] and patterns of bronchial gene expression [58,59]. It is essential and of utmost importance to determine whether exposure to END aerosol is a significant source of DNA damage, especially in oral and lung tissue, as these tissues are the primary targets of ENDS vapor. A schematic overview of the oxidative stress induced by ENDS aerosols is illustrated in Fig. 1.

2.1. Oxidative stress in *in vitro* studies

Ecig aerosols have been reported to contain toxic compounds, however it has been determined that the number of genotoxic compounds and their concentration is much lower compared to cigarette smoke [30,34,60,61]. Side by side comparison of the effects of similar doses of tobacco smoke extracts and ecig aerosols on overall DNA damage indicates that tobacco smoke causes significantly higher levels of double strand breaks (a specific type of DNA damage) compared to ecig aerosol on human bronchial epithelial cells (BEAS2Bs) [62]. Furthermore, exposure of human bronchial epithelial cells (H292) to ecig aerosol extracts resulted in negligible or no oxidative damage compared to aerosol extracts of reference cigarette, as with the former the GSH:GSSH ratio was maintained to the levels of untreated cells and the generation of intracellular oxidant species was not increased [63].

In agreement with these data, it has been shown that both short and long term exposure to ecig aerosol extract causes overall less DNA damage than the corresponding exposure to tobacco smoke [64], which, however, was significantly higher than in unexposed cells, as detected by ELISA (8-oxo-dG) and PCR based assays (qPADDA for p53), using normal human bronchial epithelial cells (Nuli1) and human oral squamous cell carcinoma cells (UMSCC1). The severity of DNA damage induced by ecig aerosols increases in a dose dependent manner, and is unaffected by nicotine levels, in accordance with the reports showing that nicotine is not likely a contributing factor in increased ROS reactivity [54].

Figure 1



ENDS aerosol is comprised of compounds that induce oxidative stress resulting in inflammation, toxicity and increased endothelial permeability in respiratory system.

In a different study, primary normal human bronchial epithelial (NHBE) cells consisting of a mixed cell population (basal cells, progenitor cell for secretory and ciliated cells) were used in order to enable the cells to fully differentiate into cultures with *in vivo* like phenotype under air lift conditions. Air lift conditions is implemented by cultivating the cells on transwells placed into air liquid interface. The cell surface is exposed to the surrounding atmosphere and the nutrients are supplied from the basal side of the cells. The exposure of cells to direct air technically simulates *in vivo* inhalation exposure as closely as possible [54,65–67]. In this setting, the cells were exposed to eliquid aerosol, reference smoke or clean air. In eliquid aerosol treatment, cell viability was significantly decreased, as the oxidative stress (intracellular H_2O_2 determined using fluorescent probes such as DCF-DA) was substantially increased compared to clean air exposed cells. In contrast, when compared to reference cigarette smoke, cell viability with eliquid aerosol treatment was 4.5–5 times higher and oxidative stress was 4.5–5 times lower [68]. Additional studies have reported that ecig exposed human lung vascular endothelial cells and umbilical vein endothelial cells develop oxidative stress [55,69] with increased inflammation, cytotoxicity and endothelial cell permeability [54,55,68–70].

Previous *in vitro* and *in vivo* studies have shown that components of tobacco smoke can disturb the function of the mitochondrial respiratory chain complexes [71,72]. Mitochondrial malfunction is recognized as a key component in acute and chronic cellular stress. This, in turn, results in significant changes in epithelial barrier function, inflammation, and lung injury, which are seen in patients with tobacco smoke induced lung disease [73]. A recent study showed that in addition to cigarette smoke, ecig aerosols and copper nanoparticles induce mitochondrial ROS production, mitochondrial stress (reduced stability of OxPhos electron transport chain complex IV subunit) and DNA fragmentation in lung fibroblasts [56].

Multiple cellular parameters (mitochondrial oxygen consumption, ROS levels, the efficiency of the antioxidant defense system, and markers of oxidative stress) were evaluated on human bronchial epithelial cells (BEAS2B) exposed to total particulate matter (TPM), a phase of mainstream cigarette smoke that excludes the gas and vapors of cigarette smoke. Cells continuously exposed to TPM from either HnB aerosol or reference cigarette smoke for short (1 week) or long term (12 weeks) exhibited alteration in mitochondrial respiratory chain, oxidative phosphorylation, gene expression and in oxidative stress related proteins, with reference cigarette smoke eliciting more pronounced consequences [74]. In the 1 week exposure, cigarette smoke TPM had a stronger inhibitory effect on mitochondrial basal and maximal oxygen consumption rates compared to TPM from HnB aerosol. It was implied that heating rather than combusting tobacco could reduce the content of harmful constituents in the inspired fraction, resulting in diminished mitochondrial dysfunction that is associated with oxidative stress related diseases [74].

When mouse lung or rat myocardial cells in culture were directly treated with the eliquid, cell viability was minimally affected [54,75,76]. A different study testing a variety of cells [hESC, mHSC and human pulmonary fibroblasts (hPF)] has demonstrated that the effects of eliquids on viability and cell toxicity are variable and mostly depend on flavor additives [77]. For example, cinnamon flavored eliquid induced cellular toxicity [75,77,78] and elicited a strong IL8 response compared to liquid tobacco smoke extract [54]. Depending on nicotine level and flavor choice, stress phenotype and inflammatory responses varied upon treatment of cells with eliquids. Cell morphology was altered especially when nicotine was present in the eliquid, as filopodia appeared to shorten in lung fibroblasts or periodontal ligament gingiva fibroblasts [54,79]. Propylene glycol and glycerin, main humectants of eliquids on the market, were associated with increased H_2O_2 (equivalent to μM levels) and alterations of lung fibroblasts morphology [54]. Accumulation of vacuoles was reported in various cells treated with nicotine containing eliquid [54,79,80]. The effects of direct exposure of lung tissue *in vivo* to eliquids and these humectants have not been well studied yet.

2.2. Effects on miRNA and gene expression in bronchial epithelium

MicroRNAs (miRNAs), small RNA molecules (21–25 nucleotides long) that regulate gene expression post-transcriptionally [81], have been reported previously as modulators of smoking induced gene expression changes in human airway epithelium [82]. The effects of ecigs on whole miRNA transcriptome and on specific miRNA expression in NHBE cells upon ecig and tobacco cigarette exposure have been assessed in pilot studies [83,84]. Liquid fractions/extracts of the aerosol were generated and the condensate was used to expose NHBE that are differentiated at air lifted conditions. It should be noted that such aerosol fractions may differ in composition from cigarette smoke and contain far fewer components. 578 miRNAs were reported to be differentially expressed between the ecig exposure (nonvaporized or vaporized and condensed) and untreated cells. Vaporized (and condensed) ecig fraction treatment resulted in differentially expression of 125 miRNAs compared to nonvaporized liquids, thus vaporization of ecig liquid was associated with a greater response. Nicotine containing ecig extract also displayed a greater effect on miRNA expression [84]. In cells exposed to vaporized ecig liquid, expression of MIR1265P was increased while its target genes *MYC*

and *MRGPRX3* exhibited reduced expression in all conditions tested [84].

Furthermore, exposure to ecig liquid (nonvaporized or vaporized and condensed) induced the expression of oxidative stress response genes such as *GCLM*, *GCLC*, *GPX2*, *NQO1* and *HO1* in NHBE cells [84]. The protein products of *GCLC* and *GCLM* catalyze the production of the cellular endogenous antioxidant glutathione (GSH), which exhibits high concentrations in the lungs and even higher expression levels in smokers [85]. In the same vein, glutathione peroxidases, the primary antioxidant enzymes that scavenge and detoxify H_2O_2 and organic hydroperoxides, were also increased in the lungs of cigarette smoke exposed mice [84,86]. For note, *Nrf2* expression was not induced in this particular experimental setting in NHBE cells although it plays an important role in the transcriptional regulation of oxidative stress response genes [84].

Zannetti et al. [87] compared the impact of nicotine containing aerosol generated by HnB tobacco product (developed by Philip Morris International) with cigarette smoke on human organotypic buccal and gingival epithelial cultures derived from the same, nonsmoker, healthy donor. Cultures established on transwells were exposed to comparable concentrations of tobacco smoke and HnB aerosol, matched by delivered nicotine dose. Minor overall morphological changes were observed in HnB aerosol treated cells compared to tobacco smoke treated cells and there were no signs of explicit cytotoxicity at even higher concentrations of HnB. The overview of differentially expressed genes and the biological interpretation using a network based analysis [88,89] revealed that pathways that were consistently impacted by both the cigarette smoke and HnB aerosol included xenobiotic metabolism, oxidative stress response and inflammation related processes [87]. At comparable concentrations, tobacco smoke had higher impact on gene expression related to oxidative stress network than HnB aerosol, and higher impact on buccal samples compared with gingival samples. *HMOX1*, *TXNRD1*, *GCLC*, *GCLM*, *SLC7A11*, *NQO1*, and *GPX2* genes, which are associated with oxidative stress, were upregulated by exposure to both tobacco smoke and HnB aerosol, with the response in HnB treated cultures being again lower.

In the same study, tobacco smoke resulted in 265 and 264 differentially expressed miRNAs in buccal and gingival cultures, respectively, whereas HnB aerosol induced only 4 and 145 differentially expressed miRNAs. Thus, HnB aerosol seems to affect less miRNA expression compared with tobacco smoke. Lastly, morphological changes and cytoskeleton reorganization were also observed at the molecular level, by considerable changes in mRNA, miRNA and protein levels of structural molecules in cigarette smoke and HnB aerosol exposed buccal and gingival cultures [87].

2.3. Oxidative stress in experimental animals *in vivo* and in humans

Ecig aerosol's major component is nicotine [90] and via nicotine metabolites, methyldiazohydroxide (MDOH), and aldehydes, nicotine can induce mutagenic DNA adducts such as O^6methyldeoxuguanosine in lung, liver, bladder and heart tissues of mice, as well as γ−OH−PdG and α-methyl-γ−OH−1,N2-PdG that are major DNA adducts in mouse models [35,40]. Nicotine metabolites are shown to inhibit DNA repair in human lung and bladder epithelial cells and ecig exposed mice exhibit significantly lower NER and BER activity in lung tissue compared to filtered air exposed mice [35]. Long time exposure (90 day) to HnB aerosol through the nasal cavity exhibited lower cellular stress and lung inflammation, compared to similar cigarette smoke exposure in Sprague Dawley rats [91].

To date, *in vivo* data on effects of ecigs on the airways are very limited. To study the influence of ecigs use on protein abundance in lung tissue, bronchial brush biopsies and lavage samples were collected from healthy nonsmokers, cigarette smokers and ecig users. Proteomic investigation by mass spectrometry (LC-MS/MS) revealed alterations in overall protein abundance between smokers and ecig users. 292 proteins were significantly up or downregulated (141 and 151 proteins, respectively) in the smokers, and the levels of 191 proteins were altered (132 up and 59 downregulated) in ecig users; 78 of these proteins were common in both groups. Pathway analysis of these proteins revealed that especially, the groups of proteins associated with membranes were altered in ecig user group [92], however the proteins or the pathways relevant to oxidative stress or antioxidant metabolism were not further studied. In a different study, proteome analysis of sputum of ecig users revealed significant upregulation of oxidative stress response proteins, such as thioredoxin (TXN) and glutathione S transferase (GSTP1) [93].

3. Concluding remarks

Airway epithelial cells reside at the interface between the host and the environment, and act as the first line of defense against microorganisms, allergens and harmful gases such as cigarette smoke and potentially ENDS aerosol. Tobacco smoke has been previously shown to reduce the antioxidant capacity of tissues and the rate of DNA damage repair [23,94]. The majority of studies cited in this review report that ENDS aerosols can induce oxidative stress, albeit much lower compared to tobacco smoke *in vitro*, however significantly higher compared to naive (unexposed control) condition, pointing to some potential toxicity whose pathophysiological importance is still difficult to evaluate.

In studies which focus on harmful effects of ENDS aerosol, different forms of aerosols/liquids are being tested. Vaporized and condensed, liquid fractions, extracts of smoke or aerosols recapitulate only partly the exposure strategies to cigarette or ENDS aerosol. The effects of direct exposure of lung tissue to eliquids and their humectants are not widely known. Analytical methodologies that precisely determine the chemical composition and the existence of specific toxic substances in the liquefied vapor, aerosol etc. would help in the generation of more trustworthy results. Regarding the dosage, this could for example be adjusted to the nicotine emission levels or cotinine plasma levels in case of *in vivo* studies. Most of the *in vitro* studies employ short time exposure that imitates acute effects, however "vapers" are exposed to cigarette smoke for much longer time periods. Data on the effects of long term exposure, which would resemble the *in vivo* situation more closely, are currently limited in number [95,96], as are the *in vivo* studies that investigate the effects of ENDS aerosols. Some particular flavor additives have been specifically reported to be stronger oxidizers than tobacco flavors [54], which makes claims about the relative safety of ENDS questionable. The cell culture conditions under which the exposure is made are also quite variable, including application of aerosols to the cell growth media, air liquid exposure at the apical side and use of transwells. Three dimensional organotypic airway cultures should also be further considered as valuable models to evaluate the effects of ENDS aerosol and efforts to use setups that resemble the actual situation in the respiratory epithelium during smoking have to be more widely used, to provide more reliable conclusions.

A more in depth assessment of ENDS aerosols in various experimental settings regarding their safety, toxicity and potential carcinogenic and oxidative effects is needed.

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Conflicts of interest

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