Graphical review: The redox dark side of e-cigarettes; exposure to oxidants and public health concerns

Hua Cai, Chen Wang

Since the initial marketing in 2005, the use of e-cigarettes has increased exponentially. Nonetheless, accumulating evidence has demonstrated the ineffectiveness of e-cigarettes in leading to smoking cessation, and decreasing the adverse health impacts of cigarette smoking. The number of adolescents adapted to e-cigarettes has been increasing substantially each year, and this adaptation has promoted openness to tobacco smoking. The present review discusses controversies regarding the smoking cessation effects of e-cigarettes, recent governmental policies and regulations of e-cigarette use, toxic components and vaporization products of e-cigarettes, and the novel molecular mechanisms underlying the adverse health impacts of e-cigarettes leading to oxidative stress in target tissues, and consequent development of cardiopulmonary diseases (i.e. COPD), neurodegenerative disorders (i.e. Alzheimer’s disease), and cancer. Health warning signs on the packaging and professional consultation to avoid adaptation in risk groups might be helpful solutions to control negative impacts of e-cigarettes. It is also recommended to further expand basic and clinical investigations to reveal more detailed oxidative stress mechanisms of e-cigarette induced damages, which would ultimately result in more effective protective strategies.
The regulatory policies on e-cigarettes have been lacking, at least in part due to the incompletely understood health impacts of the e-cigarettes. With encouragement of the government to promote self-regulation, the first National Association of Electronic Cigarettes (NAEC) was established in 2015 in China, which is a professional association with 1800 industrial participants [1]. Back in 2006, The State Tobacco Monopoly Administration (STMA) classified e-cigarettes as potentially harmful chemical products, recommending regulation under the State Administration of Work Safety [1]. Appropriate regulation is in urgent need given the potential health hazard induced by e-cigarettes, particularly considering the e-cigarette marketing to youth and non-smokers. In the UK, e-cigarettes that contain nicotine are regulated either as tobacco-related products or as licensed medicines [2]. In the US, the Food and Drug Administration (FDA) established new rules on e-cigarette regulations in 2016, with a series of timelines starting in August 2016 (http://fdaregs.info/fda-deeming-regulations/timeline/).

For habitual e-cigarette users or people who use e-cigarettes as a smoking cessation aid, the chronic use of e-cigarettes, and the use of e-cigarettes during and after attempted smoking cessation, are associated with worrisome adverse effects. Most of the e-cigarettes tested for smoking cessation contain nicotine [9,10], which is known to contribute to cardiopulmonary diseases, neurodegenerative disorders and cancer [12]. Although consumed at lower levels in e-cigarette users compared to tobacco cigarette users, this lower level of nicotine has already passed the threshold of being potentially toxic [13]. For teenagers, nicotine exposure will affect brain development and lead to nicotine addiction that has a lifelong impact. Thermal decomposition of the solvents in e-cigarettes generates an array of organic compounds including carbonyls, one of which is acrolein (from glycerol/glycerine). Of note, acrolein has been shown to be capable of inducing COPD [14], the third leading cause of death worldwide [9]. The levels of acrolein are elevated in lung fluids of COPD patients [14]. Many of the identified components released from e-cigarette vaporization are potential carcinogens, including toxic metals (cadmium, chromium, lead, manganese and nickel), acrolein, and other organic compounds such as propylene oxide formed from propylene glycol [10,15]. The latter, propylene glycol, is not present in traditional tobacco cigarettes. Direct exposure of mice to e-cigarette vapors resulted in inflammation and reduced clearance of bacteria and virus, which are key features of COPD [3]. A recent study has demonstrated that e-cigarette exposure induced DNA strand break and apoptosis regardless of nicotine contents [16]. Moreover, some of the flavoring additives have been shown to be pathogenic [8,17]. These flavorings contain cytotoxic [8,17], which are recognized as “primary irritants” of mucosal tissue of the respiratory tract [18]. Using gas chromatography/mass spectrometry (GC/MS) to analyze 28 e-cigarette liquids from seven manufacturers, Hutzler and colleagues reported that vanillin, ethyl maltol, ethyl vanillin and menthol were the four most frequently found flavor chemicals, which were present in 79%, 57%, 50% and 43% of the 28 samples, respectively [19]. Of note, Bahl et al. examined 41 e-cigarette refill fluids for cytotoxicity to human pulmonary fibroblasts, human embryonic stem cells and mouse neural stem cells, and noted that when present, the cytotoxicity was related to the flavor chemicals, especially for cinnamon-flavored refill fluids [20,21]. The potential pathological effects of these e-cigarette vaporization products and flavoring additives are summarized in Fig. 2.

Importantly, the latest studies have demonstrated that the use of e-cigarettes is associated with increased oxidative stress, which seems to mediate the adverse effects of e-cigarettes. Oxidative stress develops in e-cigarette-exposed human bronchial and lung epithelial cells [22,23], human lung vascular endothelial cells and human umbilical vein endothelial cells [24,25], resulting in inflammation, cytotoxicity and increased endothelial cell permeability [22–26]. In these studies, intracellular production of reactive oxygen species (ROS) was determined.

![Diagram of e-cigarette components and effects](image-url)
using fluorescent probes such as DCF-DA for intracellular hydrogen peroxide [23,25,27]. Exposure to nicotine that was specifically generated by the use of e-cigarettes, was shown to promote oxidative stress dependent impairment of autophagy, which in turn serves as a potential mechanism leading to development of COPD [27]. Learner and colleagues have recently shown that e-cigarette aerosols and copper nanoparticle induce mitochondrial ROS production, mitochondrial stress (reduced stability of OxPhos electron transport chain (ETC) complex IV subunit) and DNA fragmentation in lung fibroblasts [28]. Acrolein has been shown to provoke oxidative stress and inflammation to result in loss of endothelial cell barrier integrity in the lung [24]. Propylene glycol, glycerin, and methanol have all been shown to increase production of hydrogen peroxide [28]. The flavoring additive of cinnamon roll stimulated substantial increase in the production of inflammatory cytokine IL-8 in human lung fibroblasts [28].

Furthermore, recent reports have confirmed oxidative stress inducing effects of e-cigarettes in animal models in vivo [3,28], and in human e-cigarette users [10,15]. Electron paramagnetic/spin resonance (EPR/ESR) determination of free radical production indicated increased ROS release from e-cigarette vaporization in both cell-free and cell systems, which is associated with increased lipid peroxidation in the lung homogenates (assessed by thiobarbituric acid reactive substances (TBARS)) of e-cigarette exposed mice [3]. The authors also observed a 58% increase in macrophage counts in the bronchoalveolar lavage (BAL) 24 h after the final exposure (twice per day for two weeks), which was accompanied by an elevated production of IL-6 [3]. In healthy human subjects, the use of e-cigarettes stimulated oxidative stress, nitric oxide deficiency, and endothelial/vascular dysfunction that translated into impaired flow-mediated dilatation [29]. In addition, Moheimani et al. recently reported occurrence of oxidative stress and increased cardiac sympathetic activity in habitual e-cigarette users, which are known risk factors for cardiovascular diseases [30]. The role of oxidative stress in mediating adverse effects of e-cigarettes to result in cardiopulmonary pathogenesis, neurodegenerative disorders and cancer, is illustrated in Fig. 3.

One may propose that e-cigarette users could take some anti-oxidants to offset the toxicity of oxidative stress. Given the complexities of how ROS function in vivo for both physiological (low levels of ROS production are required for growth signaling) and pathological processes, and that the detailed molecular mechanisms underlying e-cigarette induction of oxidative stress have remained unclear, it is not yet possible to deliver precise anti-oxidative treatments effectively to control oxidative stress damage. The oxidase systems selectively responsible for the development of different human diseases are still under intensive investigations to enable targeted therapies. Nonetheless, recent studies have demonstrated whereas NADPH oxidase (NOX) isoform 1 (NOX1) is responsible for diabetic vascular complications [31], NOX4 activation mediates ischemia reperfusion injury in the heart [32]. Cigarette smoking has been shown to induce oxidative stress to lead to endothelial dysfunction and vascular diseases [33–35]. A role of endogenous NOX and extracellular superoxide dismutase (ecSOD) in smoking induced oxidative stress has been implicated [36]. Of note, recent studies indicate that airway smooth muscle NOX4 can be upregulated to produce ROS in COPD [37].

NOX family oxidases are known to activate other oxidase systems to result in a vicious cycle of prolonging ROS production, resulting in sustained oxidative stress and initiation of pathological processes. It would be important to investigate the oxidase networks to reveal detailed mechanisms of oxidative stress development in response to e-cigarette exposure. At the present, however, the best solution to prevent damages caused by e-cigarette-derived oxidative stress might be to avoid adaptation to e-cigarettes, particularly when their smoking cessation effects are questionable.

Therefore, it is important to give deeper consideration to the adaptation and regulation of e-cigarettes as an alternative to tobacco smoking since they introduce new toxic agents, toxicity of which is poorly understood. With the availability of objective data from human trials and basic research, appropriate health warnings could be included on the packaging alerting nicotine-dependent and independent adverse effects. Recent data indicate that only 22% of e-cigarette liquid bottles used a warning statement that indicated the product “contained nicotine” [38]. None of the statements included the information that nicotine was “addictive”. Only about half of the websites for e-cigarette marketing have a minimum age requirement barrier that prevented under-aged persons from entering [38].

Furthermore, it is probably not too early to start advising the tobacco users in the community not to rush into the harmful adaptation of e-cigarettes, and for physicians to guide populations at risk of relevant diseases, including cardiopulmonary diseases, neurodegenerative disorders and cancer, to avoid them. These recommended strategies to...
better control negative impacts of e-cigarettes are summarized in Fig. 4. Increasing the awareness of the oxidative stress aspects of the e-cigarette use may prove beneficial in promoting basic and clinical investigations to further our understandings of the adverse effects of e-cigarettes to better protect against these detrimental consequences.

Acknowledgements

This work was supported by the National Key Research and Development Program of China Grants 2016YFC1303900 (CW), 2016YFC0901102 (CW), National Institute of Health National Heart, Lung and Blood Institute (NHLBI) Grants HL077440 (HC), HL088975 (HC), HL108701 (HC, DGH), HL119968 (HC), and an American Heart Association Established Investigator Award (EIA) 12EIA8990025 (HC).

References

[1] X. Xu, X. Wang, X. Zhang, Y. Liu, H. He, J. Mackay, The debate on regulation of e-cigarettes in China, Lancet Respir. Med. 4 (2016) 856–858, http://dx.doi.org/10.1016/S2216-325X(16)30137-9 (Epub 2016 Oct 12).

[2] House of Parliament Postnote Number 533 on Electronic Cigarettes, Parliamentary Office of Science and Technology.

[3] T.E. Sussan, S. Gaigkate, R.K. Thimmulappa, J. Ma, J.H. Kim, J. Hahn, Chemical hazards present in liquids and vapors of electronic cigarettes, Arch. Toxicol. 88 (2014) 1295–1308, http://dx.doi.org/10.1007/s00204-014-1294-7 (Epub 2014 Jun 11).

[4] V. Bahl, S. Lin, N. Xu, B. Davis, Y.H. Wang, P. Talbot, Comparison of electronic cigarette refill fluid cytotoxicity using embryonic and adult models, Reprod. Toxicol. 34 (2012) 529–537, http://dx.doi.org/10.1016/j.reprotox.2012.08.001 (Epub 2012 Aug 20).

[5] P.A. Tierney, C.D. Karpinski, J.E. Brown, W. Luo, J.F. Pankow, Flavour chemicals in electronic cigarettes: a policy statement from the American Heart Association, Circulation 130 (2014) E211-E218, http://dx.doi.org/10.1161/CIR.0000000000000107 (Epub 2014 Nov 4).

[6] R.A. Arrazola, T. Singh, C.G. Corey, C.G. Huerin, L.J. Neff, B.J. Apelberg, R.E. Bunnell, C.J. Choiniere, B.A. King, Association between electronic cigarette use and openness to cigarette smoking among middle and high school students – United States, NCHS Data Brief. (2015) 1–8.

[7] S. Kalkhoran, S.A. Glantz, E-cigarettes and smoking cessation in real-world and clinical settings: a systematic review and meta-analysis, Lancet Respir. Med. 4 (2016) 116–128, http://dx.doi.org/10.1016/S2213-2600(15)00214-4 (Epub 2016 Jun 14).

[8] R.A. Arrazola, T. Singh, C.G. Corey, C.G. Huerin, L.J. Neff, B.J. Apelberg, R.E. Bunnell, C.J. Choiniere, B.A. King, S. Cox, T. McAfee, R.S. Caraballo, Tobacco use among middle and high school students – United States, MMWR Morb Mortal Wkly Rep. 2015, 64, 2011–2014, pp. 381–5.

[9] B.N. Coleman, B.J. Apelberg, B.K. Ambrose, K.M. Green, C.J. Choiniere, R. Bunnell, C.J. Carroll, B.D. Ray, W.C. Hubbard, E.S. Kim, X. Lai, M. Wang, W.D. Kranz, C.J. Forste, L.E. Crotty Alexander, K.A. Jacobson, C. Westerlund, A. Luch, Chemical hazards present in liquids and vapors of electronic cigarettes, Arch. Toxicol. 88 (2014) 1295–1308, http://dx.doi.org/10.1007/s00204-014-1294-7 (Epub 2014 Jun 11).

[10] V. Bahl, S. Lin, N. Xu, B. Davis, Y.H. Wang, P. Talbot, Comparison of electronic cigarette refill fluid cytotoxicity using embryonic and adult models, Reprod. Toxicol. 34 (2012) 529–537, http://dx.doi.org/10.1016/j.reprotox.2012.08.001 (Epub 2012 Aug 20).

[11] P.A. Tierney, C.D. Karpinski, J.E. Brown, W. Luo, J.F. Pankow, Flavour chemicals in electronic cigarettes: a policy statement from the American Heart Association, Circulation 130 (2014) E211-E218, http://dx.doi.org/10.1161/CIR.0000000000000107 (Epub 2014 Nov 4).

[12] R.A. Arrazola, T. Singh, C.G. Corey, C.G. Huerin, L.J. Neff, B.J. Apelberg, R.E. Bunnell, C.J. Choiniere, B.A. King, Association between electronic cigarette use and openness to cigarette smoking among young adults, Nicot. Tob. Res. 17 (2015) 212–218, http://dx.doi.org/10.1093/ntr/ntu211 (Epub 2014 Nov 4).

[13] J.A. Aguilera, S. Advani, L.E. Crotty Alexander, K.T. Brumund, J. Wang-Rodriguez, M. Aufderheide, Evaluation of E-cigarette liquid vapor and mainstream cigarette smoke after direct exposure of primary human bronchial epithelial cells, Int J. Environ. Res. Public Health 12 (2015) 3915–3925, http://dx.doi.org/10.3390/ijerph120403915.

[14] C.A. Lerner, L.K. Sundar, H. Yao, J. Gerlof, D.J. Ossip, S. McIntosh, R. Robinson, I. Rahman, Vapours produced by electronic cigarettes and e-juices with flavors induce toxicity, oxidative stress, and inflammatory response in lung epithelial cells and in mouse lung, PLoS One 10 (2015) e0116732, http://dx.doi.org/10.1371/journal.pone.0116732 (Ecollection 2015).

[15] K.S. Schweitzer, S.X. Chen, S. Law, M. Van Demark, C. Poirier, M.J. Justice, W.C. Hubbard, Evaluation of E-cigarette liquid vapor and mainstream cigarette smoke after direct exposure of primary human bronchial epithelial cells, Int J. Environ. Res. Public Health 12 (2015) 3915–3925, http://dx.doi.org/10.3390/ijerph120403915.

[16] J.A. Aguilera, S. Advani, L.E. Crotty Alexander, K.T. Brumund, J. Wang-Rodriguez, M. Aufderheide, Evaluation of E-cigarette liquid vapor and mainstream cigarette smoke after direct exposure of primary human bronchial epithelial cells, Int J. Environ. Res. Public Health 12 (2015) 3915–3925, http://dx.doi.org/10.3390/ijerph120403915.
C.A. Lerner, P. Rutagarama, T. Ahmad, I.K. Sundar, A. Elder, I. Rahman, Electronic cigarette aerosols and copper nanoparticles induce mitochondrial stress and promote DNA fragmentation in lung fibroblasts, Biochem. Biophys. Res. Commun. 477 (2016) 620–625, http://dx.doi.org/10.1016/j.bbrc.2016.06.109 (Epub2016 Jun 23).

R. Carnevale, S. Sciarretta, F. Violi, C. Nosella, L. LoRredo, L. Perri, M. Peruzzi, A.G. Marullo, E. De Falco, I. Chimenti, V. Valentì, G. Biondi-Zoccai, G. Frati, Acute impact of tobacco vs electronic cigarette smoking on oxidative stress and vascular function, Chest 150 (2016) 606–612, http://dx.doi.org/10.1016/j.chest.2016.04.012 (Epub2016 Apr 22).

R.S. Moheimani, M. Bhetraratana, F. Yin, K.M. Peters, J. Gornbein, J.A. Araujo, H.R. Middlekauff, Increased cardiac sympathetic activity and oxidative stress in habitual electronic cigarette users: implications for cardiovascular risk, JAMA Cardiol. 2 (2017) 278–284, http://dx.doi.org/10.1001/jamacardio.2016.5093.

J.Y. Yoon, L. Gao, H. Cai, The p47phox- and NADPH oxidase organiser 1 (NOXO1)-dependent activation of NADPH oxidase 1 (NOX1) mediates endothelial nitric oxide synthase (eNOS) uncoupling and endothelial dysfunction in a streptozotocin-induced murine model of diabetes, Diabetologia 55 (2012) 2069–2079 (Epub2012 May 2).

K.L. Siu, C. Lotz, P. Ping, H. Cai, Nitrin-1 abrogates ischemia/reperfusion-induced cardiac mitochondrial dysfunction via nitric oxide-dependent attenuation of NOX4 activation and recoupling of NOS, J. Mol. Cell Cardiol. 78 (2014) 174–185.

H. Cai, Hydrogen peroxide regulation of endothelial function: mechanisms, consequences and origins, Cardiovasc. Res. 68 (2005) 26–36.

H. Cai, NAD(P)H oxidase-dependent self-propagation of hydrogen peroxide and vascular disease, Circ. Res. 96 (2005) 818–822.

H. Cai, D.G. Harrison, Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress, Circ. Res. 87 (2000) 840–844.

A.K. Tollefson, R.E. Oberley-Deegan, K.T. Butterfield, M.E. Nicks, M.R. Weaver, L.K. Remigio, J. Decesznak, H.W. Chu, D.L. Bratton, D.W. Riches, R.P. Bowler, Endogenous enzymes (NOX and ECSOD) regulate smoke-induced oxidative stress, Free Radic. Biol. Med. 49 (2010) 1937–1946, http://dx.doi.org/10.1016/j.freeradbiomed.2010.09.022 (Epub2010 Sep 29).

F. Hollins, A. Sutcliffe, E. Gomez, R. Berair, R. Russell, C. Szyndralewiez, R. Saunders, C. Brightling, Airway smooth muscle NOX4 is upregulated and modulates ROS generation in COPD, Respir. Res. 17 (2016) 84, http://dx.doi.org/10.1186/s12931-016-0403-y.

P. Fagan, P. Pokhrel, T.A. Herzog, M.C. Guy, K.K. Sakuma, D.R. Trinidad, K. Cassel, D. Jorgensen, T. Lynch, J.Q. Felicia-Perkins, S. Palaloox, F. Hamamura, S. Maloney, K. Degree, K. Sterling, E. Moolchan, M.S. Clanton, T. Eissenberg, Warning statements and safety practices among manufacturers and distributors of electronic cigarette liquids in the United States, Nicotine Tob. Res. (2017) 18.