ORGAN TOXICITY AND MECHANISMS

J. Y. C. Ma · M. W. Barger · A. J. Kriech
V. Castranova

Effects of asphalt fume condensate exposure on acute pulmonary responses

Received: 3 January 2000 / Accepted: 22 May 2000 / Published online: 22 August 2000
© Springer-Verlag 2000

Abstract Objective: The present study was carried out to characterize the effects of in vitro exposure to paving asphalt fume condensate (AFC) on alveolar macrophage (AM) functions and to monitor acute pulmonary responses to in vivo AFC exposure in rats. Methods: For in vitro studies, rat primary AM cultures were incubated with various concentrations of AFC for 24 h at 37 °C. AM-conditioned medium was collected and assayed for lactate dehydrogenase (LDH) as a marker of cytotoxicity. Tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1) production were assayed in AM-conditioned medium to monitor AM function. The effect of AFC on chemiluminescence (CL) generated by resting AM or AM in response to zymosan or PMA stimulation was also determined as a marker of AM activity. For in vivo studies, rats received either (1) a single intratracheal (IT) instillation of saline, or 0.1 mg or 0.5 mg AFC and were killed 1 or 3 days later; or (2) IT instillation of saline, or 0.1, 0.5, or 2 mg AFC for three consecutive days and were killed the following day. Differential counts of cells harvested by bronchoalveolar lavage were measured to monitor inflammation. Acellular LDH and protein content in the first lavage fluid were measured to monitor damage. CL generation, TNF-α and IL-1 production by AM were assayed to monitor AM function. Results: In vitro AFC exposure at <200 μg/ml did not induce cytotoxicity, oxidant generation, or IL-1 production by AM, but it did cause a small but significant increase in TNF-α release from AM. In vitro exposure of AM to AFC resulted in a significant decline of CL in response to zymosan or PMA stimulation. In the in vivo studies showed that AFC exposure did not induce significant neutrophil infiltration or alter LDH or protein content in acellular lavage samples. Macrophages obtained from AFC-exposed rats did not show significant differences in oxidant production or cytokine secretion at rest or in response to LPS in comparison with control macrophages. Conclusions: These results suggest that: (1) in vitro AFC exposure does not adversely affect cell viability or induce the release of high levels of inflammatory cytokines or oxidants; and (2) exposure of rats to AFC did not cause acute pulmonary inflammation or injury, and did not significantly alter AM functions.

Key words Paving asphalt · Asphalt fume condensate · Alveolar macrophage · Pulmonary inflammation · Lung injury

Introduction

Asphalt fumes are complex mixtures of particulate and organic compounds and may pose a potential health risk to exposed workers. Approximately 300,000 workers are currently employed in the asphalt paving industry in the U.S. (Miller and Burr 1996). The current concern for these workers includes both dermal exposure to asphalts and inhalation exposure to asphalt fumes. These fumes consist of an inorganic part, dust from the mineral aggregates, and a highly complex mixture of paraffinic and polycyclic aromatic hydrocarbons (PAHs) and heteroatomic compounds containing sulfur, nitrogen, and oxygen (King et al. 1984). Due to the presence of PAHs in asphalt, most studies of the health hazards for asphalt exposure have been centered on carcinogenicity (Machado et al. 1993; Qian et al. 1998, 1999; Sivak et al. 1997), while very few studies have been devoted to characterization of the effects of asphalt fumes on non-cancerous responses in the lung.
Asphalt workers (road workers and roof layers) have reported symptoms of mucous membrane and skin irritations, but decreased respiratory function has not been demonstrated (Nyqvist 1978; Waage 1987). Norseth et al. (1991) have reported a correlation between subjective symptoms and asphalt temperature or asphalt fume concentration among road repair and construction asphalt workers. Other nonmalignant pulmonary effects, such as bronchitis, emphysema, and asthma have been reported among roofers (Hammond et al. 1976), mastic asphalt workers (Hansen 1991), and California highway workers (Maizlish et al. 1988). Recently NIOSH have conducted health assessment evaluations at several locations and showed an increased incidence of mucous membrane irritation among road pavers (Almaguer et al. 1995; Hanley and Miller 1994a, b; Kinnes et al. 1996; Miller and Burr 1995, 1996), with a few pavers exposed to a crumb rubber asphalt mix exhibiting a work-shift decline in peak expiratory flow rate (Kinnes et al. 1996; Miller and Burr 1996). However, Gamble et al. (1999) have reported that there is no consistent association between an acute reduction in lung function or the incidence of symptoms among workers exposed to asphalt fumes. The few studies reported suggest that paving asphalt fumes may exhibit pulmonary toxicity. At present, there is a serious lack of biochemical and physiological information related to the mechanisms involved in such asphalt fume-induced lung injury.

The respiratory system is considered a prime target for potential adverse effects of exposure to asphalt fumes. Alveolar macrophages (AM) play an important role in the response of the lung to inhaled environmental particles or fumes. AM are responsible for the clearance of particles and microorganisms from the distal airways and the alveolar spaces. Activated AM are known to release reactive oxygen species (ROS) and a wide variety of mediators. The ROS produced by AM are highly reactive and can interact with biological membranes to cause cell damage. Proinflammatory cytokines such as interleukin-1 (IL-1) and tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) are produced by activated AM and promote the inflammatory process by recruiting polymorphonuclear leukocytes (PMN) into the airspaces and stimulating these phagocytes to release ROS and enzymes (Laskin and Pendino 1995; Le and Vileck 1987). Activation of AM is believed to play a key role in the development and progression of pulmonary disease in response to particles such as silica or asbestos. Inhalation exposure to organic substances, such as diesel exhaust particles (DEP), has also been shown to induce pulmonary inflammation and proinflammatory cytokine production (Yang et al. 1999b). However, the depressive effect of DEP on AM responsiveness to microbial products differs from that of carbon black, particles which have a carbonaceous core similar to that of DEP but contain a markedly lower amount of adsorbed organic compounds, suggesting that the organic components adsorbed on DEP may play an important role in modulating macrophage antimicrobial function (Yang et al. 1997, 1999a, b). These studies have shown that DEP exposure suppresses proinflammatory cytokine secretion by AM in responses to LPS stimulation, which may be a contributing factor to the increased susceptibility to pulmonary infection after prolonged DEP exposure (Castranova et al. 1985; Hahon et al. 1985).

It has been shown in our laboratory as well as others that the inflammatory response of the lung on exposure to occupational or environmental agents can be readily demonstrated by analysis of bronchoalveolar lavage fluid (BALF) of the exposed animals (Driscoll et al. 1990; Ma et al. 1999; Yang et al. 1999b). Cellular changes in BALF during acute inflammation include an influx of PMN and activation of AM. The protein content and cytoplasmic enzyme activity, e.g. lactate dehydrogenase (LDH), in acellular BALF provide important information concerning the degree of acute lung injury. Secretion of oxidants and cytokines from harvested AM are important markers of macrophage functions. To date the effects of exposure to asphalt fumes on pulmonary inflammation and lung injury have not been elucidated. The present study was designed to evaluate the acute pulmonary responses to paving asphalt fume condensate (AFC) exposure. The objectives of this study were to investigate: (1) the effects of in vitro exposure to AFC on AM functions; and (2) the effects of in vivo exposure of rats to AFC on acute pulmonary inflammation and lung damage.

### Materials and methods

**Asphalt fume condensate**

AFC was collected at the top of a paving storage tank (at Asphalt Materials Indianapolis, Ind.) by cold trap using the same pumps and traps as used in the laboratory fume generator described by Sivak et al. (1997). The paving asphalt was a PG 64-22 used on the I-65 (1997) project collected at 160 °C. AFC stock solution was made up in DMSO and subsequently diluted with medium or buffer to the desired final concentration with a final DMSO concentration of <1%.

**Treatment of animals**

Male Sprague-Dawley rats (about 250 g) were obtained from Hilltop Laboratories (Scottsdale, Pa.). Rats were acclimatized for 1 week in an AAALAC approved animal facility before use. For in vivo AFC exposure studies, animals were lightly anesthetized with sodium methohexitol (Brevitol; Eli Lilly Co., Indianapolis, Ind.). Once the rats were anesthetized, intratracheal (IT) instillation was performed. The experimental design consisted of three treatment groups: (1) a saline-treated group that received an IT instillation of 0.25 ml sterile saline (control); (2) an AFC-treated group that received an IT instillation of 0.1 mg, 0.5 mg, or 2 mg AFC in 0.25 ml sterile saline; and (3) a solvent control group that received an IT instillation of 0.25 ml 1% DMSO which was equivalent to the final DMSO concentration contained in a 2-mg AFC sample. Rats from each treatment group were killed either 1 day or 3 days after exposure. To determine the cumulative exposure effects on animals, another set of rats received IT instillations for three consecutive days and were killed the following day.

**Isolation of alveolar macrophages**

Animals were anesthetized with sodium pentobarbital (0.2 g/kg body weight) and exsanguinated by cutting the renal artery.