Acute Effects of COREXIT EC9500A on Cardiovascular Functions in Rats

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These studies characterized cardiovascular responses after an acute inhalation exposure to COREXIT EC9500A, the oil dispersant used in the Deepwater Horizon oil spill. Male Sprague-Dawley rats underwent a single 5-h inhalation exposure to COREXIT EC9500A (average exposure level 27.12 mg/m^3) or air. On d 1 and 7 following the exposure, rats were implanted with indwelling catheters and changes in heart rate and blood pressure were assessed in response to increasing levels of adrenoreceptor agonists. A separate group of rats was euthanized at the same time points, ventral tail arteries were dissected, and vascular tone along with dose-dependent responses to vasoconstricting and dilating factors were assessed in vitro. Agonist-induced dose-dependent increases in heart rate and blood pressure were greater in COREXIT EC9500A-exposed than in air-exposed rats at 1 d but not 7 d after the exposure. COREXIT EC9500A exposure also induced a rise in basal tone and reduced responsiveness of tail arteries to acetylcholine-induced vasodilation at 1 d but not 7 d following the exposure. These findings demonstrate that an acute exposure to COREXIT EC9500A exerts transient effects on cardiovascular and peripheral vascular functions.

Dispersants are commonly used after oil spills to accelerate the biodegradation of oil. Most dispersants are composed of two components: a solvent that breaks up the oil, and a surfactant that emulsifies the oil (Chapman et al. 2007). In the Deepwater Horizon oil spill in the Gulf of Mexico, approximately 1.8 million gal of the dispersant COREXIT EC 9500 A (COREXIT; Nalco Inc, Sugar Land TX) was sprayed from planes and boats to control the spread and speed the degradation of the oil (Guard 2011). Aerial application of the dispersant, and aerosols generated by waves, created a potential inhalation exposure for cleanup workers and subjects living along the Gulf during dispersant application.

COREXIT and other dispersants may exert adverse effects on a number of physiological systems, including the cardiovascular system. For example, exposure to dispersant-treated oil was shown to induce cardiovascular damage in developing fish by increasing absorption of polycyclic aromatic hydrocarbons (PAH) (Ramachandran et al. 2004). Absorption of PAH results in cardiac arrhythmias and interferes with cardiac tissue development in

embryonic fish (Carls et al. 2008; Incardona et al. 2009; Pollino and Holdway 2002). However, it is difficult to predict the effects of dispersants on human cardiovascular function from these studies because the adverse effects of COREXIT alone on cardiovascular function were not assessed, the route of exposure was different, and the effects on adult fish were not examined.

COREXIT contains high concentrations of propylene glycol (PG) and thus, based on its chemical composition, inhalation of COREXIT may induce pulmonary edema, injury, and dysfunction (ATSDR 1997; 2008), and these actions on the pulmonary system may in turn act indirectly to disrupt cardiovascular function (Forman 1988). For example, inhalation of titanium dioxide (TiO_2) nanoparticles results in a mild and transient irritation in the lungs of rats characterized by potentiation of blood polymorphonuclear leukocytes (PMNL) (LeBlanc et al. 2009; Nurkiewicz 2006). PMNL adhere to systemic microvascular walls and generate reactive oxygen species (ROS) which in turn disrupt cardiovascular function by altering the responsiveness of coronary and resistance vessels to endothelial cell-induced vasodilation (LeBlanc et al. 2009; Nurkiewicz et al. 2004; 2006). Pulmonary exposure to TiO_2 nanorods was also found to produce changes in heart rate and blood pressure at high doses (Nemmar et al. 2011). Therefore, it is possible that inhalation of COREXIT might disrupt cardiovascular function indirectly by actions on the respiratory/pulmonary system.

In these studies a rat model was used to determine if an acute inhalation exposure to COREXIT results in alterations in cardiovascular and peripheral vascular functions in rats. In vivo measures of heart rate and blood pressure, along with in vitro measures of peripheral vascular functions, were conducted 1 and 7 d after a single inhalation exposure to COREXIT. Based on previous studies, it was postulated that exposure to COREXIT may result in transient changes in blood pressure and heart rate, and that these changes may be associated with a reduced sensitivity of peripheral vessels to endothelial-mediated vasodilation.

METHODS

Animals

Male Sprague-Dawley rats (Hla: SD CVF, 8–10 wk old, weighing approximately 300 g) obtained from Hilltop Labs (Scottdale, PA) were housed in AAALAC-accredited facilities under a 12-h light/dark cycle, with food (Teklad 2918 irradiated) and tap water available ad libitum. Rats were allowed to acclimate to the facilities for 1 wk before exposures were performed. All procedures and surgeries were approved by the National Institute for Occupational Safety and Health (NIOSH) Animal Care and Use Committee and were in compliance with the Centers for Disease Control (CDC) and National Institutes of Health (NIH) guides for Care and Use of Laboratory Animals.

Exposure

An automated whole-body inhalation exposure system was used to expose individually housed rats to COREXIT or air (control). The oil dispersant aerosol was generated with a collision-type atomizer (TSI, 3076) and regulated with software feedback loops to maintain a constant concentration throughout exposures. A description of the exposure system can be found in Goldsmith et al. (2011, this issue). Rats were placed in the inhalation chamber and exposed to air or COREXIT (at approximately 27.12 mg/m^3) for 5 h (see Goldsmith et al. for details regarding dose and dose measurement). All animals were returned to the colony room immediately following the exposure. Rats were anesthetized with pentobarbital (100 mg/kg, ip) and euthanized by exsanguination for microvessel studies 1 or 7 days following exposure. Rats used for the measurement of in vivo hemodynamics underwent surgery to implant catheters 1 or 7 d following the exposure.

In Vivo Hemodynamic Measurements

Rats (7–8/group) were anesthetized with inhaled 2% isoflurane mixed with oxygen at a flow rate of 2 L/min. Using aseptic technique, a custom catheter made according to the method

described by Wang et al. (2004) was inserted into the left ventricle through the carotid artery. The correct position of the catheter tip in the left ventricle was confirmed by the waveform of left ventricular pressure visualized on a computer monitor. To study vascular function, in vivo systemic arterial blood pressure (BP) was determined by using a fluid-filled arterial catheter that was placed in the femoral artery and connected to a pressure transducer coupled to a computerized cardiovascular continuous monitoring system (a PowerLab/4SP analog-to-digital converter; AD Instruments, Chalgrove, UK). Another catheter (polyurethane, 2 French size) was inserted into the jugular vein for the administration of isoproterenol (ISO) or norepinephrine (NE). Both chemicals were purchased from Sigma-Aldrich (St. Louis, MO). All three catheters were exteriorized through subcutaneous tunneling and suturing on the back. Left ventricular function and BP were measured in unrestrained, conscious rats while ambulating within a small cage. The arterial catheters were connected to a fluid-filled pressure transducer for at least 20 min prior to measurements being made (or until rats display stable heart rate and blood pressure measures). Heart rate (HR) and BP were recorded and analyzed using cardiovascular continuous monitoring software (PowerLab/4SP, AD Instruments, Colorado Springs, CO).

In Vitro Microvessel Measurements

Tails were dissected from rats (8 rats/group) after exsanguination and placed in cold Dulbecco's modified Eagle's medium with glucose (Invitrogen/Gibco; Carlsbad, CA). Ventral tail arteries from the C14–C15 region of the tail were dissected shortly after euthanasia, mounted on glass pipettes in a microvessel chamber (Living Systems; Burlington, VT), and perfused with biocarbonated-HEPES buffer warmed to 37°C. Arteries were pressurized to 60 mm Hg and allowed to equilibrate for approximately 1 h. After 1 h the chamber buffer was changed and the pressure was lowered to 10 mm Hg. Pressure was gradually increased (5 mm Hg every 10 s until reaching a final pressure of 110 mm Hg) and changes in the internal diameter of the artery were recorded. Arteries were repressurized to 60 mm Hg and allowed to stabilize, and then phenylephrine (PHEN, α-1 adrenoreceptor agonist)-mediated vasoconstriction and acetylcholine (ACh)-mediated redilation were assessed. PHEN was applied to the chamber in half-log increments (-8.5 to -6 M) and the internal diameter was recorded after vessels stabilized (approximately 5 min between application of doses). Because ventral tail arteries usually display little basal tone, endothelial-mediated redilation was assessed after constriction by adding ACh in half-log increments (-8 to $-5M$) using the procedures that were used to apply PHEN.

Nitrate/Nitrite (NO_x) and Hydrogen Peroxide (H_2O_2) Assays

Ventral tail artery samples from the C12–C14 region were dissected and immediately frozen. Tissue samples were homogenized in 200 µl lysis buffer (10 mM Tris base, 1 mM ethylenediamine tetraacetic acid [EDTA], 1 mM EGTA, 150 mM sodium chloride, 1% Triton-X 100), and NO_x and H_2O_2 concentrations were measured using the NO_x colormetric assay (Caymen Chemical Company; Ann Arbor, MI) and the Fluoro H_2O_2 assay (Cell Technology, Inc., Mountain View, CA) following the manufacturers' protocols. Protein concentrations were analyzed using the bicinchoninic acid (BCA) assay (Pierce, Rockford, IL).

Statistical Analyses

The analysis of measures of BP, HR, and dose-dependent changes in internal vascular diameter to PHEN and ACh was generated using SAS/STAT software, version 9.1, of the SAS system for Windows (SAS Institute, Inc., Cary, NC) or Jmp 5.1 (SAS Institute, Inc., Cary, NC). PROC MIXED was utilized to run a two-way factorial analysis of variance with concentration of ISO, NE, PHEN, or ACh as a repeated measure to account for multiple measures in individual animals. Treatment comparisons

were then calculated at each level of a drug utilizing the "slice" option. Baseline diameter, H_2O_2 and NO_x concentrations, and percent change in internal diameter as a result of increasing pressure were analyzed using Student's t-test. Measures collected from rats 1 and 7 d after exposures were performed separately. All differences were considered significant at $p < .05$.

RESULTS

In Vivo Hemodynamics

Baseline measures of HR were similar in all groups of rats. However, 1 d after exposure to COREXIT, ISO induced increases in HR were greater in exposed than in control animals, and the differences were significant at the highest doses of ISO (Figure 1A). Although baseline
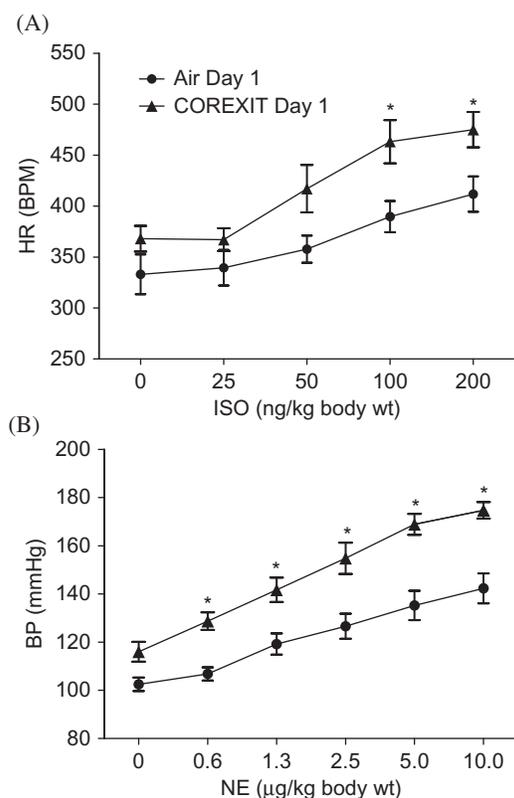

FIGURE 1. Dose-response curves for (A) HR and (B) mean BP changes in response to adrenoreceptor agonists ISO and NE, respectively, at 1 d postexposure to COREXIT. Values are means ± SEMs; asterisk indicates significantly different from controls, $p < .05$, $n = 7$–10/condition.
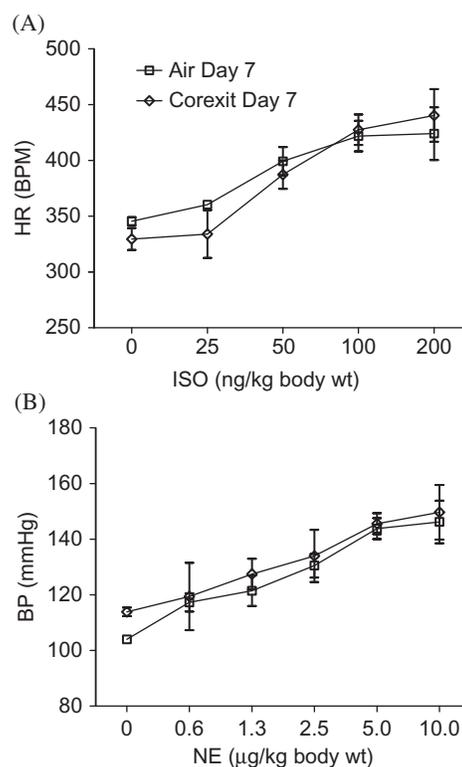

FIGURE 2. Dose-response curves for (A) HR and (B) mean BP in response to adrenoreceptor agonists ISO and NE, respectively, at 7 d postexposure to COREXIT. Values are means ± SEMs; $n = 5$–6/condition.

measures of BP appear to be numerically elevated in COREXIT-treated rats 1 d following the exposure, this difference was not significant. However, COREXIT-exposed rats did display significantly greater elevation in BP in response to NE infusion compared to the control (Figure 1B). BP in COREXIT-treated rats was higher than in control rats at all doses of NE. In rats examined 7 d after the exposure, increases in HR and BP in response to treatment with adrenoreceptor agonists were similar in exposed and control animals (Figures 2A and 2B).

Peripheral Vessel Physiology

COREXIT exposure did not exert a significant effect on baseline diameters of arteries maintained at a constant pressure of 60 mm Hg (Figure 3A). However, pressure-induced increases in the diameter of vessels
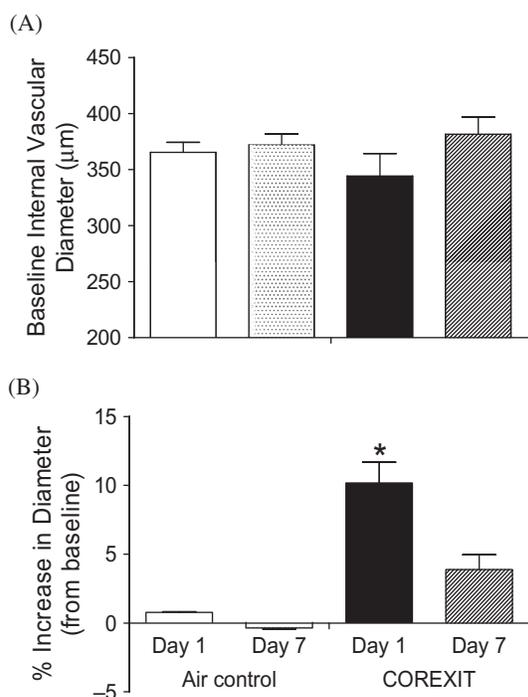
(A)

(B)

FIGURE 3. (A) Internal baseline diameters and (B) basal tone in ventral tail arteries were measured in rats 1 or 7 d after exposure to air (control) or COREXIT. Baseline diameters were not different between the groups. However, 1 d following exposure, the basal tone in arteries from COREXIT-treated rats was greater than the tone in rats exposed to control conditions. Values are means ± SEMs; asterisk indicates significantly ($p < .05$) greater than air d 1 treated rats, $n = 8$ rats/condition.
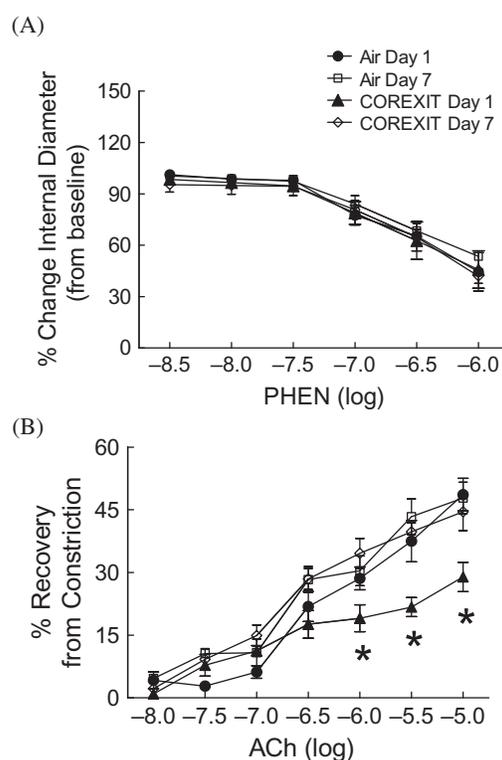
(A)

(B)

FIGURE 4. The effects of COREXIT exposure on adrenoreceptor-mediated vasoconstriction (A; PHEN-induced) and endothelial-mediated vasodilation (B; ACh-induced) measured in ventral tail arteries. COREXIT exposure did not affect PHEN-induced vasoconstriction, but arteries collected from rats 1 d after COREXIT exposure displayed a reduced sensitivity to ACh-mediated vasodilation. Values are means ± SEMs; asterisk indicates significantly ($p < .05$) less than air d 1 treated rats, $n = 8$ rats/condition.

were significantly greater in arteries from COREXIT-exposed rats than in controls 1 d after the exposure (Figure 3B), indicating that COREXIT-exposed arteries display elevated basal tone. Although arteries from rats collected 7 d after COREXIT exposure also displayed a rise in diameter in response to increasing pressure, the response was not significantly different than controls.

Ventral tail arteries did not display a marked difference in responsiveness to PHEN-induced vasoconstriction at 1 or 7 d after the exposure (Figure 4A). However, 1 d following exposure to COREXIT, arteries displayed a reduced responsiveness to ACh-induced vasodilation (Figure 4B). This change was no longer present in arteries collected 7 d after exposure. Changes in vascular sensitivity to ACh 1 d following the exposure were not associated with marked alterations in NO_x or H_2O_2

concentrations in artery segments (data not shown).

DISCUSSION

Little is known regarding the potential toxicity associated with inhalation exposure workers in the Gulf received during the cleanup efforts after the oil spill. This study and the others in this issue are the first to examine the physiological effects of COREXIT inhalation. Data demonstrated that inhalation of COREXIT results in transient increases in responses of the cardiovascular system to adrenoreceptor-mediated changes in HR and BP, elevation in peripheral vascular tone, and reduced responsiveness of peripheral arteries to ACh-induced vasodilation.

Previous studies demonstrated that inhalation of pulmonary irritants resulted in changes in cardiovascular function, primarily by altering endothelial-mediated vasodilation in coronary and peripheral arteries (LeBlanc et al. 2009; Nurkiewicz et al. 2008). These studies showed that exposure does not need to result in overt pulmonary inflammation (as determined by bronchoalveolar lavage markers) or gross changes in morphology to induce changes in vascular function. The study by Roberts et al. (2011, this issue) did not find evidence of overt pulmonary inflammation or damage in response after a single exposure to COREXIT. However, Roberts et al. (2011, this issue) found a change in respiratory compliance indicating that there was evidence of a pulmonary response to the exposure. Such a response may have been sufficient to induce the transient changes in cardiovascular function observed in this study.

Baseline measurements of cardiovascular function such as HR and BP were not markedly altered in COREXIT-treated rats at either time point. However, COREXIT-treated rats displayed greater increases in HR and BP in response to adrenoreceptor-agonist infusion than control rats 1 d after exposure. These changes may be attributed to an increased sensitivity of the cardiovascular system to adrenoreceptor-mediated actions, a reduction in ACh-induced vasodilation (Granger et al. 2010), or an elevation in sympathetic tone. However, data from in vitro concentration-response studies indicated that there was a disruption in endothelial-mediated vasodilation in COREXIT-exposed rats. Inhalation of COREXIT did not significantly affect PHEN-induced vasoconstriction 1 or 7 d after exposure. However, arteries from COREXIT-treated rats were less sensitive to ACh-induced vasodilation than arteries from controls. Thus, although COREXIT may exert mild effects on adrenoreceptor-mediated vasoconstriction, it appears that a transient disruption in endothelial-induced vasodilation is the primary effect following COREXIT exposure. These findings are consistent with other studies demonstrating that cardiovascular changes that occur in response to pulmonary irritants are the result of disruptions in endothelial cell function (LeBlanc et al. 2009; Nurkiewicz et al. 2004).

The mechanisms by which COREXIT induces these changes in endothelial cell function are not known. However, previous studies examining the effects of pulmonary irritants on cardiovascular function demonstrated that the deposition of irritants in the lungs resulted in pulmonary irritation, which may or may not be accompanied by pulmonary inflammation as measured by bronchoalveolar lavage (LeBlanc et al. 2009; Nurkiewicz et al. 2008). This irritation in turn results in potentiation of blood PMNL and generation of ROS. ROS disrupt both ACh and prostaglandin-mediated vasodilation in nanoparticle-exposed animals (LeBlanc et al. 2009; Nurkiewicz et al. 2008). PMNL act by adhering to the walls of peripheral and coronary blood vessels, releasing ROS, which disrupt nitric oxide-mediated vasodilation. Treatment with anti-oxidants or depletion of PMNL eliminates these alterations in vascular function (LeBlanc et al. 2010; Nurkiewicz et al. 2006; 2008; 2009). In the current study, significant differences in NO_x or H_2O_2 concentrations in tail arteries were not observed. However, these factors were measured in intact vessels of animals. This method may not have been sensitive enough to detect changes in oxidant levels within the vessel induced by PMNL.

Pulmonary irritants may also interfere with vascular function via neurogenic mechanisms. Inhalation of ultrafine TiO_2 nanoparticles resulted in an increase in the phosphorylation of p38 mitogen-activated protein kinase and cardiac troponin I in heart tissue 0 and 24 h following a single 4-h exposure. These changes in phosphorylation were associated with an elevation in substance P (SP) in the nodose ganglia (Kan et al. 2011). SP-containing neurons receive sensory input from the lungs, and transmit information from the lungs to the medullary cardiovascular regulatory center in the brainstem, where these neurons might act to modulate cardiovascular function (Armour 1999; Kosta et al. 2010). Sriram et al. (2011, this issue) also demonstrated that

COREXIT exposure produced an increase in L-type voltage-gated calcium channels in select brain regions. Similar changes in cardiac or peripheral vascular tissues may also affect heart rate, blood pressure, and peripheral vascular sensitivity to modulatory inputs. Additional studies examining the effects of COREXIT on oxidative activity and neurogenic influences need to be performed to determine whether these mechanisms underlie the alterations seen in these studies.

These studies are the first to demonstrate that inhalation of COREXIT exerts effects on the cardiovascular system. This study assessed the influence of a single exposure to the dispersant, and demonstrated that there are transient effects on the cardiovascular system. These acute changes in HR and BP and vascular functions may have contributed to the headaches and dizziness reported by workers (Chen et al. 2010; Karadzinska-Bislimovska et al. 2010). However, individuals living in the Gulf region most likely experienced repeated exposures to COREXIT. Repeated exposures may potentially result in prolonged changes in cardiovascular function and ultimately contribute to the development of hypertension and other cardiovascular diseases (Bartolomucci et al. 2009; Granger et al. 2010; Mizuno et al. 2010). Additional research examining the effects of repeated exposures to COREXIT needs to be undertaken to determine potential adverse health risks of this dispersant.

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