Electronic cigarettes: The nicotyrine hypothesis

Aaron Abramovitz [a], Amy McQueen [a], Raul E. Martinez [b], Brent J. Williams [b], Walton Sumner [a,*]

[a] School of Medicine, Washington University in St. Louis, St. Louis, MO, USA
[b] School of Engineering & Applied Science, Washington University in St. Louis, St. Louis, MO, USA

ABSTRACT

There are conflicting reports about the efficacy of electronic cigarettes (e-cigs) as nicotine delivery devices and smoking cessation products. In addition, smokers' responses to some nicotine dependence questions often change as they transition to exclusive e-cig use. Nicotyrine may explain these observations. Nicotyrine forms by the gradual oxidation of nicotine in e-liquids exposed to air. E-cigs aerosolize nicotyrine along with nicotine. Nicotyrine inhibits the cytochrome P450 2A family of enzymes (CYP2A) in airways and liver. These enzymes metabolize nicotine to cotinine, and then cotinine to *trans* 3-hydroxycotinine. In humans, nicotine is metabolized primarily by hepatic CYP2A6.

We propose that e-cig users (vapers) achieve measurable serum nicotine levels when they inhale nicotine and nicotyrine together, because nicotyrine reversibly inhibits nicotine metabolism by CYP2A13 in airways. Consuming nicotyrine by any route should irreversibly inhibit hepatic CYP2A6. When CYP2A6 is substantially inhibited, nicotine clearance is delayed and nicotine withdrawal symptoms are attenuated. Small, relatively infrequent nicotine doses can then sustain satisfying nicotine doses.

This theory has numerous implications for e-cig research and tobacco control. Behavioral and pharmacokinetic e-cig studies should be interpreted with attention to likely levels of nicotyrine delivery: e-cig studies may need to routinely measure nicotyrine exposure, assess CYP2A6 activity, confirm nicotine delivery, or deliberately compare unoxidized and oxidized e-liquids. The risks of nicotyrine exposure include impaired clearance of all CYP2A substrates and any effects of the metabolic products of nicotyrine. CYP2A inhibitors like nicotyrine may be useful for future smoking cessation therapy.

Introduction

Electronic cigarettes (e-cigs) aerosolize a nicotine solution (e-liquid) for inhalation (vaping) by users (vapers). E-cig use is increasing rapidly, raising questions about safety and utility for smoking cessation. E-cig designs have evolved from low power, cigarette-shaped first generation designs, to higher power, typically refillable second generation designs, to very powerful, adjustable, refillable, and customizable third generation designs [1]. E-liquids are comprised of propylene glycol (PG) and/or vegetable glycerin (VG), nicotine, and artificial flavoring. Most flavorings are chemicals that are Generally Regarded As Safe (GRAS) for external use or ingestion, but these chemicals may not be safe to inhale. The manufacturing of e-liquids and e-cigs is unregulated, leading to further safety concerns among public health officials and vapers. Health advocates also have raised concerns about the potential for e-cigs to aerosolize illicit drugs, cause fires, re-normalize

smoking type behavior, and act as a gateway drug, addicting non-smoking youth to nicotine and leading them to become smokers. On the other hand, e-cig aerosols clearly contain fewer and less concentrated noxious compounds than cigarette smoke, and first and second generation e-cigs seem to reduce conventional measures of dependence [2–5].

Investigators report many contradictory observations about the efficacy of first and second-generation e-cigs as nicotine delivery devices. One group reported that e-cigs did not deliver nicotine to smokers [6,7], but later found that vapers using their own e-cigs did absorb nicotine [8]. Many vapers report a learning curve with e-cigs. This may not be just a matter of technique: experienced vapers may find new e-liquids unsatisfying for a few days [2]. One research group reported that smokers can reduce or stop smoking with e-cigs [9–11], yet these and other researchers have found modest cessation rates in randomized controlled trials, with little difference between e-cigs with and without nicotine, or between e-cigs and conventional nicotine replacement products [12,13]. E-cigs and cigarettes relieve nicotine cravings at different speeds: smokers get relief in a few seconds, implying alveolar absorption of nicotine, while vapers using their preferred devices report relief taking several minutes [2]. This suggests nicotine

* Corresponding author at: School of Medicine, Washington University in St. Louis, St. Louis, MO 63110, USA. Tel.: +1 314 454 8164.
E-mail address: wsumner@dom.wustl.edu (W. Sumner).

absorption through airways, as with nicotine inhalers, yet e-cigs relieve craving more quickly and are viewed more favorably than inhalers [2,14].

Substantial variability in aerosol chemistry is expected due to formulation details, but one compound is noteworthy. Nicotyrine is usually not detected in e-liquids or e-cig aerosols, but was reported in an early analysis by the FDA [15,16]. We found that this nicotine oxidation product [17] accumulates in e-liquids over time, but only after exposure to air (Fig. 1) [18]. We have identified no other nicotine analogs in e-liquids or e-cig aerosols. Nicotyrine is a potent reversible inhibitor [19] of human cytochrome P450 2A13 (CYP2A13) found in the nasal and respiratory epithelium [20] and is an irreversible, tight-binding inhibitor [19] of CYP2A6, which is found in the liver [21] and possibly respiratory epithelium [22]. CYP2A enzymes oxidize nicotine to cotinine and then cotinine to *trans* 3-hydroxycotinine [23]. CYP2A6 activity is highly correlated with nicotine clearance and smoking behavior [24,25] and inversely correlated with smoking-related exposures and risks [26–28].

The nicotyrine hypothesis

We propose that first and second generation e-cigs deliver nicotine most effectively when aerosolized nicotyrine facilitates absorption of aerosolized nicotine through airways by reversibly inhibiting airway epithelial CYP2A13, and then substantially slows hepatic nicotine clearance by irreversibly inhibiting CYP2A6. Using e-liquids with high nicotine and nicotyrine concentrations therefore may lead to higher and more sustained serum levels of nicotine, compared to e-liquids with high nicotine and low nicotyrine levels.

In contrast to conventional thinking about e-cigs, this hypothesis anticipates that nicotyrine is not an incidental contaminant, but an important component in many e-cig aerosols that successfully deliver nicotine to users. To achieve a satisfying serum nicotine level in vapers with normal CYP2A13 activity, e-cigs must do at least one of the following: (1) aerosolize partially oxidized e-liquids; (2) oxidize some nicotine in the process of aerosolization; (3) overwhelm CYP2A13 in airways with nicotine, or (4) generate nicotine-laden particles that deposit in alveoli. We believe that in first and second-generation e-cigs, the primary mechanism is aerosolization of partially oxidized e-liquids.

If e-cigs deliver enough nicotyrine to impair CYP2A6, then we expect smokers with highly active CYP2A6 genotypes to develop a much lower and less frequent need for nicotine. This should markedly reduce exposure to compounds associated with smoking. However, e-cig users will be exposed to possible health risks inherent in having diminished CYP2A6 activity and from any toxicity due to nicotyrine or other components of aerosolized e-liquids.

Evaluation of the nicotyrine hypothesis

Supporting evidence

The hypothesis could succinctly explain the seemingly contradictory observations described above. Investigators testing e-cigs in controlled studies are almost certain to have acquired new devices and e-liquids rather than aged e-liquids. New e-cigs containing new e-liquid would not deliver nicotine very effectively to smokers, but vapers using their own refillable e-cigs would use partially oxidized solutions that co-deliver nicotyrine and nicotine. The hypothesis would explain why new e-liquids and disposable e-cigs are initially unsatisfying and why so many vapers report a "learning curve" lasting several days [2]. It also could explain the popularity of deliberate aging of e-liquids by vendors and steeping by vapers seeking "to improve the flavor" [29,30]: these activities must oxidize some nicotine prior to vaping. Finally, short-term changes in nicotine dependence measures could primarily reflect slowed nicotine clearance rather than a lasting change in nicotine dependence.

At least two mouse models support the hypothesis. A 1982 report found that nicotyrine inhibits the metabolism of nicotine without increasing its toxicity in albino mice [31]. A recent study [32] demonstrates behavioral changes in mice exposed to methoxsalen, a CYP2A5 inhibitor (CYP2A5 is the murine analog of human CYP2A6 and CYP2A13). Mice given methoxsalen preferred lower doses of nicotine, had more sustained plasma nicotine levels, and had decreased plasma cotinine levels compared to untreated mice. In addition, treated mice required lower doses of nicotine to reverse nicotine withdrawal, compared to untreated mice. Analogous human CYP2A6/13 inhibition by nicotyrine could allow low and infrequent doses of nicotine to relieve craving. The mouse models also suggest that nicotine toxicity can develop with lower nicotine exposure when CYP2A enzymes are impaired by nicotyrine.

This hypothesis mirrors other tobacco-related hypotheses. Nicotyrine was suggested to be the agent in tobacco smoke that slows nicotine metabolism [23,33,34]. Nicotyrine also may be a minor product of CYP2A6 nicotine metabolism, so that nicotine inhibits its own metabolism [35]. Nicotyrine has been used as a model for a potential class of smoking cessation aids based on CYP2A inhibition [35–37].

Predictions

In vitro studies can demonstrate the effects of nicotyrine on nicotine metabolism by purified CYP2A6 and CYP2A13 variants. We are investigating the effects of nicotyrine on primary human tracheal epithelium to test whether nicotyrine inhibits CYP2A13 function in these cells: inhibition should lead to higher concentrations of nicotine relative to cotinine and 3-OH-cotinine in cell media if the hypothesis is correct.

Mouse studies could compare the behavioral and metabolic effects of nicotine administration (as in Bagdas et al. [32]). Wild type mice dosed with nicotyrine and CYP2A5 knockout mice should have similarly slow metabolism and related behaviors: wild type mice not dosed with nicotyrine should have fast metabolism. The hypothesis suggests that long-term vapers will experience chronic nicotyrine exposure, and might not suffer important harms
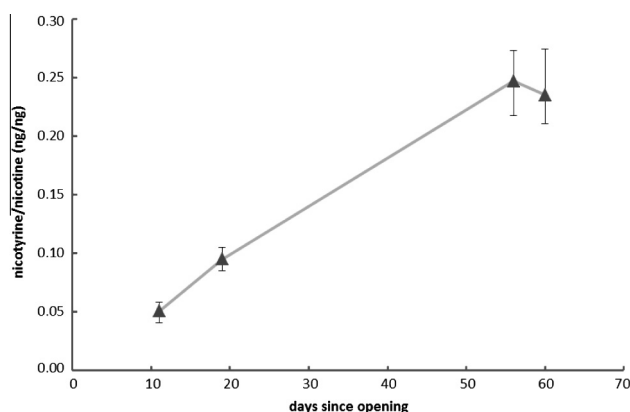
Fig. 1. Nicotyrine to nicotine ratios in aerosol following exposure to air. The fraction of nicotine oxidized to nicotyrine in e-cig aerosols increases steadily for at least 55 days after commercially available e-liquid refill bottles are opened and partially emptied, under a variety of storage conditions [18].

aside from increased susceptibility to nicotine toxicity. Mouse models can evaluate the consequences of prolonged nicotyrine exposure.

Several human study designs can test the hypothesis. The hypothesis suggests that CYP2A genotypes and aerosol nicotine and nicotyrine content should predict serum nicotine levels, nicotine clearance, metabolite formation, and short-term behavior changes. In addition to these factors, nicotinic acetylcholine receptor alleles will influence long-term e-cig use.

- Smokers with high CYP2A6 and CYP2A13 activity often smoke at least one-half pack per day: daily nicotine metabolite excretion and serum nicotine levels after smoking can be measured. When these smokers switch to first and second-generation e-cigs without nicotyrine, the novice vapers should have low serum nicotine levels, high excretion of cotinine and *trans* 3-hydroxycotinine, and low excretion of other nicotine metabolites, due to large amounts of nicotine being metabolized in airways. As their aging e-liquids accumulate nicotyrine, serum nicotine levels should rise after vaping; cotinine and *trans* 3-hydroxycotinine excretion should fall; relative excretion of other nicotine metabolites should rise, and total nicotine metabolite excretion could fall.
- Vapers with high CYP2A6 and CYP2A13 activity should have nicotyrine dose-dependent increases in total nicotine metabolite excretion. These metabolites could include *cis* 3-hydroxycotinine [38,39], 5-hydroxy-1-methyl-5-(3-pyridinyl)-3-pyrrolin-2-one [35], possibly 5-hydroxycotinine [35], and corresponding glucuronides.
- Smokers with low CYP2A13 activity should show little change during the transition from smoking to vaping, but nicotine consumption and metabolite excretion should decline and shift away from CYP2A products as nicotyrine accumulates in e-liquids and inhibits CYP2A6.
- Smokers with normal CYP2A13 but low CYP2A6 activity probably will smoke less than one-half pack of cigarettes daily, and initially should have relatively low excretion of CYP2A6 products. Cotinine and *trans* 3-hydroxycotinine excretion may increase when they switch to vaping, and then fall back toward baseline as accumulating nicotyrine inhibits CYP2A13.

Cross-sectional studies may support some of these longitudinal predictions. For instance, current 1 pack per day smokers should have genetics similar to vapers who formerly smoked 1 pack per day, but these groups should have markedly different nicotine metabolite excretion patterns, with the vapers excreting smaller amounts of CYP2A6 products. Whether vapers eventually quit using nicotine altogether should be more closely related to nicotinic acetylcholine receptor genotypes than to CYP2A alleles.

Similarly, an established vaper who switches to conventional NRT for several days should exhibit a slowly increasing pattern of nicotine consumption as CYP2A6 activity recovers. Recovery of CYP2A6 function also should result in a gradual increase in the fraction of excreted nicotine metabolites represented by cotinine and 3-OH cotinine glucuronides.

E-cig users in blinded studies should exhibit different vaping behaviors and report satisfaction correlating with the manipulated nicotyrine content in e-liquids. Vaping should be less frequent and satisfaction should increase as nicotyrine exposure increases. Furthermore, e-cig devices and vaping techniques that generate nicotyrine should be more satisfying than those that do not.

E-cigs that relieve nicotine craving without nicotyrine should have a demonstrable capacity to deliver nicotine to alveoli, to overwhelm CYP2A13 with nicotine, or to deliver another CYP2A inhibitor. Third generation e-cigs already have features that suggest alveolar nicotine delivery [40,41].

Discussion

The hypothesis prompts numerous research questions. Answering these questions would inform use and regulation of e-cigs, as well as the development of NRT products enhanced by CYP2A inhibitors.

Nicotine and tobacco exposure markers

Investigators may need to measure serum nicotine and multiple nicotine metabolites to obtain a complete picture of nicotine absorption and metabolism in vapers. Three products of CYP2A enzymes – cotinine, *trans* 3-hydroxycotinine, and NNAL – are common measures of exposure and risk in smoking research. Falling levels and reduced excretion of these products in exclusive vapers and smoking vapers could reflect either reduced exposure to or slowed metabolism of the corresponding CYP2A substrates, nicotine and NNK. The potentially very complex relationship between cotinine levels and psychoactive nicotine doses is reflected in the mouse model that found reduced cotinine formation with increased nicotine levels following CYP2A5 inhibition [32]. In brief, nicotine delivered without nicotyrine may be metabolized to cotinine by a CYP2A enzyme before reaching the blood, resulting in high cotinine levels without systemic nicotine exposure. Conversely, nicotine delivered with or after nicotyrine might be metabolized entirely by alternative pathways, resulting in low cotinine levels in spite of very high systemic nicotine levels.

Behavioral research

Vapers' extensive discussions of products and behaviors are a rich source of raw data for behavioral research. Existing online records describe the trial-and-error discovery of e-liquid handling techniques that "improve taste," likely by increasing nicotyrine delivery, and may describe other methods to raise or maintain serum nicotine levels. Laboratory and clinical studies should examine these accounts in detail, and attempt to include common behaviors that might affect aerosol physics and chemistry. These accounts also document information dissemination, social networks, political activism, and changes in risk perceptions, social norms, and nicotine consumption [42]. Changes in these accounts are likely to reflect new information, new e-cig models, and major tobacco companies' sophisticated marketing efforts for new e-cig products.

Conventional questions that infer smoker's nicotine dependence by assessing frequency of cigarette smoking will require careful refinement if e-cigs can change nicotine metabolism. New vapers' answers to questions about daily vs. non-daily use, uses per day, or time to first use after waking may change quickly because vapers' nicotine clearance is slowing, not because nicotine dependence is diminished. Among smokers, these measures may conflate effects of genetic variation and expression of both nicotine receptors and nicotine metabolism. In contrast, vapers could have uniformly impaired nicotine metabolism, leaving nicotine receptors as the main determinant of dependence. High nicotine dependence might then manifest as inability to quit, rather than frequency of use. The peculiar behavioral profile and physiological exposures of vaping compared to other tobacco product use reinforces the call for changes in the conceptualization and measurement of nicotine and tobacco dependence [43].

Existing studies are difficult to compare due to the use of different e-cig models, e-liquids, number and duration of puffs, and experience of the vaper. Future studies could also report levels of nicotine and nicotyrine in e-liquids for more informative comparisons of results across studies. Studies of nicotine and nicotyrine

delivery with e-cigs could be complicated further by the mixture of first, second, and third generation e-cigs in the marketplace. If third generation products deposit nicotine in alveoli, users may revert to behavior patterns that are much more like smoking.

Aerosolized drug delivery

E-cigs raise complex questions about aerosolized drug delivery generally, and specifically regarding the dose of nicotyrine that vapers receive. E-cigs convert e-liquids into a mixture of gases and particles. Nicotyrine and nicotine are semivolatile compounds, nicotine being the more volatile of the two, and therefore are present in both the gas and particle phases in e-cig emissions. The composition of each phase changes constantly during inhalation and determines where nicotyrine and nicotine will deposit and how much will be absorbed. The gas phase fraction of these emissions may begin deposition in the upper respiratory tract, governed by high diffusion and solubility with the lining of the epithelial layer. E-cig aerosol particles generated in vitro are similar in size to those of cigarette smoke, large enough that they should not experience major diffusional losses, and small enough that they should not experience significant gravitational settling and impaction losses [44]. Thus, chemicals in the particle phase could deposit deep in the lungs.

Accurately modeling the deposition of nicotine and nicotyrine in the respiratory system requires a quantitative measure of both the gas and particle phase fractions as a function of vaping behavior, e-cig hardware, e-liquid age, and e-liquid solvent (glycerin and/or propylene glycol). All of these parameters are certain to alter the total amount of nicotyrine produced, subsequent partitioning to the particle phase, and distribution of each compound in the airways. Systemic absorption of inhaled nicotine may require that absorbing epithelium have near simultaneous deposition of both nicotine and nicotyrine. For example, an upper airway region that favors diffusion from gas phase over particle deposition might receive much more gas phase nicotine than particle-bound nicotyrine. CYP2A enzymes in such an area could promptly metabolize nicotine to cotinine. Recent advances in online aerosol measurement techniques allow for quantitative measurements of the partitioning of semivolatile species [45] and are well suited for e-cig aerosol measurements. Sampling of the gas and particle fraction of nicotine and nicotyrine at the inlet and outlet of a series of models constructed to replicate various fractions of the human airway will offer greater insight on associated phase partitioning and deposition locations.

Risk assessment

The risks of chronic nicotyrine exposure are not known, but several observations suggest that the risks are low. We do not know of any reports that nicotyrine is psychoactive. Nicotyrine could pose a toxic risk, have a toxic metabolite, be mutagenic, or pose indirect risks through CYP2A inhibition. Evidence of toxicity and mutagenicity has been sought but not found. For instance, nicotyrine was briefly examined as a potential murine carcinogen but was abandoned as uninteresting [46]. Animals do not suffer acute toxicity from nicotyrine. Rabbits excrete nicotyrine metabolites (e.g. *cis*-3′-hydroxycotinine) in urine [39]. Reactive intermediate compounds have been suspected and sought with little success.

Nicotyrine has not yet been associated with any smoking-related diseases in humans. Nicotyrine is mentioned in 4474 Legacy Tobacco documents: to our knowledge, none of these associate nicotyrine with smoking risks. All tobacco products contain some nicotyrine. Smoking generates more, and researchers have known for decades that smokers inhale nicotyrine. Furthermore, to the extent that nicotyrine is a CYP2A6 product of

nicotine metabolism [35], even NRT users are chronically exposed to nicotyrine.

The risks of inhibiting CYP2A enzymes may be small under usual circumstances. CYP2A enzyme deficiencies are common in some populations and are associated with reduced smoking and reduced risk of emphysema and lung cancer [25,28,47,48]. Some adults are homozygous for CYP2A6 deletions without reported ill effect. Similarly, reduced CYP2A13 activity has been associated with lower lung cancer risk [49]. Accordingly, much of the literature on CYP2A family enzymes relates to activation of carcinogens [50,51], and indirectly suggests that inhibition might *lower* cancer risk. The International Agency for Research on Cancer classifies methoxsalen, the CYP2A inhibitor used in some mouse studies, as a Group 1 carcinogen, but it is carcinogenic when combined with UVA radiation, and not due to its effects on CYP2A6 [52]. CYP2A family enzymes are not strongly expressed in fetal liver [53,54], limiting concerns about fetal hepatic anomalies.

CYP2A6 inhibition risks

Nicotyrine could increase risks from dietary toxins that CYP2A6 metabolizes. These substrates notably include nicotine and aflatoxin. Mouse models have not reported toxicity from nicotyrine itself, but do suggest that sustained nicotine levels make toxicity possible with relatively low nicotine exposures. Nicotine and aflatoxin are almost non-existent in the food supplies of developed countries, but acute and fatal poisonings occur when, for instance, molds invade corn stockpiles [55,56]. Ironically, the main modern source of environmental nicotine exposure is probably e-liquid. The importance of preventing accidental ingestions and skin exposures, especially of partially oxidized e-liquids, should be emphasized while this risk is evaluated. Patients suffering nicotine poisoning with concurrent nicotyrine exposure may require prolonged medical support.

Nicotyrine could increase risks of liver injury following various liver diseases or in hyperbilirubinemia. Increased CYP2A6 expression occurs in some conditions causing cellular injury in the liver, presumably due to oxidative stress [57]. Hepatocytes produce bilirubin from biliverdin during oxidative stress. Bilirubin scavenges reactive oxygen species, protecting cells from oxidative stress, but it is toxic to cells at high concentrations. Bilirubin induces CYP2A6 [58], so that when oxidative stress is relieved, CYP2A6 converts bilirubin back to biliverdin, eliminating risks of bilirubin toxicity [57]. Artificially lowered CYP2A6 activity in this setting, and possibly in the setting of neonatal hyperbilirubinemia, might therefore pose risks of bilirubin toxicity. There are no reports yet of adverse effects due to altered bilirubin metabolism in populations with CYP2A6 deficiency or in vapers.

Nicotyrine interferes with metabolism of only a few drugs. One is valproic acid, an anticonvulsant and mood stabilizer with a narrow therapeutic window. CYP2A6 metabolizes about 10% of ingested valproic acid [21]. We are looking for evidence of toxic valproic acid levels associated with initiation of vaping, or sub-therapeutic levels associated with cessation of vaping among individuals being treated with valproic acid at our institution.

CYP2A13 inhibition risks

The biologic functions of CYP2A13 are not clear, but expression of the gene begins in olfactory mucosa midway through fetal development [53]. This suggests that pregnancy outcomes, especially airway development, should be evaluated in vapers and animal models. Frequent, transient inhibition of CYP2A13 should be considered when analyzing studies of vapers' respiratory and sinus health.

Risk–benefit balance

In spite of the theoretical risks enumerated above, the facts that nicotyrine exposures and inactive CYP2A6 alleles are not yet associated with health risks, and that CYP2A13 inhibition is transient are tentatively reassuring. In spite of concerns about e-cigs, accumulating evidence suggests to some investigators that, in general, switching from cigarettes to e-cigs reverses pulmonary injuries caused by smoking [59]. However, given the heterogeneity of e-cig products, the global safety and efficacy of e-cig use will remain a subject of research and debate. Furthermore, e-cigs could deliver a higher dose of nicotyrine than any other product, and therefore warrant specific evaluation of this risk. Nevertheless, if the nicotyrine hypothesis is correct, it suggests a much safer approach to smoking cessation: For smokers who have failed to quit with conventional nicotine replacement therapies (NRT), the benefits of CYP2A6 inhibition as an adjunctive smoking cessation aid could outweigh harms.

Consequences of the hypothesis

A CYP2A inhibitor like nicotyrine might improve smoking cessation rates achieved with conventional NRT [37]. Rapid clearance of nicotine is routine with all current NRT; only transdermal patches provide a sustained, but generally low, nicotine level. Inhibitor-augmented NRT could allow users to achieve and maintain higher nicotine levels with relatively little effort while mitigating many risks. For instance, a combination nicotine and nicotyrine lozenge would not only provide predictable nicotyrine dosing, but could avoid many known and potential problems with e-cigs. Although these problems are small compared to the relentless risks of smoking conventional cigarettes, some problems with e-cigs that such lozenges would obviate include:

- Expensive hardware.
- Technique-dependent nicotine and nicotyrine delivery.
- Hardware design errors, such as exposing e-liquid to soldered connections [60].
- Hardware materials errors, such as plastics or metals that contaminate e-liquids or aerosols [61].
- Incendiary or explosive battery failure [61,62].
- Hardware degradation over time, and use past its expected lifetime [61].
- Constantly changing and unpredictable nicotine and nicotyrine levels in e-liquids exposed to air [18].
- Inhalation of e-liquid ingredients [63], hardware contaminants [64], and reaction products formed during storage and vaporization [65].
- Accidental exposures to potentially lethal doses of nicotine through spills [66]: ingestion remains a risk.
- Any risks related to frequent CYP2A13 inhibition.
- Exposure of bystanders to exhaled aerosols [67].
- Environmental impact of hardware disposal [68].

Conclusion

Nicotine in solution slowly oxidizes to nicotyrine. Nicotyrine in e-cig aerosols may improve absorption of intact nicotine and sustain serum levels by inhibiting CYP2A enzymes. Genetically low CYP2A6 enzyme activity is associated with a low level of smoking and low risk of related disease, and has not been linked to offsetting risks. Switching from smoking to exclusive vaping can immediately stop exposure to many definitely harmful compounds in tobacco smoke and may facilitate complete cessation of all nicotine products for some, but not all, users. Conventional biochemical markers of smoke exposure could be misleading during this transition. The potential for harm reduction is not proven, but should not be ignored. The hypothesized role of nicotyrine in e-cigs suggests nicotine replacement products that combine nicotine with CYP2A6 inhibitors, potentially avoiding pulmonary exposure and complex devices altogether. These products could be much less expensive than e-cigs, pose even less risk, and could be consistently and continuously satisfying to people dependent on nicotine.

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