TOXICOLOGICAL HIGHLIGHT

To Breathe or Not to Breathe: Negative Data on Ozone and Vascular Function in an Established Research Model

Matthew J. Campen

Department of Pharmaceutical Sciences, University of New Mexico, 1 University of New Mexico MSC09 5360, Albuquerque, New Mexico 87131-0001

To whom correspondence should be addressed. Fax: (505) 272-6749. E-mail: mcampen@salud.unm.edu.

Received July 29, 2013; accepted July 29, 2013

In speaking with people outside of the air pollution field, the question often arises “How bad is air pollution where I live?” and my first response is invariably, “Well, don’t stop breathing …” (followed by sage medical and toxicological advice, naturally). The article by Barath et al. (2013) in this issue of Toxicological Sciences presents largely negative vascular outcome data from a well-executed clinical exposure study with ozone. Scientists at the Universities in Edinburgh and Umeå have a decade-long history of conducting rigorous clinical assessments of vascular consequences of inhaled pollutants, including diesel emissions, concentrated particulate matter (PM), and nitrogen dioxide (NO₂; Mills et al., 2007; Langrish et al., 2010; Törnqvist et al., 2007). The present randomized double-blind crossover study examines the impact of inhaled ozone at 0.3 ppm for 2h in healthy human subjects. Similar to exposure to nitrogen dioxide (Langrish et al., 2010), another highly reactive gaseous pollutant, ozone induced negligible changes in most outcomes measured, including acetylcholine-mediated vasodilation and release of cytokines, and possibly improved vasodilation at certain time points. Although numerous recent studies demonstrate or infer a potential cardiovascular impact of ozone, the present study gives pause to this assertion and forces the field to reconsider the weaknesses of animal work (high concentration, vulnerability) and population studies (delineating causality among covarying components of complex mixtures).

The high concentrations typically used in rodent studies are frequently justified by the relative species insensitivity owing to more complex turbinates in the nares and a different anatomical bifurcating pattern of the airways (humans would asphyxiate if they attempted to sniff all day long like rats; Wiester et al., 1996). Recent rodent studies with concentrations from 0.5 to 1.0 ppm ozone have revealed substantial vascular impacts. Chuang et al. (2009) observed endothelial dysfunction in C57BL/6 mice exposure for 5 days to 0.5 ppm ozone for 8h/day. Additionally, ozone-related loss of antioxidant capacity and increased mitochondrial DNA damage was observed in both mice and nonhuman primates. More recently, a single exposure to a higher concentration (1 ppm × 4h) replicated such endothelial dysfunction and moreover showed that the effects are likely carried in the circulation, as serum from exposed mice induced vasorelaxation impairments in a CD36-dependent but pulmonary inflammation–independent manner (Robertson). Interestingly, in this study ozone exposure improved endothelial-independent vasodilation (i.e., smooth-muscle-cell response to a direct nitric oxide donor), similar to some of the findings by Barath et al. (2013). Observing the circulating vasoactive factor/s—generated by a pollutant that reacts out in the surfactant layer of the lung and penetrates no more than 0.1 micron into biological fluids (Pryor)—may help explain other systemic effects of ozone, such as exacerbated acetaminophen liver toxicity (Aibo et al., 2010) or hippocampal oxidative stress (Rivas-Arancibia et al., 2010). Additionally, the identification of a circulating vasoactive factor confirms earlier work on serum obtained from humans exposed to NO₂, wherein endothelial cells incubated with serum obtained after exposures induced inflammatory activation, characterized by adhesion molecule and chemokine upregulation (Channell et al., 2012).

Very few published controlled clinical exposure studies have examined the cardiovascular impact of ozone. A small cohort exposed to 0.12 ppm ozone revealed no changes in heart rate and only a slight, transient increase in T-wave alternans, an electrocardiographic index of arrhythmic risk (Kusha et al., 2012). Devlin et al. (2012) from the U.S. Environmental Protection Agency recently observed physiologically modest but statistically significant changes in heart rate variability, certain circulating cytokines, and serum coagulation indices following 0.3 ppm ozone for 2h. Of importance, many of the significant findings occurred 18h after exposures. Similarly, many of the rodent studies examined systemic outcomes in this time frame (Chuang et al., 2009; Robertson et al., 2013), whereas the current study by Barath et al. (2013) opted for more acute outcome assessments at up to 6-h postexposure.
However, the relatively high levels in these controlled exposure studies do little to bridge with epidemiologically observed associations at environmentally relevant ozone concentrations. Ozone has been associated with increased carotid intima-media thickness in a cohort of young adults wherein the average ambient concentrations of ozone were 0.023±0.005 ppm (Breton et al., 2012). In examining the associations between multiple pollutants and circulating markers of cardiovascular inflammation, Bind et al. (2012) found ozone to be associated with circulating C-reactive protein and intracellular adhesion molecule-1. However, in this study, the mean ambient ozone levels were 0.024 ppm with the highest 95th percentile only achieving 0.049 ppm. These are just two recent examples but serve to emphasize how far the short-term rodent studies are from explaining epidemiological associations. For reference, the current U.S. National Ambient Air Quality Standard is 0.075 ppm averaged for an 8-h period; efforts to lower this to 0.065 ppm have stalled in the current administration. The European Commission has set a standard at 120 \( \mu g/m^3 \) (0.057 ppm). Although a number of regions in the United States and Europe will exceed these standards, they will come nowhere near the levels used in rodent and human controlled exposure experiments. The associative nature of the linkages between ambient O\(_3\) and vascular outcomes, however, must be more critically considered in light of the present controlled exposure study.

Although most scientists end the discussion section of manuscripts with the eminently self-serving conclusion that “further research is warranted,” a careful consideration should be made for the case of negative findings of environmental hazards. Were the findings negative because the model and/or outcomes were not adequately sensitive or because there is really no health concern? Healthy male subjects are certainly not representative of the overall population by any stretch, but diesel emissions have routinely elicited impairments in endothelial function in similar cohorts stemming from the Umeå/Edinburgh collaborations. Interestingly, “sensitive” subjects with diagnosed coronary artery disease did not exhibit vasodilatory alterations with the same exposure to diesel that elicited effects in healthy volunteers, although the electrocardiographic findings during a stress test were certainly remarkable (Mills et al., 2007). Future studies on ozone and vascular should carefully consider whether the motivation is really an important biological research question or whether the motivation is biased toward “seeing effects.”

It must be clearly understood that respiratory health effects, with specific attention to sensitive populations (asthmatics), drive the current regulatory policy for ozone and not cardiovascular toxicity, which factors prominently in particulate matter standards. The studies from the past few years by investigators at Umeå and Edinburgh underpin many regulatory decisions by establishing biological plausibility for particulate matter air pollution and vascular disease. Given their present results with regard to ozone and human health effects, the emphasis for ozone regulatory justifications will remain on the respiratory side. Regardless of whether one worries about respiratory or cardiovascular health, the advice of “don’t stop breathing” remains a good idea, but it does not hurt to stay indoors during peak hours of those high ozone days.

REFERENCES


