Biomarkers of Human Cardiopulmonary Response After Short-Term Exposures to Medical Laser-Generated Particulate Matter From Simulated Procedures

A Pilot Study

Ramon Lopez, PhD, Mark O. Farber, MD, Vincent Wong, MD, and Steven E. Lacey, PhD, CIH, CSP

Objective: We conducted an exposure chamber study in humans using a simulated clinical procedure lasing porcine tissue to demonstrate evidence of effects of exposure to laser-generated particulate matter (LGPM). **Methods:** We measured pre- and post-exposure changes in exhaled nitric oxide (eNO), spirometry, heart rate variability (HRV), and blood markers of inflammation in five volunteers. **Results:** Change in pre- and post-exposure measurements of eNO and spirometry was unremarkable. Neutrophil and lymphocyte counts increased and fibrinogen levels decreased in four of the five subjects. Measures of HRV showed decreases in the standard deviation of normal between beat intervals and sequential 5-minute intervals. **Conclusion:** These data represent the first evidence of human physiologic response to LGPM exposure. Further exploration of coagulation effects and HRV is warranted.

BACKGROUND

In the United States, an estimated half million health care professionals were exposed to surgical smoke in 2008,[1] and the rapid development of new clinical laser technologies and their applications, as well as growth in the sale of medical lasers[2] promises continued growth of human exposure. Medical laser-generated aerosol is created from the heating of the target tissue leading to the vaporization, pyrolysis, and combustion of cellular material, and the release of steam, cell content, and combustion by-products.[3,4] Our preliminary work measuring the concentration of gases and particulate matter (PM) present in the laser-generated aerosol has directed us to focus specifically on the PM fraction,[5,6] and we have demonstrated that operational parameters (eg, power, beam diameter) impact size-specific laser-generated PM (LGPM) emission rates[6] that may be important in determining possible health implications and designing control strategies.

The current evidence-based understanding of LGPM exposure health effects has been limited to animal studies, which have demonstrated inflammatory responses in pulmonary tissue defined by interstitial pneumonia, bronchiolitis, and emphysema.[7–10] One study noted a decreased response with increased air filtration, indicating a potential dose-response relationship.[7] Long-term outcomes of human exposure associated with the chronic inhalation of the aerosol have not been studied. This pilot study measured for the first time the human physiological response to LGPM aerosol exposure by measuring exhaled nitric oxide (eNO), spirometry, heart rate variability (HRV), and blood biomarkers, including white blood cell (WBC) counts, fibrinogen, and platelets. A symptom survey was also used to document any noticeable effects the participants may have experienced.

Measures of Cardiopulmonary Response to Particulate Matter Exposures

Nitric oxide is produced by the endothelial and epithelial cells in lung tissue, and its concentration in exhaled breath has been demonstrated to increase with airway inflammation.[11,12] Real-time eNO analyzers have been developed, tested, and approved for medical use,[13,14] and studies examining the repeatability of eNO measurements have shown low variability not impacted by season, food intake, body weight, or height.[15] The utility of eNO for measuring PM-associated airway inflammation has been demonstrated in studies of ambient air pollution in which increased eNO was measured after short trips along high-density roadways, after exercise activities in urban locations, and after normal activities on days with high ambient PM concentrations.[14,16–20]

Spirometry studies have measured forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) and demonstrated an inverse relationship with PM concentration in susceptible populations, including elderly and asthmatic individuals, after short-term ambient PM exposures such as walking in urban settings during heavy traffic.[21–23]

Although heart rate remains fairly constant in most normal individuals, the time between two successive beats can vary significantly.[24] Time-domain measures of HRV have been established to measure response to environmental exposures, including the standard deviation of all normal RR intervals (SDNN, representing overall HRV); standard deviation of sequential 5-minute intervals (SDANN, long-term changes in HRV); and root mean square of the successive differences (RMSSD, short-term changes in HRV).[25] HRV is a predictor of cardiovascular mortality and morbidity after exposure to high levels of ambient PM[26–29]; disruption of the autonomic nervous system, activation of pro-inflammatory pathways, and accelerated atherosclerosis are linked to HRV and the increased risk of cardiovascular events. Reductions in HRV from PM exposure have been demonstrated in animals and humans, even when signs of hypoxia and respiratory distress are absent.[30,31]

It has been proposed that ultrafine particles may penetrate into the blood stream and cause a systemic inflammatory response that can be measured by biomarkers present in blood.[32] The particles interact with platelets in the bloodstream, increasing coagulability.[33] It has also been demonstrated that changes occur in plasma fibrinogen[34–37] and peripheral neutrophils[38–40] in animals and humans; both conditions are associated with coronary heart disease and myocardial infarction[41,42] and are believed to be

From Indiana University, Fairbanks School of Public Health, Department of Environmental Health Science (Drs Lopez, Lacey); Indiana University Health, Pulmonology-Critical Care (Dr Farber); and Indiana University, Krannert Institute of Cardiology, Department of Medicine, Indianapolis (Dr Wong).

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Address correspondence to: Steven E. Lacey, PhD, CIH, CSP, Indiana University, Fairbanks School of Public Health, Department of Environmental Health Science, 1050 Wishard Blvd. (RG 6086), Indianapolis, IN 46202 (selacey@iu.edu).

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responses to oxidative stress and systemic inflammation caused by exposure to ambient PM.[33,37,43,44] Other studies have demonstrated increases in lymphocytes and eosinophils in animals with exposure to outdoor PM[45]; the increases were noticed 6 hours after exposure and peaked at 12 hours post-exposure before declining.[45]

METHODS

We measured pre- and post-exposure changes in eNO, spirometry, HRV, and blood markers of systemic inflammation in five volunteer participants under controlled laboratory conditions. Study subjects were healthy men and women with no current pulmonary or cardiovascular illness or disease, self-described as nonsmokers, sedentary, and between 35 and 55 years of age. We used an Ultra MD 60 Laser System (max power = 60 W, $\lambda = 10{,}600$ nm, pulsed) (Laser Engineering Inc., Franklin, TN) in a simulated laser clinical procedure, and lased porcine skin in the hood of an exposure chamber in a controlled manner. Size-selective particle concentration was measured. The study design was reviewed and approved by the Indiana University Institutional Review Board, protocol #1505847764.

Exposure Chamber

The exposure chamber was a hybrid design of the emission chamber described in the study by Lippert et al[46] and an exposure chamber designed by Morawska et al[47] (Fig. 1). The chamber system is composed of a rectangular glass hood, connected via the transition section to an aluminum duct. An opening in the glass hood allows participants to comfortably position their heads in the system and maintain eye contact with the research team.

Exposure Concentrations

Two previously reported field studies that simulated medical laser procedures in hospital operating rooms documented concentrations of LGPM ranging from 590 to 1690 $\mu g/m^3$.[48,49] We used 1690 $\mu g/m^3$ as upper limit to establish the range of exposures in the chamber study, performing our evaluations at 0%, 50%, and 100% of 1690 $\mu g/m^3$. Our earlier work demonstrated that our system is capable of reliably generating predictable mean concentrations inside the exposure chamber through manipulation of operational parameter settings (Power: 2 and 3 W; PRF: 5 Hz; pulse duration: 0.1 seconds; beam diameter: 1.0 mm). Real-time monitoring of LGPM concentration in the chamber was measured using an Aerotrak 9306 (TSI Inc. Shorewood, MN), and a P-Trak 8525 (TSI Inc. Shorewood, MN) as described in the study by Lopez et al.[6] At the start of each day, we verified background concentration of PM and air-flow rate in the exposure chamber. We recognize that gases and vapors are also present in laser-generated aerosol, but our previous work has demonstrated that these contaminants are transient and, when present, have extremely low concentrations, so we have focused on the PM fraction.[50]

Human Exposure Events

As cardiopulmonary response to LGPM exposure has never been measured systematically, it was unclear at what level of exposure a response would be noticed. We used an accelerated study design with two phases (phase 1 and phase 2), with a new set of study participants in each phase. Five participants (one male, four female) comprised the study group. All participants wore laser eye protection during the lasing procedure.

In phase 1, we performed a response range-finding experiment with two subjects. In their first session, each participant sat in the exposure chamber for 15 minutes, but no lasing was performed (zero exposure). Three days later, study subjects returned and were exposed to four sequential 15-minute sessions, with a 10-minute break between sessions; the first two sessions were at ~850 $\mu g/m^3$ (50% exposure) and the latter two sessions were at ~1690 $\mu g/m^3$ (100% exposure). The initial range-finding experiment in phase 1 was intended to scale up exposure concentrations and to monitor for any unanticipated acute adverse reaction as a safety measure. Exposure time of 15 minutes for each session was chosen because it is considered a typical duration for many clinical laser procedures,[51] and it is typical for several procedures to be performed in a single day.

Similarly, in phase 2, three new subjects were first exposed to a control session (zero exposure), and then three days later, study subjects returned and were exposed to four sequential 15-minute sessions, with a 10-minute break between sessions, at ~1690 $\mu g/m^3$ (100% exposure).

Measuring a Response

Exhaled NO

Participants provided pre- and post-exposure exhaled breath samples using a Niox Vero eNO monitor (Aerocrine, Solna, Sweden).[15] Participants had 20 minutes to adjust to the laboratory environment before two pre-exposure eNO measurements were made. Post-exposure eNO measurements were made immediately after the exposure event, and then 20 minutes after the event concluded. In phase 2, additional post-exposure measurements were collected every 20 minutes for 1 hour and 20 minutes.

Spirometry

Pulmonary function testing was performed in accordance with the guidelines of the American Thoracic Society/European

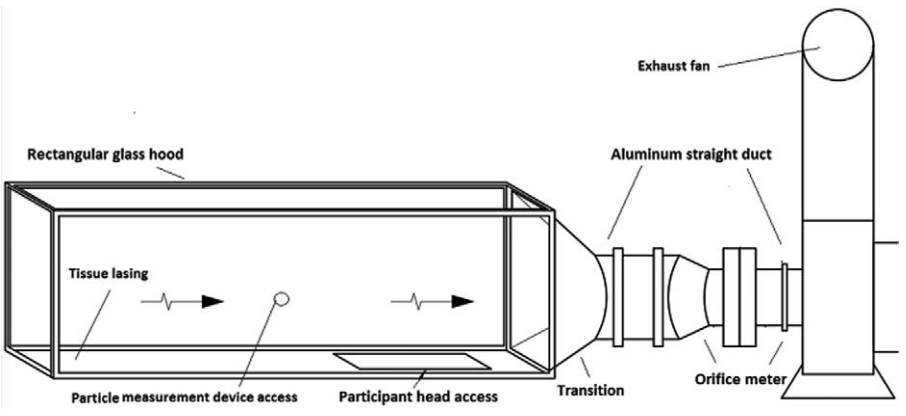

FIGURE 1. Glass exposure chamber for lasing of tissue and exposure to study participants, air is pulled through the exposure chamber and exhausted to a fume cabinet.

TABLE 1. Pre- and Post-Exposure Levels of Exhaled NO in PPB

	Participant ID	Pre	Post	20 min Post	40 min Post	60 min Post	80 min Post
Phase 1	1	19	17	16	—*	—	—
	2	29.5	32	31	—	—	—
Phase 2	3	21	21	20	20	21	20
	4	34.5	29	29	32	28	31
	5	36	33	33	28	29	38

*Post-exposure measurements after the first 20 minutes were not made during phase 1.

Respiratory Society Task Force: Standardization of Spirometry.[52] A Spirodoc Spirometer (MIR, Waukesha, WI) with logging capabilities was used to measure FVC and FEV_1, and the study participants were coached by our team pulmonologist. Each participant completed the spirometry tests immediately before the exposure event(s), and again 5 minutes post-exposure.

Heart Rate Variability

HRV was measured using a Datrix VX3 Holter (Biomedical Systems, Brussels, Belgium) with seven lead attachments. Our team cardiologist prepped and connected the monitors and participants wore the devices for two consecutive 24-hour monitoring sessions that represented pre- and post-exposure event. Post-exposure monitoring began at the start of the exposure event.

Blood Analysis

Participants provided peripheral venous blood 20 minutes before the exposure event, and three hours post-exposure. Samples were collected by a certified phlebotomist and analyzed by the Indiana University Health Pathology Laboratory for WBCs (total, monocytes, neutrophils, lymphocytes, eosinophils, and basophils), fibrinogen, and platelets.

Symptom Survey

A short survey was verbally administered to participants pre- and post-exposure, and in the evening after the exposure event. The questionnaire was based on a tool developed by the National Institute for Occupational Safety and Health to assess possible occupational exposure health outcomes from medical laser aerosol exposure.[53] Participants were asked whether they were experiencing symptoms, including irritation, headache, dizziness, cough, and noticeable lung problems.

RESULTS

For all measures of response, no material changes were observed in the control (zero-exposure) sessions. Tabulated results present pre- and postmeasures of response from actual exposure sessions.

TABLE 2. Pre- and Post-Exposure Levels of Spirometry in Liters

		FEV_1		FVC	
	Participant ID	Pre	Post	Pre	Post
Phase 1	1	2.93	2.86	3.4	3.3
	2	2.18	2.07	2.84	2.82
Phase 2	3	2.15	2.54	2.92	2.56
	4	4.35	4.39	4.49	4.47
	5	4.43	4.42	5.21	5.0

FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity.

Exhaled Nitric Oxide

There was no notable change in eNO measurements for any participant (Table 1); change in post-exposure measures varied within ±4 ppb, similar to measures observed in control exposure sessions. During phase 2, additional post-exposure eNO measures were made, but again no material change was observed.

Spirometry

Spirometry results were unremarkable for all participants (Table 2). The data demonstrate no change from pre- to post-exposure for all five study participants.

Heart Rate Variability

Three measures of HRV were calculated for each 24-hour time interval: SDNN, SDANN, and RMSSD.[25] All participants demonstrated a post-exposure decrease in SDNN and SDANN, and three of five participants demonstrated a post-exposure decrease in RMSSD (Table 3). Control session measurements showed greater change both above and below baseline measurements.

Biomarkers in Blood

There was a general increase in WBC counts from pre- to post-exposure in four of the five participants; three participants demonstrated increases between 20% and 25% (Table 4). Neutrophils and lymphocytes had the greatest post-exposure change. Changes in monocyte and eosinophil counts were unremarkable.

Post-exposure fibrinogen levels decreased between 6% and 10% in four out of five participants. One participant experienced a 22% post-exposure increase in fibrinogen during the control exposure.

Post-exposure platelet counts increased from 5% to 12% in three out of five participants; the two other participants showed modest decreases.

Symptom Survey

One participant reported feeling dizzy and lightheaded for 3 to 5 minutes immediately post-exposure. No other participants reported any noticeable effects.

DISCUSSION

eNO is an effective measure of inflammatory response in asthmatic patients, but studies related to ambient air pollution have been inconclusive, with increases in eNO demonstrated in some studies, and no change in other studies. Most of these investigations involve longer exposure periods than our study.[17–19,54] Our investigation looked at short exposures of 15 minutes, with a total 1 hour of exposure, for a single day. We were uncertain whether a change would occur instantaneously, or if there was lag time between exposure and outcome. During phase 2, participants provided exhaled breath samples every 20 minutes post-exposure for 80 minutes, but no change was observed. Future exploration to determine whether a response is dose- or time-dependent is warranted.

TABLE 3. Pre- and Post-Exposure Levels of HRV Measures

		SDNN		SDANN		RMSSD	
	Participant ID	Pre	Post	Pre	Post	Pre	Post
Phase 1	1	154	134	140	120	33	28
	2	139	135	120	119	61	42
Phase 2	3	110	97	99	79	27	35
	4	116	101	106	85	35	44
	5	110	92	87	68	35	33

RMSSD, root mean square of the successive difference; SDANN, standard deviation of sequential 5-minute intervals; SDNN, standard deviation of all normal RR intervals.

In our study, concentration of fibrinogen decreased and platelet count increased post-exposure for most subjects. In a previous chamber study, human subjects were exposed to airborne particle concentrations up to 200 $\mu g/m^3$ for 2 hours; measures of fibrinogen showed an increase in 18 hours post-exposure, but measures immediately after exposure showed no change.[34] A second study measured fibrinogen after exposure to welding fume and noticed a significant decrease in fibrinogen concentration 6 and 24 hours post-exposure.[55] In our study, it was unclear when a response began or when it peaked since we collected a single blood sample 3 hours post-exposure. The change in levels of fibrinogen and platelets may be explained by the coagulation cascade. Endothelial damage activates the increased synthesis of platelets and fibrinogen, and the conversion of fibrinogen to fibrin fibers, to reduce blood loss and stabilizes platelet plugs.[56] As this is a time-dependent process, when a post-exposure blood sample is collected may affect the observed fibrinogen and platelet count. In future studies, additional blood samples may elucidate time-dependent effects.

We noted post-exposure increases of neutrophils, lymphocytes, monocytes, and total WBC, similar to increases seen in ambient PM studies as an inflammatory response from oxidative stress.[26,38,43,55,57] In our study, one participant (#4) demonstrated a high pre-exposure WBC count and a post-exposure decrease in WBC count; in a follow-up conversation with the participant, he suggested that he was developing a cold at that time, which may explain this specific result.

Other biomarkers in blood exist that may also be helpful in determining response to PM exposures. C-reactive protein is believed to be a marker of inflammation and is associated with risk of myocardial infarctions,[42] and in some studies has been found to be elevated with high PM exposures.[58–60] Interluekin-6 is believed to be stimulated as an immune and inflammatory response, and also has been demonstrated to increase with high levels of PM.[60,61] Future studies should explore these additional potential indicators of response.

A high degree of HRV is normal for healthy individuals. The observed decline in HRV measures, including SDNN and RMSSD, is similar to those found in other studies comparing changes in HRV to levels of PM in ambient air, or from activities at home that produce PM, and they generally noted decreases in measures of HRV with increasing PM exposure.[62–65]

The purpose of our study was to determine a human exposure-response from LGPM exposure, and the efficacy of respiratory protection was outside the scope of this pilot study, so participants did not use respiratory protection during exposure events. It has been reported that health care professionals do use surgical masks during laser use, but these are not filtering respirators.[66,67]

CONCLUSION

Our pilot study is the first attempt to measure a response to short-term LGPM exposure in humans. The clearest evidence of response was demonstrated by decreased HRV, increased WBC counts, decreased fibrinogen, and limited evidence of increased platelets. Under our experimental conditions, eNO and spirometry did not prove to be effective measures of response. These lines of investigation were highly exploratory, and the limits of our study design mean we may have missed detecting a response that may be time- or dose-dependent. Further exploration of eNO and spirometry is warranted, as if we determine a study strategy that demonstrates a response, these measures are relatively inexpensive and non-invasive. Other blood biomarkers should also be explored.

These results warrant further investigation in exploring human responses to workplace LGPM exposure as the implications for risk management of the health effects are critical for ensuring safe work environments. Improved understanding of human health implications will make more compelling risk communication and the need for improved control strategies.

TABLE 4. Pre- and Post-Exposure Levels of Fibrinogen and White Blood Cell Counts on Days With an Exposure

		Fibrinogen, mg/dL		Total WBC (#/μL)		Neutrophils (#/μL)		Lymphocytes (#/μL)		Platelets (# K/μL)*	
	Participant ID	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Phase 1	1	295	263	5300	6600	3300	4300	1600	2000	244	262
	2	333	311	5600	6700	3600	4400	1400	1700	215	227
Phase 2	3	302	342	6700	7300	4400	4500	1200	2000	201	226
	4	414	378	9700	8300	5800	5000	2700	2300	279	257
	5	440	406	5900	7300	3100	4200	2100	2200	254	245

*Platelet count in [# * 1000].

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