

Fourteen-day E-Cigarette Exposure Disrupts Ventilation Patterns and Serum IL-1 β Levels in Adolescent Rats

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ABSTRACT

E-cigarettes are devices used to deliver vaporized liquids often containing nicotine and other chemicals. The purpose of this study was to investigate the effects of 14 days of e-cigarette vapor exposure in adolescent rats on lung function and inflammatory cytokine expression. Seventeen male Long-Evans rats were assigned to vape or air groups. The animals were exposed to either air or 5% nicotine vapor using a whole-body exposure chamber, once a day for ten minutes for fourteen consecutive days. Ventilation recordings were completed on day 0 (before exposure) and day 15 (after exposure) using unrestrained whole-body plethysmography. Both lung tissue and blood was collected for molecular assays. Baseline and post-exposure ventilation data were compared between air and vape groups across three different parameters: frequency, tidal volume, and minute ventilation. These parameters were compared resulting in three distinct 2x2 (time x treatment) Mixed Model ANOVAs. Between baseline and post-treatment measurements there was a significant decrease in values in minute ventilation ($p=0.0312$) and tidal volume ($p=0.0031$). Between treatment groups there was a significant difference in minute ventilation ($p=0.0128$) and frequency ($p=0.0042$). There were also significant alterations to the presence of IL-1 β in the serum ($p=0.0003$), but not lung tissue ($p=0.6525$). In conclusion, following two weeks of e-cigarette vapor exposure, ventilation patterns were altered in the vapor exposed animals which decreased in tidal volume and minute ventilation suggesting possible impairment of lung function and e-cigarette exposure modified the expression of pro-inflammatory cytokine, IL-1 β .

Introduction

Electronic cigarettes are devices used to vaporize liquids often containing nicotine and other potentially harmful chemicals. Research shows that vaping causes injury and damage to the airways and lungs, including inflammation in the lungs, which overtime can damage the lung tissue (Tarran et al., 2021, review). Adolescent attitudes towards vaping seem to be very misconstrued – vaping in these younger generations has been on the rise in the past few years, and those who have used e-cigarettes tend to ignore the risks or know very little about them. For example, 19.05% of those surveyed in one study believed that the vapor in e-cigarettes was just water, while 23.03% also believed that e-cigarettes weren't even a tobacco product (Gorukanti et al., 2017). Adolescents also seem to hold more positive attitudes towards vaping, as about 43.13% believed the health effects of e-cigarettes are less severe than the effects found with traditional cigarettes (Gorukanti et al., 2017). Electronic cigarettes are not the same as traditional cigarettes. For starters, while most electronic cigarettes do contain nicotine, most also contain other chemicals not typically found in traditional cigarettes, such as glycerin, propylene glycol, and flavor additives (Gorukanti et al., 2017). E-liquids can also contain substances called nicotine salts, which lowers the pH of the liquid allowing the user to increase their tolerance of high nicotine concentrations (Mishra et al., 2015). This then allows an increased consumption of higher nicotine concentrations, which can lead to an increase in airway and alveolar cell death along with increased inflammation in the lungs (Mishra et al., 2015).

Electronic cigarettes were invented in the early 2000s, so the health effects of these devices are still unknown and this area of research is relatively new. Research indicates that negative effects can happen fairly quickly. For example, in Tsai et al., 2020's study with humans, they found that after just five minutes of e-cigarette exposure, there was an increase in airway resistance in the lungs, meaning there could be some inflammation getting in the way. This study also saw diminished lung function after thirty minutes of e-cigarette exposure (Tsai et al., 2020). While human studies like these are very beneficial to vaping research, they are also very hard to come by due to ethical reasons. Therefore, animal models are more commonly used instead, which is the model that was chosen for the present study.

In many animal vaping studies, there have been both short term and long-term effects found. In one acute exposure study, rats were exposed to e-cigarettes for only 15 minutes and they found that there was an increase in plasma cotinine in animals exposed to the nicotine aerosols, confirming exposure to e-cigarette vapors by way of nicotine metabolite detection (Ahmad et al., 2019). In these same animals, inflammatory cytokines in the lungs were found to have increased as well (Ahmad et al., 2019). This showed that even after just one exposure, vaping seems to cause some sort of acute lung inflammatory response. Such responses may include inflammation and increased epithelial cell permeability, which allows toxins to pass through more easily (Chun et al., 2017). A similar pattern was also seen in other acute studies, especially the increase in lung inflammatory cytokines (Ahmad et al., 2019; Gotts, 2019; Tsai et al., 2020). Similar findings in lung tissue cytokine expression and stunted growth, were also found in long term studies that ranged from 2 to 4 weeks (Crotty Alexander et al., 2018; Glynos et al., 2018; Hwang et al., 2016; Larcombe et al., 2017). Inflammatory cytokines are used to help signal the body to produce an inflammatory response when there is injury or disease, but too much inflammation can be very damaging to the tissue (Lerner et al., 2015). Changes to these cytokine levels is concerning as this is a similar pattern to what we see in tissue leading up to some pathologies and diseases (Toews, 2001).

In this study we aimed to investigate the effects of fourteen consecutive days of a ten minute e-cigarette vape exposure on tidal volume, breathing frequency, and minute ventilation in adolescent male rats. There is a scarcity of research into the effects of vaping in adolescents. This age group is very important to study, as this generation seems to be increasing the usage of these devices in the adolescent years (Gorukanti et al., 2017). We hypothesize impairment in the lung function due to what has been seen in previous human studies showing increased airway resistance and inflammation after just 30 minutes of exposure (Gotts, 2019) and mouse studies showing impaired lung function after 8 weeks of exposure (Larcombe et al., 2017). Cytokine levels in the blood and/or lung tissue may also fluctuate based upon how long the animals are exposed to the e-cigarette vapors. In the present study, we chose to focus on the cytokine IL-1 β , as this cytokine was seen to increase after 3 days of exposure and decrease after 4 weeks of exposure (Masso-Silva et al., 2021) and this 14-day exposure study falls right in between the two studies.

Methods

Animals

All animal protocols were approved by the Southwestern University Institutional Animal Care and Use Committee (IACUC) under protocol Stokes_0721. Male Adolescent (PD30 to PD50) Long-Evans rats were chosen for this experiment. Rats were weaned at PD 23 and were split up into two experimental groups: vape (n = 9) and air control (n = 8). Rats were housed 2-3 per cage based on group assignment in hanging plastic cages on a metal cage rack, with aspen shavings used for bedding. Food and water were readily available *ad libitum* and daily humidity and temperature in the animal room were monitored. Animal colony lighting was set at an automatic 12:12 h reversed dark/light cycle, where the lights were turned off at 10:00 a.m. Recordings and acclimation all took place during the dark cycle under dim red light.

Study Timeline

Rats were acclimated to the plethysmography chambers starting at the age PD 25 for seven consecutive days. Baseline ventilation recordings were measured on PD 32, study day 0. Vape or air exposure was performed on study days 1-14 (PD 32-46). Prior to post-exposure ventilation recordings on study day 15, rats were reacclimated to the plethysmography chamber on exposure days 11-14. On study day 15 (PD 47), post-exposure ventilation recordings were measured followed by blood and lung tissue collection.

Whole-Body Plethysmography

Whole-body plethysmography was performed in awake animals using Data Sciences International Buxco whole-body plethysmography system plus rat chambers, during the rat's dark cycle under dim red light. Gas (room air) flow rate was set to 3.0 L/min throughout the duration of the experiment and animals were monitored continuously when in the chamber. Prior to the experimental start date, subjects were acclimated in the plethysmograph chambers for seven consecutive days, 10 minutes each day, before baseline testing. During acclimation the plethysmography system was set up and animals were placed in their assigned chambers, with the lids sealed and room air flowing as described above, but recording was not enabled. Ventilation recordings were obtained the day immediately before the start of exposure (Baseline, study day 0), and at the end of exposure (Post-Treatment, study day 15). Subjects were weighed immediately before each recording. Temperature and humidity were continuously monitored throughout the recording using a DSI manufactured temperature/humidity probe which was connected to the Buxco system. Each chamber was calibrated on recording days per the manufacturer's instructions. Each plethysmography session lasted 30 minutes and was divided up into two phases: 10 minutes of acclimation and 20 minutes recording, with data recording during post-acclimation phase only. Subjects were placed back in their cage after recording. All ventilatory parameters were recorded and analyzed using the DSI FinePoint software (breathing frequency, tidal volume, and minute ventilation) using waveform calculations based on the Drorbaugh and Fenn equation (Drorbaugh & Fenn, 1955). Tidal volume was normalized to 100 grams of body weight. Minute ventilation (volume of breath per minute per 100 grams) was also calculated, as it is a product of the aforementioned parameters. In post-evaluation, in order to eliminate any data segments including sniffing or movement, the recordings were visually inspected for a smooth waveform representing a calm inhale and exhale and 30 second clips were extracted from the exported data to use for statistical analysis.

Vape Exposure

Rats were placed with cagemates (2/chamber) into a vape or air chamber based on study assignment. The vape animals were placed into the vape chambers, and were exposed to the vaporized e-liquids (commercially available JUUL, 5% nicotine) for 10 minutes. The vape system, a slightly modified version of (Frie et al., 2020), ran on a continuous program of a 2 second draw of vape, followed by 4 seconds off. After 4 minutes, the system pumps were turned off, and the rats remained in the chambers for an additional 6 minutes, to total the 10 minutes of exposure per day. JUUL pods were weighed before and after use to allow for estimation of nicotine content of the vape in the chamber. This was done once a day for 14 consecutive days. After exposure, the vape chamber was vented under a draft hood to allow for safe escape of the vapor. Rats were returned to home cages following vape exposure and monitored for any adverse acute effects of exposure. Subject weights were collected every two days from the start of acclimation to monitor health since it is known that nicotine exposure can affect growth and development (McGrath-Morrow et al., 2015). JUUL pod weights were recorded before and after individual vape sessions. The amount (in grams) of vape liquid used during the sessions was recorded before and after each session and used to calculate approximate vape liquid exposure and nicotine (as measured by weight difference). Our calculations indicate that each exposure (of about 4 minutes on) resulted in an average of 0.11 grams of JUUL vape liquid which is about 5.6 mg of nicotine (5%

nicotine by weight, approximately 59 mg/mL) assuming a vape solution density of 1.15 g/mL as previously published (Ranpara et al., 2021).

Terminal Procedures and Blood Collection

At the end of study day 15, animals were first anesthetized with vaporized isoflurane (induction chamber settings 4% isoflurane with room air at 2.0 ml/min flow rate) to minimize stress of euthanasia injection. Animals were euthanized with Euthazol (pentobarbital sodium, Virbac) according to their body weight (90 mg/kg). Lack of response to a distal foot and toe compression using a hemostat and lack of response to eyelid touching and whisker stimulation was used to confirm deep anesthesia. The torso was then opened up to expose the heart and lungs. Immediately prior to tissue collection, a cardiac puncture via the left ventricle was performed to collect approximately 1.0 mL of whole blood.

Serum Processing

Blood was collected from the heart via cardiac puncture and blood samples sat at room temperature for 30-60 minutes. After coagulation, the blood samples were centrifuged at 2,500 rpm for 15 min at 4°C and supernatant (serum) was extracted to a clean tube. After centrifugation, a properly spun sample had a clear to light-yellowish serum sample separated from the buffy coat and cellular elements. Serums were stored at -80 °C until analysis.

Lung Tissue Collection and Processing

Immediately following blood collection, the lungs were harvested and perfused intratracheally with ice cold PBS using a blunt 21G needle and syringe. Perfused lungs were then flash frozen in liquid nitrogen and stored at -80 °C until homogenization. Portions of frozen right and left lung tissue was crushed into a powder on a metal plate over ice and approximately 35 mg of lung tissue was placed into a bead homogenization tube (Benchmark Scientific D1032-30 3.0mm Zirconium Bead, pre-filled 2ml tube) pre-filled with 750 µL of ice cold tissue extraction buffer (Tissue Extraction Reagent I, diluted to 1x, with 1 Pierce Protease Inhibitor Mini Tablet dissolved for every 10 mL of 1x buffer and 100 µL of 100mM PMSF; all Fisher Scientific). The bead homogenization tube with tissue and buffer was homogenized using a BeadBug Benchtop Homogenizer (Benchmark Scientific) at 4000 x 30 seconds for three rounds. The sample tube was placed on ice in between rounds. Sample tubes were then centrifuged for 5 min at 12,000 rpm at 4°C. The resulting supernatant was removed and placed in a clean 1.5 mL tube. Lung homogenate sample protein concentrations were determined using a BCA assay (Prometheus Protein Biology Products 18-440 BCA Protein Assay Kit; Genesee Scientific) to ensure adequate protein concentrations were met for the subsequent ELISA. Manufacturer instructions were followed.

Cotinine and Cytokine Assays

Levels of cotinine (a nicotine metabolite) in blood serum were measured using a Cotinine enzyme-linked immunoassay (ELISA) kit (Origene, Rockville, MD) Manufacturer instructions were followed. Blood cotinine levels were measured to confirm nicotine exposure. Levels of IL-1 β cytokine in serum and lung tissue were measured using Interleukin 1 Beta enzyme-linked immunoassay (ELISA) KIT from MyBioSource (San Diego, CA). Serum samples were diluted 2-fold and lung tissue homogenates were diluted 4-fold before addition to the assay. Manufacturer instructions were followed.

Statistical Analysis

Baseline ventilation data collected on day 0 and post-exposure ventilation data collected on day 15 were compared between air and vape groups across three different parameters: minute ventilation, frequency, and tidal volume. These parameters were compared resulting in three distinct 2x2 Mixed Model ANOVAs comparing the variables time and treatment. Weight was compared between air and vape exposure groups using a 2x10 Mixed Model ANOVA. Serum and lung tissue cytokine expression levels were compared between air and vape exposure groups using an independent sample t-test. All statistical analysis and graphical representation were performed using GraphPad Prism, Version 9 (San Diego, CA).

Results

Weight

Over time a 2x10 (Treatment x Time) Mixed Model ANOVA found no significant interaction between treatment and time ($F(9,135) = 1.430$, $p = 0.1811$), therefore showing that both groups behaved similarly across time (Figure 1). A significant difference in weights over time was found ($F(1,15) = 1180$, $p < 0.0001$) and accounted for about 86.27% of the variation seen in the weights ($p^2 = 0.8627$). There was no significant difference in weight between the Air group and Vape group ($F(1,15) = 0.0856$, $p = 0.7739$).

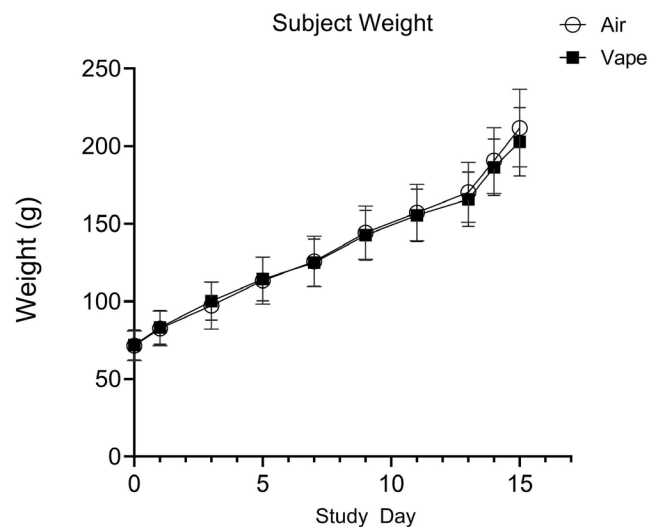


Figure 1: Subject weight over time. Weight was recorded periodically throughout the study to monitor growth and health in the animals. A significant difference was found in weight over time ($p < 0.0001$) but no significant difference was found between the groups ($p = 0.7739$).

Serum Cotinine Levels

The mean (\pm sd) cotinine levels for air and vape groups, respectively, were $0.007 (\pm 0.019)$ ng/mL and $20.71 (\pm 6.875)$ ng/mL. The results of the independent samples t test suggest that the cotinine levels for the vape group were significantly higher than the air group ($t(15) = 8.488$, $p < 0.0001$). Further there was a large effect size attributed to the presence of cotinine in air versus vape groups (Cohen $d = 4.25$), with approximately 80.69% of the variation in cotinine

levels being attributed to the difference in the type of treatments ($\Omega^2 = 0.8069$). This confirms the delivery of the e-cigarette vapor to the vape group only.

Ventilation Data

Frequency

The 2x2 (Treatment x Time) mixed model ANOVA showed there was a significant difference in frequency between treatment groups ($F(1,15) = 11.35$, $p = 0.0042$) with 33.88% accounting for the change seen in frequency ($p^2 = 0.3388$). The vape group at baseline had a 19.5% higher breath frequency than the air group at baseline, while at post-treatment the vape group was 13% higher than the air group. No significant difference was found between the baseline and post-treatment times ($F(1,15) = 1.778$, $p = 0.2023$).

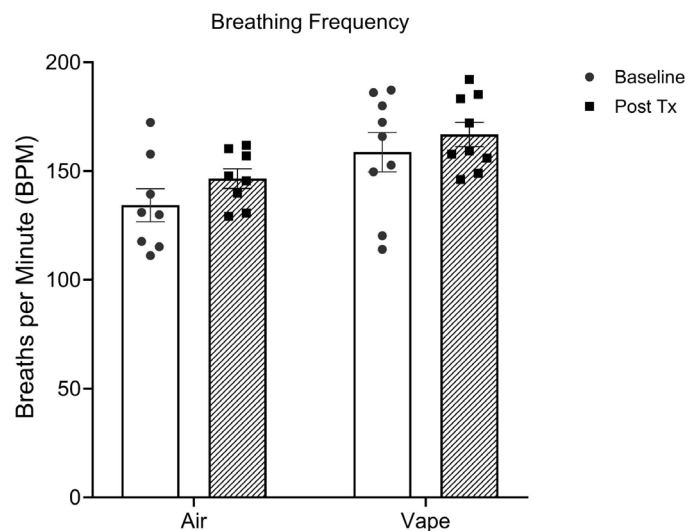


Figure 2: Breathing frequency before (baseline) and after (post-treatment; post tx) air or vapor exposure. There was a significant difference in frequency between air and vape groups ($p = 0.0042$). The vape group breath frequency at baseline was 19.5% higher than that of the air group at baseline; at post-treatment, the vape group was 13% higher than the air group. There was no significant difference between groups over baseline and post-treatment times ($p = 0.2023$).

Tidal Volume

The 2x2 (Treatment x Time) mixed model ANOVA showed the vape exposure significantly decreased tidal volume/100g levels between baseline and post treatment times ($F(1,15) = 12.38$, $p = 0.0031$) and accounted for about 27.64% of the change seen in tidal volume levels ($p^2 = 0.2764$). For the air group, the post-treatment tidal volume measures were 10% lower than the baseline values. For the vape group, these values at post-treatment were also lower by 18.91% in comparison to their baseline values. There was no significant difference between treatment ($F(1,15) = 0.2403$, $p = 0.6311$); however, the vape group had a greater percentage decrease in tidal volume after treatment.

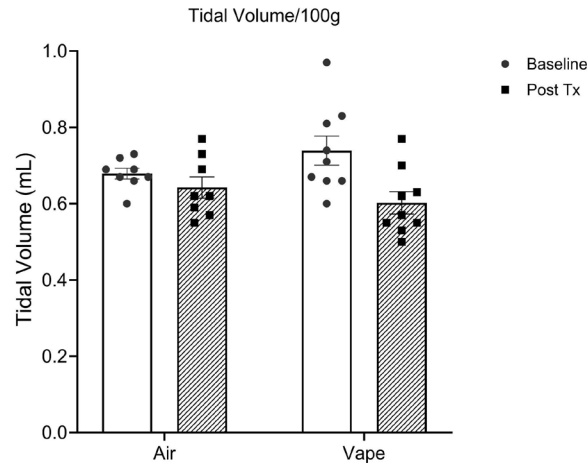


Figure 3: Tidal volume before (baseline) and after (post-treatment; post tx) air or vapor exposure. There was a significant difference in tidal volume/100g between baseline and post-treatment times ($p = 0.0031$); 27.64% of the change seen in tidal volume levels is accounted for by vape exposure ($p^2 = 0.2764$). Post-treatment tidal volume was 10% lower in the air group and 18.91% lower in the vape group in comparison to the baseline.

Minute Ventilation

The 2x2 (Treatment x Time) mixed model ANOVA showed the vape exposure significantly decreased minute ventilation/100g levels between baseline and post treatment times ($F(1,15) = 5.647$, $p = 0.0312$) and accounted for about 10.18% of the change seen in minute ventilation levels ($p^2 = 0.1018$). The air minute ventilation measurements from baseline to post-treatment were decreased by 4.3%, while the measurements for the vape group from baseline to post-treatment were decreased by 18.96%. There was also a significant difference between treatment groups on minute ventilation ($F(1,15) = 7.979$, $p = 0.0128$), and this accounted for about 19.74% of the change seen in minute ventilation levels ($p^2 = 0.1974$).

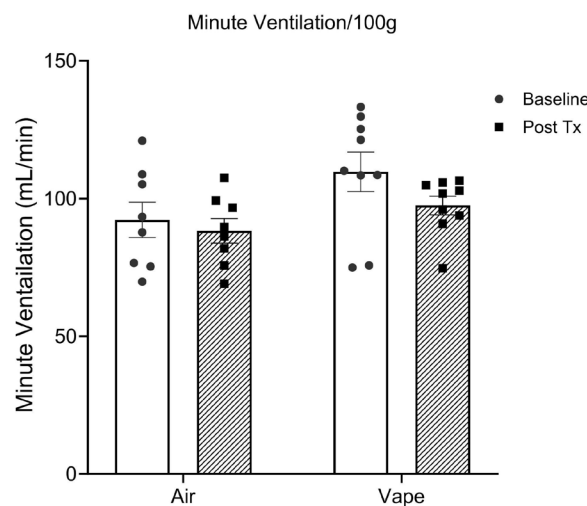


Figure 4: Minute ventilation before (baseline) and after (post-treatment; post tx) air or vapor exposure. Minute ventilation/100g levels significantly decreased between baseline and post treatment times ($p = 0.0312$), with vape accounting for 10.18% of the change ($p^2 = 0.1018$). There was a significant difference between treatment groups on minute ventilation ($p = 0.0128$), accounting for about 19.74% of the change seen in minute ventilation ($p^2 = 0.1974$).

Inflammatory Cytokines

Serum was evaluated for the presence of inflammatory cytokine IL-1 β . The mean (\pm sd) IL-1 β levels were 15.88 (\pm 7.33) pg/mL and 3.28 (\pm 3.52) pg/mL for air and vape groups, respectively. The results of the independent samples t test suggest that the IL-1 β levels for the vape group were significantly lower than the air group ($t(15) = 4.605$, $p = 0.0003$). Furthermore, there was a large effect size attributed to the presence of IL-1 β in vape versus air groups (Cohen $d = 1.315$), with approximately 28.62% of the variation in clotting time being attributed to the difference in the type of treatments ($\Omega^2 = 0.2862$).

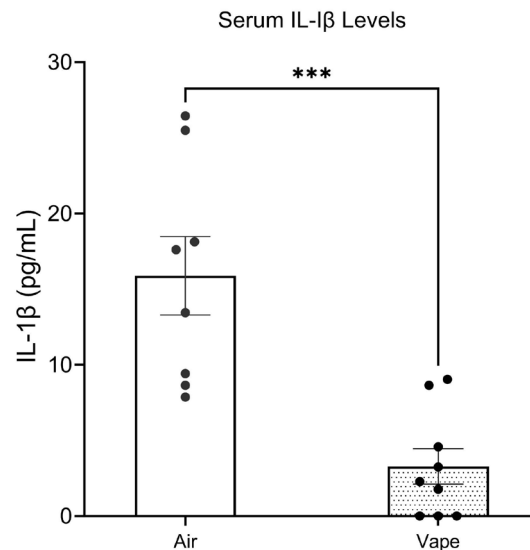


Figure 5: Serum IL-1 β levels. A significant difference was found between the air (15.88 ± 7.33 pg/mL) and vape (3.28 ± 3.52 pg/mL) exposure groups in IL-1 β levels found in the blood plasma ($p = 0.0003$, t-test).

Lung tissue homogenate IL-1 β levels were also assessed. The mean (\pm sd) IL-1 β levels were 27.8 (\pm 12.8) pg/mL and 30.65 (\pm 12.77) pg/mL for air and vape groups, respectively. The results of the independent samples t test suggest that there is no significant difference between the IL-1 β levels in the lung homogenates of the air and vape groups ($t(15) = 0.4595$, $p = 0.6525$). Graph not shown.

Discussion

The full effects of vaping are still largely unknown. However, this study and other studies show evidence of lung function impairment and proinflammatory cytokine dysregulation. The purpose of this study was to obtain data on the effects of a 14-day, one time per day, e-cigarette vapor exposure on ventilation functions and levels of circulating pro-inflammatory cytokines, both of which were hypothesized to change after exposure.

Effects of Treatment on Animal Health

The adolescent age is a time designated for growth in the human body. Decreased or stalling of growth in animals could mean something else is going on, such as stress or sickness. In many studies done with animals, vaping was shown to stunt the growth in both the long- and short-term courses (Crotty Alexander et al., 2018; Glynos et al., 2018; Hwang et al., 2016; Larcombe et al., 2017; McGrath-Morrow et al., 2015). However, in the present study, the weight was found to still significantly increase over the course of the study, showing no ill effect due to vaping or other outside factors. Weight is also typically a health indicator in studies with animals, so a steady increase in weight is a

good indicator. Throughout the study the animals showed no signs of aggression or unusual behaviors, both of which are also indicators of good health. Since the animals were only exposed to vapor once per day for a relatively short amount of time (10 minutes) and then immediately returned to their home cage, this may not have been enough cumulative exposure to induce weight-loss or extreme stress, compared to other studies with much longer vapor exposure time frames. We were able to confirm nicotine exposure with the vapor exposed group only via cotinine serum analysis. In acute studies with animals, plasma cotinine levels were found to increase when exposed to e-cigarette vapors (Chun et al., 2017). Similar results were found in the present study, confirming nicotine exposure was only associated with the vape group and not the air group.

Effects of Vaping on Lung Function

There is minimal research on the effects of vaping and lung functional changes, especially with the use of unrestrained, awake plethysmography. Using anesthetized, tracheostomized, and ventilated mice, Glynos et al. 2018 found that after four weeks of e-cigarette exposure tissue elasticity and airway resistance were negatively affected (Glynos et al., 2018). Using similar methods Larcombe et al. 2017, found a significant lung function impairment and hyperreactivity in anesthetized mice after eight weeks of e-cigarette vapor exposure (Larcombe et al., 2017). In our study done with awake animals, lung function impairment is implied given that both tidal volume and minute ventilation significantly decreased in the vape groups after treatment. The vapor-exposed animals showed a decrease in tidal volume without a change to frequency which resulted in a decrease in minute ventilation, which is a product of both frequency and tidal volume. This could indicate a difficulty in moving air in and out of the lungs after repeated exposure, possibly due to a difference in lung mechanics or a different sensation experienced during lung tissue expansion (e.g., pain). The minute ventilation results showed a significant difference between treatment groups, even with the vape exposure group having both a higher baseline and post-treatment value in comparison to the air group. This is also the first report of vape induced changes in ventilation data in the Long-Evans adolescent rat. While the results from these values could indicate that vaping diminishes lung functions over time, the major differences in baseline values makes it hard to determine this outcome (see Limitations below). Given the lack of research present on the effects of e-cigarette vapor on awake, unanesthetized subjects, this remains an area of research warranting more investigation.

Effects of Nicotine Exposure on Circulating Proinflammatory Cytokines

The present study also looked into the level of circulating inflammatory cytokines, specifically IL-1 β . In the same acute studies that found an increase in cotinine levels, there was also an increase in inflammatory cytokines in those same animals (Ahmad et al., 2019; Gotts, 2019; Tsai et al., 2020). This is concerning as the increase in inflammation, when not in response to injury or disease, can be very damaging to tissue (Lerner et al., 2015). In chronic studies, however, some cytokines such as CXCL-1 and IL-1 β have been shown to decrease after a few weeks of exposure, which could indicate an altered and diminished immune response (Masso-Silva et al., 2021; Moshensky et al., 2022). The present study focuses on the pro-inflammatory cytokine, IL-1 β . IL-1 β was selected based on a study done by Glynos et al, which performed a 3 day and 4-week e-cigarette exposure. After 3 days of exposure, they found that IL-1 β , in circulating blood, increased but then decreased after 4 weeks (Glynos et al., 2018). In the present study, a 14-day exposure resulted in a decrease in IL-1 β presence in the serum, but no change in the lung tissue, indicating that a once per day two week vapor exposure is long enough to induce a decrease in circulating levels of IL-1 β . Nicotine has been shown to decrease levels of proinflammatory cytokines, including IL-1 β in different tissues (Ni et al., 2011; Revathikumar et al., 2016); however this may be the first to show this effect with a whole-body vapor exposure model. The decrease in IL-1 β could also be indicative of an increase in an anti-inflammatory marker IL-10 which was seen to increase in other long term exposure studies (H. Chen et al., 2022). IL-10 is an anti-inflammatory cytokine whose

function is to inhibit the production of pro-inflammatory cytokines like IL-1 β (L. Chen et al., 2018). However, additional studies would need to be done to see if the increase in anti-inflammatory markers after vapor exposure could be part of the cause for the decrease in the levels of pro-inflammatory cytokines such as IL-1 β .

Conclusion

This 14-day exposure shows that vaping truly isn't as harmless as some may choose to believe. In terms of ventilation, there were some significant changes both over time and between treatment groups for tidal volume and minute ventilation, suggesting that vapor exposure may alter lung function. These data also show that e-cigarette exposure may lead to the inhibition of the pro-inflammatory cytokine IL-1 β , possibly causing dysregulation in the cytokine pathways and innate immune response.

Limitations

Our subjects were exposed to e-cigarette vapor using full-body exposure which is limited in its external validity because vaping in humans is carried out via direct inhalation; therefore, the amount of vapor inhaled by the subjects in our experiment is less controlled and subject to rat breathing rate inside the chamber. Another limitation is that the baseline values for frequency for the vape group were significantly higher than the air group which may mask additional ventilatory changes that may have occurred due to treatment. Due to the time required for analysis of the breathing data parameters, the baseline values and experimental values are evaluated at the same time following the conclusion of the study by two independent investigators and compared. This process also reduces bias in the analysis by having two individuals, unaware of treatment evaluate the ventilation data. However, differences within the baseline data were only obvious after the conclusion of the study. Finally, the relatively small sample size (17 subjects total) and inherent variation present in animal behavior studies may also affect the results. Future work will increase the total number of subjects.

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