Biological effects of inhaled crude oil vapor. III. Pulmonary effects

Protocol and Methodology Details

Animals

All studies were conducted in facilities accredited by AAALAC International, were approved by the Institutional Animal Care and Use and were in compliance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals and the NIH Guide for the Care and Use of Laboratory Animals. Male Sprague-Dawley rats were free of viral pathogens, parasites, mycoplasm, Helioptacter and cilia-associated respiratory bacillus. Animals were acclimated for one week and provided tap water and autoclaved chow ad libitum under controlled light cycle and temperature conditions.

Crude oil vapor

The crude oil used in this investigation to generate vapor was reference material associated with the 2010 Deepwater Horizon spill from the Macondo Well in Mississippi Canyon Block 252, i.e., “surrogate oil” that is similar to the Macondo Well crude oil.

Crude oil vapor inhalation exposures

Rats were placed in a whole-body exposure chamber and exposed either to a single 6-h inhalation exposure to 300 ppm total volatile organic compounds (VOC)(acute exposure) or to a sub-chronic exposure to 300 ppm total VOC 6 h/d, 4 d/wk for 4 wks. Control animals were exposed to filtered air. End points were measured at 1 d, 28 d, and 90 d post-exposure. Total VOC concentration was measured in real time and adjustments were automatically made to maintain a constant total VOC level.

In vivo pulmonary mechanics

The purpose of these experiments was to determine the effects of COV inhalation on pulmonary mechanics. Pulmonary input impedance was assessed using a small animal ventilator system permitting forced oscillation in anesthetized rats for the measurement of respiratory system resistance (Rrs), elastance (Ers), tissue damping (G), tissue elastance (H), Newtonian resistance (Rn) and hysteresivity ($\eta$).

In vivo airway reactivity to MCh

The purpose of these experiments was to determine whether COV inhalation affects airway reactivity to inhaled methacholine (MCh), a bronchoconstricor. After anesthesia rats a cannula was advanced into the trachea lumen and placed into a plethysmograph to measure lung resistance (RL) and compliance (Cdyn). After recording baseline values of RL and Cdyn and delivery of saline vehicle as a baseline, aerosols of increasing MCh concentration were delivered by inhalation. RL and Cdyn responses to MCh were logged. Dose-response curves were
In vitro reactivity to MCh: isolated, perfused trachea

The purpose of these experiments was to determine whether airway smooth muscle reactivity to MCh and the modulatory effect of the airway epithelium on reactivity were altered by COV inhalation using the isolated, perfused trachea preparation, which is used to measure contractile responses of airway smooth muscle to MCh and epithelium function. Dose-response curves for MCh were constructed. Contractions to MCh were not affected by COV exposure of rats, but the epithelium acquired greater inhibitory activity.

Electric field stimulation (EFS) of effector nerves

In the rat, airway diameter is under the regulation of cholinergic motor nerves. The purpose of these experiments was to ascertain whether COV exposure affected effector nerve function by eliciting excitatory neurotransmitter release with EFS. Rats were given an overdose of anesthetic and euthanized by exsanguination. A segment of trachea was removed from anesthetized rats and prepared for in vitro measurement of contractile responses of smooth muscle in response to EFS delivered by passing electric current across the tissue. Frequency-response curves were constructed.

Epithelial ion transport in isolated tracheal segments

In order to ascertain whether exposure to COV interfered with airway epithelial ion transport, after sacrifice, tracheal segments were mounted in Ussing chambers in order to measure transepithelial potential difference (Vt; mV), transepithelial resistance (Rt), and short-circuit current ($I_{sc}$).

To investigate the possible involvement of COV-induced changes in epithelial Na+ and Cl− channels and the Na+,K+-pump, the effects of the following inhibitors were evaluated: apical amiloride to block apical membrane Na+ channels, apical 5-nitro-2-(3-phenylpropylamino) benzoic acid to block apical membrane Cl− channels, and ouabain to block the basolateral membrane Na+,K+-pump. The responses to these agents were quantified with regard to their effects on $I_{sc}$ and Rt.

Measurement of vascular permeability using Evans blue dye

The purpose of these experiments was to investigate the effects of COV exposure on vascular permeability of airway blood vessels using capsaicin-induced Evans blue dye extravasation. Evans blue dye binds tightly to albumin after i.v. injection and appears in extravascular tissues if vascular permeability is increased; Evans blue normally remains in the vascular compartment after it is injected intravenously.

Following anesthesia, Evans blue dye was infused i.v. followed by an i.v. injection of capsaicin. In the acute COV study, control animals did not receive capsaicin; in the sub-chronic study, control animals received capsaicin to determine if basal permeability was affected by COV. After capsaicin was given, the rat was exsanguinated, and the thoracic aorta was catheterized retrogradely. The animal was perfused with saline to flush excess dye from the
bronchial and pulmonary vasculature. The trachea, intrapulmonary airways and lung were removed, the Evans blue was extracted chemically, and the Evans blue level was measured in a spectrophotometer.