Quantification of Regional Murine Ozone-Induced Lung Inflammation Using [^{18}F]FDG MicroPET/CT Imaging

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Abstract

Purpose Ozone (O_3) is a highly potent and reactive air pollutant, which is formed from human-related activities. It has been linked to acute and chronic respiratory diseases in humans by inducing inflammation. Our studies have found evidence that 0.05 ppm of O_3 , within the threshold of air quality standards, is capable of inducing acute lung injury. This study was undertaken to examine O_3 -induced lung damage using [^{18}F]FDG (Fluorodeoxyglucose) microPET/CT imaging in wild-type mice. [^{18}F]FDG is a known PET tracer for inflammation.

Methods Sequential [^{18}F]FDG microPET/CT imaging was performed at baseline (before O_3 exposure), immediately following 2 h of 0.05 ppm O_3 exposure (0 h), at 24 h and at 28 h post-exposure. The images were quantified to determine spatial standard uptake values of [^{18}F]FDG in relation to tissue density and compared with baseline values.

Results Immediately after O_3 exposure, we detected a $72.21 \pm 0.79\%$ increase in lung [^{18}F]FDG uptake ratio when compared to baseline measures. At 24 h post- O_3 exposure, the [^{18}F]FDG uptake becomes highly variable (S.D. in FDG = 0.0005174 units) with a $42.54 \pm 0.33\%$ increase in lung [^{18}F]FDG compared to baseline.

Conclusion We have developed a microPET/CT imaging protocol to quantify regional lung uptake of [^{18}F]FDG to understand lung response to O_3 exposure.

Introduction

Ozone (O_3) is a toxic and highly reactive gaseous oxidizing chemical with well-documented adverse health effects in humans. On the basis of animal and human data, environmental guidelines and air quality standards recommend a threshold for exposure of no more than 0.063 ppm of O_3 (daily concentrations). Experiments done in animal models have shown that O_3 induces acute lung injury, albeit at much higher and longer O_3 exposures (near 2 ppm for 3–6 h). Our research has standardized a sensitive model of sterile murine lung inflammation by exposing mice for two hours at 0.05 ppm O_3 , a level below the current recommendations for what is considered a safe or “ambient” ozone concentration for humans (1, 2). 0.05 ppm O_3 exposure causes immediate lung neutrophil recruitment, release of IL-1 β dependent cytokines in broncho-alveolar lavage and bone marrow mobilization of pan-leukocyte chemokine, SDF1a (2). Thus, it is imperative to understand the biological effects of O_3 at concentrations feasible in the environment.

[^{18}F]FDG (Fluorodeoxyglucose) microPET/CT imaging is a sensitive method to detect lung cancer and study various inflammatory diseases. The current quantification methods focus on tracer kinetics and compartment modelling with methods such as the Patlak method (3). For understanding disease progression and regional tracer localization in relation to specific organs such as lungs, we developed an

imaging protocol cum image analysis strategy to quantify the sequential uptake and distribution of [^{18}F]FDG in murine lungs. We applied this novel methodology to track changes in the lung [^{18}F]FDG uptake before as well as after O_3 exposure by sequential [^{18}F]FDG microPET/CT imaging.

Our results indicate that O_3 induces higher and heterogeneous lung [^{18}F]FDG uptake, which was analyzed in detail by guided region of interest (ROI) analysis of the PET image slices that were co-registered to X-ray CT ortho-slices. The resulting CT attenuation grey values were plotted against the percent (%) lung [^{18}F]FDG SUR (standard uptake ratio). These plots provide quantifiable spatio-temporal lung FDG distribution pattern.

Methods

Mice: The study design was approved by the University of Saskatchewan's Animal Research Ethics Board (AUP 20170016) and adhered to the Canadian Council on Animal Care guidelines for humane animal use. Six-eight week old male C57BL/6NJ (**Stock No. 005304**) mice were procured from Jackson Labs (CA, US).

Ozone exposures

For O_3 exposures, mice were continuously exposed in an induction box for 2 h (1, 2). These mice had free access to food and water while housed in the custom induction box. O_3 (0.05 ± 0.02 ppm) was generated, at 3 litres/minute, from ultra-high-purity air using a silent-arc discharge O_3 calibrator cum generator (2B Technologies, CO, USA). Constant chamber air temperature ($72 \pm 3^\circ\text{F}$) and relative humidity ($50 \pm 15\%$) were maintained. O_3 concentrations were calibrated in small box using a real-time O_3 monitor (2B Technologies, CO, USA).

Experiment design

Three pre-weighed mice were acclimatized to the cyclotron facility, overnight, and fasted for at least 4 hours before imaging. For a simpler experiment schematic, please refer to Fig. 1. At the beginning of the experiment, mice were prepared for baseline (i.e. without any treatment) imaging. At any given time point, imaging was performed on a single mouse. Immediately after inducing isoflurane (5%) anesthesia, mice were maintained under 1.5–2.5% isoflurane on a warm water circulated heating pad and monitored for vital signs (*BioVet*, breathing rate 40–100 breaths per min, body temperature $36.5\text{--}38^\circ\text{C}$ and blood oxygen saturation by pulse oximetry i.e. SpO_2 98–100%). Mice were injected with 2–5 MBq of [^{18}F]FDG via a tail vein. At 15–30 min post injection, mice were imaged for 3–5 full-body multiple time-frames (800 μm voxel size), where each frame spanned 5 min (*VECTor⁴CT, MI Labs*), for over 25 minutes. [^{18}F]FDG microPET imaging was followed by a 2 minute X-ray CT (2 bed positions). After baseline imaging, the mice were continuously exposed for 2 h at 0.05 ppm O_3 as explained above. Immediately after O_3 exposure (i.e. 0 h post- O_3 exposure), the mice underwent similar full-body microPET/CT imaging. The next day, at 24 h after O_3 exposure, the mice were again injected i.v. with 2–5 MBq of [^{18}F]FDG and

imaged for 3–5 full body [^{18}F]FDG PET-CT scans. Four hours later, i.e. at 28 h post- O_3 exposure, mice were imaged for any residual [^{18}F]FDG activity with similar full-body [^{18}F]FDG scans.

Image processing and analysis: The acquired X-ray and PET-CT image data sets were processed for flat and dark current normalization, reconstruction (MI Lab reconstruction software), co-registration and quantification by Pmod (pmod.com). Thereafter, the images were quantified and analyzed on Image J (<https://fiji.sc/#>). The image stacks from X-ray CT were threshold-selected to segment out the lungs. Depending upon the data-set, anywhere from 130–160 ortho slices spanned the entire lung region. The selected regions of interest (ROIs) were then copied on to the corresponding [^{18}F]FDG PET image slices across multiple frames (F0-F4). The ROIs, from X-ray CT as well as the PET images, were simultaneously quantified for the various image parameters such as the area, perimeter, mean, median, mode, standard deviation (SD), range and integrated counts. After exporting the data to excel file, data was sorted, filtered and analyzed for corresponding mouse time-points, frames and/or CT parameters. Finally, a SUM of the full-body image Z-stacks, from the multiple frames, was analyzed for the same parameters in order to calculate the full body [^{18}F]FDG SUV (Standard uptake value). Thus, [^{18}F]FDG is quantified for lung slice(s) as well as the full-body and the ratio of these values is termed as the standard uptake ratio (SUR). All scans (baseline and post- O_3 exposure) were acquired using the same imaging protocol. The [^{18}F]FDG counts were decay corrected in order to account for time-dependent [^{18}F]FDG decay. The lung CT grey values were plotted against “lung [^{18}F]FDG SUR”, which was overlaid for multiple frames on the same graph. These plots were then smoothened using the second polynomial function (Savitsky-Golay), in order to compute trends in lung [^{18}F]FDG SUV or S.D. and subsequently, % increase in lung [^{18}F]FDG post- O_3 exposure. Please refer to Fig. 2 for flow-chart of the image processing.

Results

At baseline (i.e. 15–30 min after [^{18}F]FDG injection), [^{18}F]FDG is extracted (1.90–2.28%) by lungs (Fig. 3, Movie 1) compared to the full-body uptake (Fig. 4A), with majority excreted through the kidneys (data not shown). The physical half-life of [^{18}F]FDG decay is 109.8 min and biological half-life of 16 min, which means that > 50% of [^{18}F]FDG was decayed by the time of the imaging, which is immediately after O_3 exposure (at 0 h i.e. 2 h after baseline imaging). However, because we took account of full-body counts at every imaging time-point, regional lung [^{18}F]FDG SUR was expressed as a percent (%) normalized lung [^{18}F]FDG, which is irrespective of the time of imaging. Lung FDG SUV (units) varied from 0.019 to 0.0228, at baseline. The % regional lung FDG SUR varied from 7.42×10^{-5} to 0.043%, at baseline. Immediately after O_3 exposure (O_3 -0 h), mid-level lung slice shows large alveolar regions devoid of [^{18}F]FDG uptake (Fig. 3). The FDG SUV (units) varied from 0.0147 to 0.0164, at 0 h. The % regional lung FDG SUR varied from 7.42×10^{-6} to 0.032, at 0 h. Data from all the selected lung regions (Figs. 4A-D)) was smoothened (Figs. 4E-H) and then the SUR from individual time frames were pooled to evaluate cumulative % lung [^{18}F]FDG uptake SUR (Fig. 4I). It must be noted that O_3 induces instant lung tissue damage, as indicated by attenuated lung CT grey values, at 0 and 24 h post-exposure, compared to baseline values (Fig. 4J).

Despite the loss of lung tissue, O₃ induced a $72.21 \pm 0.79\%$ increase ($p < 0.05$) in lung [¹⁸F]FDG at 0 h compared to baseline (Figs. 4A, B, E, F, M). This is not very obvious in the single ortho-slice shown but evident in the volume rendered [¹⁸F]FDG uptake in lungs shown in Fig. 3 and Movie 2. The FDG SUV (units) varied from 0.0123 to 0.0166, at 24 h. The % regional lung FDG SUR varied from 1.51×10^{-4} to 0.032, at 24 h. At 24 h post-O₃ exposure, [¹⁸F]FDG was $42.54 \pm 0.33\%$ higher ($p < 0.01$) compared to baseline (Figs. 3, 4C, G, Movie 3). There were multiple areas of high concentration near the higher attenuating lung regions. The FDG SUV (units) varied from 0.0158 to 0.0183, at 28 h. The % regional lung FDG SUR varied from 3.64×10^{-5} to 0.041, at 28 h. Thus, at 28 h post-O₃ exposure, there wasn't a significant change ($p > 0.05$) in lung [¹⁸F]FDG compared to baseline (Figs. 3, 4D, H, Movie 4), but the pattern of [¹⁸F]FDG uptake shifted towards the lower attenuating lung regions. The lung CT grey values also showed higher values, indicating inflammatory influx, at 28 h post-O₃ exposure.

The regional lung [¹⁸F]FDG distribution standard deviation (S.D.) values indicate the variation in FDG uptake across the analysed lung regions i.e. low vs high grey value regions. At baseline, the [¹⁸F]FDG S.D. ranged from 0.000153 to 0.000958. We initially observed an attenuation in the lung [¹⁸F]FDG S.D. (1.78×10^{-5} to 0.0000362) at 0 h ($p < 0.01$) (Fig. 5A, B, E, F, I) but at 24 h, we observed a heterogeneous distribution (higher FDG S.D. ranging from 0.000265 to 0.000807, $p < 0.01$) when compared to baseline (Figs. 3, 5A, C, E, G, I). At 28 h post O₃ exposure, lung [¹⁸F]FDG S.D. distribution was not significantly high (ranging from 0.000112 to 0.000404, $p > 0.05$), when compared to the baseline S.D. (Figs. 3, 5A, D, E, H, I). However, there were specific areas of high [¹⁸F]FDG uptake (Fig. 3).

Discussion

Positron emission tomographic (PET) imaging with [¹⁸F]FDG is a promising technique that may serve as a more sensitive outcome measure for pulmonary inflammation. The advantages of PET imaging include its noninvasiveness, ease of quantification, and ability to assess the entire lung. [¹⁸F]FDG-PET exploits the “Warburg effect”, the observation that many cancers use aerobic cytoplasmic glycolysis as opposed to mitochondrial glucose oxidation as a major energy source, a process that requires increased cellular glucose uptake. Aerobic glycolysis is also a characteristic of nonmalignant proliferating cells and is observed in acute lung inflammation (4, 5). Evidence to date suggests that neutrophils contribute primarily to the increased uptake of [¹⁸F]FDG in lung inflammation and that the [¹⁸F]FDG-PET signal correlates with the presence of activated neutrophils (6). Clinical studies have also demonstrated that [¹⁸F]FDG-PET imaging can be used to assess the neutrophilic inflammatory burden in the lungs in cystic fibrosis, pneumonia, and experimentally induced lung inflammation (6–9). These results together indicate that [¹⁸F]FDG-PET imaging can potentially be used to measure changes in pulmonary inflammation in response to anti-inflammatory treatments.

We set out to explore 2 questions: (1) the utility of dynamic [¹⁸F]FDG-PET acquisition in discriminating before and after O₃ exposure; (2) the feasibility of [¹⁸F]FDG-PET in tracking the pathology of O₃ induced

lung inflammation in *in vivo* O₃ models and their response over time (0, 24 and 28 h). Our data demonstrate time-dependent variability in [¹⁸F]FDG uptake in O₃ exposed lungs. Dual-time [¹⁸F]FDG imaging has been done in the past to evaluate pulmonary fibrosis (10) and idiopathic interstitial pneumonia (11), where positive lung [¹⁸F]FDG retention is predictive of earlier deterioration of pulmonary function and mortality. However, these studies quantified [¹⁸F]FDG in a few 2–5 ROIs in lungs, whereas our study evaluates whole lung [¹⁸F]FDG distribution and provides both fractional as well as total lung [¹⁸F]FDG distribution over time. We observed a differential pattern of lung [¹⁸F]FDG distribution immediately after O₃ exposure. The immediate (0 h) increase in O₃-induced lung [¹⁸F]FDG was detectable as multiple spikes in the regions with lowest CT attenuation number near cranial and caudal regions. The increase in lung FDG uptake was observed despite O₃ induced lung damage, as indicated by lowered CT values, at 0 and 24 h post O₃ exposure. Some ortho-slices showed large gaps in [¹⁸F]FDG distribution, which also indicate the immediate alveolar damage by O₃. Thus, the strength of our systematic regional as well as gross lung [¹⁸F]FDG analysis lies in the fact that our analysis could detect O₃-induced metabolic activation of different alveolar regions, as a result of inflammation, despite alveolar damage. At 24 h after O₃ exposure, the opposite pattern was observed i.e. the [¹⁸F]FDG spikes were smaller and observed in the high attenuation regions or the mid-lung regions. At 28 h after O₃ exposure, there was an overall reduction in the lung [¹⁸F]FDG uptake. However, the distribution pattern was homogenous, indicating that the pulmonary circulation homogeneously extracts [¹⁸F]FDG.

Thus, our sequential [¹⁸F]FDG imaging strategy is capable of objective analysis of the lung [¹⁸F]FDG retention before as well as up to 28 h after O₃ exposure.

Conclusion

Thus, our study reveals that [¹⁸F]FDG-PET may offer a tool for deep phenotyping of lung inflammation to understand the response to new targeted treatments in animal models and later in clinical trials.

Declarations

Ethics approval and consent to participate: The current animal study design was approved by the University of Saskatchewan's Animal Research Ethics Board (AUP 20170016) and adhered to the Canadian Council on Animal Care guidelines for humane animal use.

Consent for publication: Not applicable

Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

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Authors' contributions: GKA, BK, HF designed the study and the imaging protocol. VB, SE and GKA executed the experiments. MK, GKA and HF analysed the results and wrote the manuscript. All the authors have read and edited the manuscript.

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Figures

Figure 1

Experiment design and FDG PET-CT imaging protocol: Three pre-weighted fasted wild-type (WT) C57BL/6J mice were injected intravenously (i.v.) with 2-5 MBq of [^{18}F]FDG. Each mouse was then prepared for isoflurane anaesthesia and imaged. After baseline imaging, mice were exposed to 0.05 ppm of ozone (O₃) for 2 h. After exposure i.e. O₃-0 h, the mouse was prepared for follow-up imaging of residual [^{18}F]FDG activity. The mice were brought back to their cages and left in room-air. At 24 h after O₃ exposure, the mice were injected with another intravenous dose of 2-5 MBq of [^{18}F]FDG and imaged (O₃-24 h). After imaging, mice were returned to their cages. After 4 h, i.e. O₃-28 h, the mice were imaged again for residual [^{18}F]FDG activity.

Figure 2

Image processing and analysis for sequential lung FDG SUR determination: The acquired X-ray and PET-CT image data sets were processed for flat and dark current normalization, reconstruction (MI Lab reconstruction software), co-registration and quantification by Pmod (pmod.com). Thereafter, the image sequences were quantified and analyzed on Image J (<https://fiji.sc/#>). The image stacks from X-ray CT were threshold-selected to segment out the lungs. The selected lung regions of interest (ROIs) were then copied on to the corresponding [18F]FDG PET image slices across multiple frames (F0-F4). The ROIs, from X-ray CT as well as the PET images, were simultaneously quantified for the various image parameters such as the area, perimeter, mean, median, mode, standard deviation (S.D.), range and integrated counts. After exporting the data to excel file, data was sorted, filtered and analyzed for corresponding mouse time-points, frames and/or CT parameters. Finally, a SUM of the full-body image Z-stacks, from the multiple frames, were analyzed for the same parameters in order to calculate the

[18F]FDG SUV (i.e. ratio of standard uptake values (units) for lung slice(s) to full-body, which is termed as standard uptake ratio or SUR). As all the time-points, baseline and post-O3 exposure, were acquired through the same imaging protocol, the FDG counts were decay corrected in order to account for time-dependent [18F]FDG decay. The lung CT grey values were plotted against “lung [18F]FDG SUR” or “lung [18F]FDG S.D.” which was overlaid for multiple frames on the same graph. These plots were then smoothened using the second polynomial function (Savitsky-Golay), in order to compute trends in final lung [18F]FDG SUR or S.D. and subsequently, % increase in lung [18F]FDG post-O3 exposure.

Figure 3

Sequential lung FDG distribution using X-ray region-of-interest: Representative lung X-ray CT slice, FDG slice in the fourth frame (F4) and a 3-d render of the merged lung [18F]FDG volume at baseline, O3-0, 24 and 28 h. The 3-d volume rendered view shows color-coded sequential frames (F0 in blue, F1 in green, F2 in red and F3 in magenta). Please note that yellow indicates a merge of green and red and white indicates a merge of all the colors. The yellow arrow-heads indicate regions of high lung [18F]FDG uptake.

Figure 4

Quantification of lung FDG Standard Uptake Ratio (SUR) and CT parameters: Representative SUR data from a mouse quantified for each 5 minute imaging time-frame A-D) Percent (%) lung [18F]FDG SUR plotted against corresponding lung X-ray CT units (in arbitrary grey values), E-H) Smoothened % lung [18F]FDG SUR plotted against the lung X-ray CT units (grey values), I-L) Lung [18F]FDG standard deviation (S.D.) plotted against the lung X-ray CT units (grey values) for each imaging time-frame. Please note that F0 is shown in blue, F1 in red, F2 in green, F3 in purple and F4 is shown in orange data points and line plots. I) Three imaging time-frames were then pooled to plot the % Lung [18F]FDG at baseline, as well as 0, 24 and 28 h post-O3 exposure. J) Regional lung mean, upper and lower range CT grey (arbitrary) values shown at baseline, as well as 0, 24 and 28 h post-O3 exposure. Baseline data is shown in blue, O3-0 h in red, O3-24 h in green and O3-28 h in purple scatter plots. Data from figures I and J were analysed by one-way ANOVA, * represents significance at $p < 0.05$ and ** at $p < 0.01$.

Figure 5

Quantification of lung FDG Standard Deviation (S.D.) parameters: Representative S.D. data from a mouse quantified for each 5 minute imaging time-frame A-D) Percent (%) lung [18F]FDG S.D. plotted against corresponding lung X-ray CT units (in arbitrary grey values), E-H) Smoothened % lung [18F]FDG S.D. plotted against the lung X-ray CT units (grey values). Please note that F0 is shown in blue, F1 in red, F2 in green, F3 in purple and F4 is shown in orange data points and line plots. I) Three imaging time-frames were then pooled to plot the Lung [18F]FDG S.D. at baseline, as well as 0, 24 and 28 h post-O3 exposure. Baseline data is shown in blue, O3-0 h in red, O3-24 h in green and O3-28 h in purple scatter plots. Data from figure I was analysed by one-way ANOVA, * represents significance at $p < 0.05$ and ** at $p < 0.01$.

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