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Measuring Acute Pulmonary Responses to Occupational Wildland Fire Smoke Exposure Using Exhaled Breath Condensate

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Abstract

Wildland firefighters are directly exposed to elevated levels of wildland fire smoke (WF smoke). Although studies demonstrate WF smoke exposure is associated with lung function changes, few studies that use invasive sample collection methods have been conducted to investigate underlying biochemical changes. These methods are also either unrepresentative of the deeper airways or capable of inducing inflammation. In the present study, levels of biomarkers of oxidative stress (8-isoprostane) and pro-inflammatory response (interleukin-6 [IL-6], interleukin-8 [IL-8], C-reactive protein [CRP], and soluble intercellular adhesion molecule-1 [sICAM-1]) were determined in exhaled breath condensate (EBC) samples that were collected from firefighters before, after, and next morning of prescribed burn and regular work shifts. Results show only a marginal cross-shift increase in 8-isoprostane on burn days ($0.05 < p\text{-value} < 0.1$), suggesting WF smoke exposure causes mild pulmonary responses.

Keywords

Wildland fire smoke; wildland firefighter; exhaled breath condensate; acute pulmonary response; oxidative stress; inflammation

Introduction

Wildland firefighters are repeatedly exposed to elevated levels of wildland fire smoke (WF smoke) while protecting lives and properties from wildfires. WF smoke contains various air pollutants such as carbon monoxide, respirable particulate matter, and other chemical compounds.[1-3] These pollutants can induce acute respiratory symptoms and spirometric

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changes following exposure.[1, 4] Increased minute ventilation due to high physical exertion, lack of respiratory protection, and prolonged work shifts potentially worsen this occupational exposure scenario.[5] Although exposure to WF smoke has been associated with lung function decline among wildland firefighters,[6, 7] a limited number of studies directly evaluating pulmonary biochemical changes underlying such physiological response has been conducted. The association between WF smoke exposure and markers of respiratory inflammation in nasal lavage (eosinophilic cationic protein and myeloperoxidase) and induced sputum (granulocytes) among wildland firefighters has been reported in two observational studies.[8, 9] However, nasal lavage is more representative of upper airway responses,[10] while sputum induction is capable of inducing inflammation.[11]

Exhaled breath condensate (EBC) obtained from airway lining fluids during spontaneous breathing contains large amounts of compounds that are measurable for elucidating ongoing biochemical responses in the lungs.[12] EBC collection is non-invasive and therefore suitable for investigating acute pulmonary effects with repeated sample collections in short time periods. Studies evaluating pulmonary effects by measuring non-specific cytokines in EBC show the potential of using EBC for detecting acute oxidative stress and inflammatory responses among wildland firefighters who repeatedly experience high levels of WF smoke exposures.[13] In the present study, healthy wildland firefighters were recruited and their EBC was collected before, after, and the next morning following prescribed burns. An oxidative stress biomarker, 8-isoprostane, and pro-inflammatory biomarkers including interleukin-6 (IL-6), interleukin-8 (IL-8), C-reactive protein (CRP), and soluble intercellular adhesion molecule-1 (sICAM-1) were measured in EBC so as to determine acute pulmonary responses among the firefighters following the WF smoke exposure.

Materials and methods

Detail of the recruitment procedure has been previously reported.[14] Briefly, twelve healthy wildland firefighters (9 males and 3 females with an average age of 33 years) were recruited from US Forest Service–Savannah River Site, SC during January to July 2015. The subjects were briefed face-to-face on the purpose, design, and sample collection procedures of the study. All subjects voluntarily participated in the study and informed consent was obtained from each of them. The study was approved by Institutional Review Boards in both the University of Georgia (UGA) and The Ohio State University (OSU).

EBC was collected before (pre-shift), immediately after (post-shift), and the morning following each of 7 prescribed burn shifts (burn days) as well as 3 regular work shifts (non-burn days) using RTube™ breath condensate collection device (Respiratory Research, Inc., Austin, TX). Each firefighter was instructed about the collection procedure, and then proceeded to breathe spontaneously for 10 min into a mouthpiece connected by a one-way valve into the collection tube. The collection tube was surrounded by an aluminum sleeve that was pre-cooled in −80 °C freezer and put on dry ice while in the field. The volume of EBC collected from the firefighters was 1500-2000 μl. The RTube™ were stored in containers with dry ice immediately after the collection and subsequently transported to UGA and later to OSU. The samples were stored at −80 °C until analyses.

Oxidative stress biomarker (i.e. 8-isoprostane) was assayed in duplicate using enzyme-linked immunosorbent assay (ELISA) kit (Cayman, Ann Arbor, MI) in accordance with the manufacturer's instruction. Four pro-inflammatory biomarkers, including IL-6, IL-8, CRP, and sICAM-1 in EBC were analyzed in duplicate using Human V-plex Ultra-Sensitive Kit designed by Meso Scale Discovery (MSD) (Rockville, MD) in MSD multiplex electrochemiluminescent immunoassay system. Two or three positive controls for each pro-inflammatory biomarker were also included in each immunoassay analysis (11 positive controls in total for each biomarker). All the controls were detectable except for one control each for IL-6 and IL-8 that were below the detection range. The analysis was performed in accordance with the manufacturer's instruction.

Since only 3 of 142 EBC samples had detectable IL-6 levels, no further analysis was conducted on IL-6. Non-burn day EBC samples were not analyzed for all pro-inflammatory biomarkers due to high degree of undetectable levels observed in the burn day samples. Measures below the limit of detection (LOD) were substituted with the LOD divided by square root of 2. Descriptive statistics was performed and the mean and standard deviation of the ratios of pro-inflammatory biomarkers collected at the three-time points compared to each other (ratios of post- to pre-shifts [Post / Pre], next morning to pre-shifts [MA / Pre], and next morning to post-shifts [MA / Post]) were reported.

In order to test whether WF smoke exposure was associated with increases in the EBC biomarkers across burn day work-shifts, the concentrations of biomarkers were log-transformed because the original data was not normally distributed. Since normality was not achieved by log-transformation for IL-8, CRP, and sICAM-1, the effect of the exposure on the cross-shift changes in the biomarkers on burn days was examined using clustered Wilcoxon signed-rank test.[15] Normality was achieved for 8-isoprostane following log-transformation and the concentration of EBC 8-isoprostane was detectable on non-burn days. Therefore, linear mixed effect model (LMM) was used to test: 1) whether the cross-shift changes of 8-isoprostane concentrations on burn days is significant, and 2) whether the cross-shift changes on burn days are significantly different from non-burn days. Results of the LMM were back transformed to obtain estimated ratios of the three-time EBC collection points compared to each other on burn days and ratios of the ratio on burn days to non-burn days. The statistical analysis was conducted using SAS version 9.4 (Cary, NC) and differences were considered significant at p-value less than 0.05.

Results

None of the firefighters reported having any cardiovascular (e.g. elevated blood pressure) and respiratory (e.g. asthma) disease. Typical of wildland firefighters,[5, 16] no respiratory protection was used by any of our subjects during the study. The average levels of IL-8, CRP, sICAM-1, and 8-isoprostane in EBC collected on burn days were in the range of 0.02-0.03 pg/ml, 2.40-2.56 pg/ml, 2.39-2.59 pg/ml, and 3.51-3.80 pg/ml, respectively. The descriptive statistics of the cross-shift ratios of the inflammatory biomarkers on burn days (Post / Pre, MA / Pre, and MA / Post) are presented in Table 1. The relative changes across the time-points from the statistical models are also shown in Table 1. The post-shift 8-isoprostane concentrations were marginally higher than the pre-shift concentrations on burn

days (p-value = 0.06). No other significant change across the prescribed burn shifts was observed.

EBC concentrations of 8-isoprostane on non-burn days were slightly lower (2.86-3.20 pg/ml) compared to burn days. Table 2 shows the ratios of 8-isoprostane levels in EBC collected at three different time-points on burn days compared to non-burn days. There was no significant difference in the ratios between burn and non-burn days.

Discussion

Our results show no sign of airway inflammation after occupational exposure to WF smoke (Table 1). The level of IL-6 was barely detected in EBC (2%) and the other inflammatory biomarkers were detectable in only about half of the samples (48-56%). These results could be due to the lower intensity and shorter duration of WF smoke exposure of the wildland firefighters. The exposure level and duration of WF smoke in this study are 354 μg/m^3 and 265 min, respectively. Both are at least 20% lower than what has been reported previously and are more than 40% lower in most cases.[5, 16-19] Studies of less intensive smoke PM exposure have similarly reported no significant acute pulmonary inflammatory effects among healthy subjects.[20, 21] Nonetheless, detectable levels of inflammatory biomarkers in EBC have been reported in previous studies using the multiplex immunoassay technology such as we performed in this study.[22, 23]

In contrast to the pro-inflammatory biomarkers, 8-isoprostane could be detected in all EBC samples. No significant change, however, was found among the three different time-points as well as when comparison was made between burn and non-burn days (Table 1 and 2). Similar results held true in a controlled human exposure study, in which EBC 8-isoprostane levels did not significantly change immediately following a 3-hour exposure to either low (200 μg/m^3) or high (400 μg/m^3) wood smoke particle concentration.[20] These results might be attributable to a mismatch between sample collection time points and the peak 8-isoprostane expression in EBC. A recent study of experimental wood smoke exposure (90 min, 250 or 500 μg/m^3 of $PM_{2.5}$) with simulation of wildland firefighter activities reported that a significant increase of EBC 8-isoprostane concentrations was not found immediately after exposure but was observed at 1-hour post exposure, presumably because of a delayed 8-isoprostane onset in the lungs.[21] This could indicate that the time of maximum oxidative responses is missed in our study due to the choice of sampling time points. Another possible explanation for these results could be the moderate levels of WF smoke exposure among the wildland firefighter working at the prescribed burns. The average exposure levels of $PM_{2.5}$ was in the range of $PM_{2.5}$ exposure concentrations used in the controlled exposure studies.[20, 21] EBC samples were analyzed 606-1021 days after collection. Knowledge about the stability of EBC components is limited. However, cytokines have been reported to be stable for up to 1 year in storage.[24, 25] On the other hand, storage stability information for isoprostanes is limited to 2 weeks.[24] Nonetheless, we did not observe any independent effect of the length of storage time on cross-shift changes of 8-isoprostane in the linear mixed effect model.

Conclusions

Results of this study indicate that there is a limited effect of WF smoke exposure on acute pulmonary responses among the wildland firefighters. No significant change in cytokine levels in EBC was observed, possibly due to mismatch between time of sample collection and maximum pulmonary 8-isoprostane response, and the moderate WF smoke exposure levels.

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Table 1.

Estimated burn day cross-shift ratios (post- to pre-shifts [Post / Pre], next morning to pre-shifts [MA / Pre], and next morning to post-shifts [MA / Post]) of pro-inflammatory (i.e. IL-8, CRP, sICAM-1) and oxidative stress (i.e. 8-isoprostane) biomarkers' levels in the EBC collected from the wildland firefighters (n =12).

Ratio	Post / Pre		MA / Pre		MA / Post	
	Mean (95% CI)[a]	*p*-value[b]	Mean (95% CI)[a]	*p*-value[b]	Mean (95% CI)[a]	*p*-value[b]
Descriptive statistics and clustered Wilcoxon signed-rank test						
IL-8	1.01 (0.94 - 1.09)	0.46	0.97 (0.89 - 1.05)	0.05	0.97 (0.91 - 1.03)	0.21
CRP	0.98 (0.94 - 1.03)	0.08	1.02 (0.98 - 1.07)	0.88	1.06 (1.00 - 1.13)	0.12
sICAM-1	1.01 (0.95 - 1.08)	0.74	1.08 (0.96 - 1.20)	0.87	1.08 (1.00 - 1.17)	0.19
Linear mixed effect model						
8-isoprostane	1.11 (1.00 - 1.25)	0.06	1.08 (0.93 - 1.25)	0.33	1.02 (0.91 - 1.13)	0.76

[a] 95% CI: 95% confidence interval.

[b] *p*-value was given by clustered Wilcoxon signed-rank test.

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Table 2.

Estimated cross-shift ratios of 8-isoprostane levels in EBC collected from the wildland firefighters (n=12) working at prescribed burn shifts (burn days) compared to regular work shifts (non-burn days) using linear mixed effect model.

Ratio	Post / Pre[a]		MA / Pre[b]		MA / Post[c]	
	Mean (95% CI)[d]	*p*-value	Mean (95% CI)[d]	*p*-value	Mean (95% CI)[d]	*p*-value
8-isoprostane	1.08 (0.86 - 1.35)	0.50	1.09 (0.78 - 1.50)	0.61	1.01 (0.78 - 1.31)	0.94

[a]Ratios of post- to pre-shifts on burn days compared to ratios of post- to pre-shifts on non-burn days.

[b]Ratios of next morning to pre-shifts on burn days compared to ratios of next morning to pre-shifts non-burn days.

[c]Ratios of next morning to post-shifts on burn days compared to ratios of next morning to post-shifts non-burn days.

[d]95% CI: 95% confidence interval.

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