Analysis of Short-Term Effects of World Trade Center Dust on Rat Sciatic Nerve

Mark Stecker, MD, PhD, Jacqueline Segelnick, and Marc Wilkenfeld, MD

Objective: The purpose of this study was to investigate the short-term effects of residual dust from the World Trade Center (WTC) on rat sciatic nerve.

Methods: Nerve action potentials were recorded in nerves exposed to dust from the WTC as well as control nerves. Results: There was a reduction in the conduction velocity of nerves exposed to a high concentration of the dust from the WTC when compared with controls. Conclusions: Although there are statistically significant reductions in conduction velocity when exposed to the WTC dust in this pilot study, additional studies both clinical and basic will be needed to further understand the significance of these results.

The World Trade Center (WTC) Medical Monitoring and Treatment Program serves more than 67,000 patients, many of whom, as a result of the attack of 9/11, are suffering from a wide range of health problems. These include respiratory, gastrointestinal, and sinus issues. A list of covered conditions has been created by the National Institute for Occupational Safety and Health, and only those conditions on the list can be treated through the program. Since the inception of the program in 2011, additional conditions have been added to the list of covered conditions following the submission of petitions with scientific evidence showing causation.

Anecdotally, there has been an increase in patients experiencing neuropathy, and given the wide range of neurotoxins responders were exposed to it is likely that the neuropathy is directly related to dust exposure post-9/11. There have been no epidemiologic studies of neuropathy in responders, and the incidence of neuropathy in this group is unknown. We hypothesize that neuropathy might be caused by some of the components of the dust that are known to be neurotoxic. Previous studies exist demonstrating the toxic properties of the dust from the WTC. Data from the study by Lioy et al indicate the numerous toxins that were present in the dust, including arsenic, lead, lithium, thallium, and numerous hydrocarbons. All of these substances are known to cause neuropathy. We report on a pilot investigation to determine whether there are any short-term effects of residual dust from the WTC collapse on a rat sciatic nerve in vitro model.

METHODS

The in vitro sciatic nerve model is described in previous papers. The nerve contains both sensory and motor fibers. This model has been used to study the response of peripheral nerve to anoxia, hypothermia, hyperthermia, and hyperglycemia. Under a protocol approved by the Institutional Animal Care and Use Committee (Winthrop University Hospital Protocol, WUH-MS#1), a total of six nerves from three Sprague-Dawley rats (Hilltop, Scottsdale, PA) were exposed to dust from the WTC. The data from these exposed nerves were compared with appropriate control nerves as detailed below. Each sciatic nerve was dissected and placed into a perfusion chamber and stimulated using stainless steel subdermal electrodes. The stimulus consisted of paired unipolar pulses separated by 4 ms, each with a 15 mA peak current, a duration of 0.01 ms, and an overall pair repetition rate of 5 Hz. Bipolar recordings of the nerve action potential (NAP) were made and digitized at 99 kHz. After 20 recordings were made the average was stored. The perfusate temperature was maintained at 36°C. The perfusion solution (“artificial cerebrospinal fluid” or aCSF) was composed of 10 mM HEPES, 110.2 mM NaCl, 17.8 mM NaHCO3, 4.0 mM MgSO4, 3.9 mM KCl, 3.0 mM KH2PO4, and 1.2 mM CaCl2 as in previous studies. The dextrose concentration was 100 mg/dL. pH at the beginning of the experiment averaged 7.4 and was measured before and after each experiment and did not vary by more than 0.15.

There were two types of experiment. During the stability experiments, the nerve remains perfused by oxygenated solutions throughout the experiment. In the anoxia experiments, after a 30-minute equilibration period, the nerve was subjected to 90-minute periods of full oxygenation followed by 90 minutes of complete anoxia. In these experiments, each nerve is subjected to five cycles of oxygenation/anoxia. Each experiment was then broken into phases, such that during phases 2, 4, 6, 8, and 10 the nerve was anoxic and in phases 3, 5, 7, 9, and 11 the nerve was allowed to recover from anoxia being exposed to a fully oxygenated perfusate. Phase 1 is the baseline oxygenated state prior to the first period of anoxia. Although there is no anoxia during the stability experiments, the phase designations described above are used to describe the corresponding times in these experiments as well.

Dust from the WTC collapse was obtained from the study by Dr. Lioy. This dust was collected by Dr. Lioy in Lower Manhattan in the days following 9/11. The dust has been stored since that time for use in studies, which have been conducted by various researchers. The constituents of the dust are the same that 9/11 responders would have been exposed to. Three separate studies were performed. In the first study, in which the nerve remained fully oxygenated, 1 g of dust was placed in 10 cc of perfusate (aCSF) and agitated on a tube rotator for 24 hours. The solution was then centrifuged at 4000 rpm for 10 minutes, and the supernatant was removed and mixed with additional aCSF to obtain a total volume of 125 cc. In this condition, two nerves were exposed to the dust and results were compared against eight nerves that were not exposed to the dust. For the second study, in which the nerve was exposed to anoxia, the preceding weights and volumes were doubled (2 g in 20 cc—agitated for 24 hours—supernatant removed and mixed to a total volume of 250 cc aCSF) because two reservoirs were needed—one to deliver oxygenated and one to deliver deoxygenated perfusate. The responses of the nerves from these experiments were then studied and compared with an existing database of 27 nerves studied according to the same protocol without the WTC dust.

From the Department of Neurosciences (Dr. Stecker) and Division of Occupational and Environmental Medicine (Dr. Wilkenfeld), Winthrop University Hospital; and Queens College and Winthrop University Hospital (Ms. Segelnick), Mineola, N.Y. We declare no conflict of interests and no funding has been provided for this study.

Address correspondence to: Marc Wilkenfeld, MD, Division of Occupational and Environmental Medicine, Winthrop University Hospital, Mineola, NY 11530 (mwilkenfeld@winthrop.org).

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DOI: 10.1097/JOM.0000000000000296
10 mL of methanol was then added. This methanol/dust mixture was agitated for 10 minutes followed by centrifugation at 4000 rpm for 10 minutes. The methanol supernatant was then mixed with the original supernatant and, as previous, this was then mixed with ACSF to obtain a total volume of 125 cc. The responses from two nerves exposed to this solution were compared with four nerves exposed to a similar amount of methanol but no WTC dust.

The NAP peak-to-peak amplitude, conduction velocity, duration, and the area under the curve (AUC) for the response to the first stimulus are the primary measured parameters, which are abstracted automatically with manual supervision. The ratio of the peak-to-peak amplitude of the conditioned (second) response to the unconditioned (first) response for each stimulus is recorded as the conditioned stimulus response. To compare the results from different nerves, the values of each of the abstracted parameters are normalized so that their mean values in the baseline oxygenated state (phase 1) are equal to 1.

The statistical analyses are based on repeated-measures analysis of variance. These results are described by the repeated-measures variables CYCLE and TYPE and the between-measures variable DUST. The variable CYCLE refers to the data from specific time points during the experiment. In the anoxia experiments, each cycle refers to the period of time of reoxygenation following a period of anoxia. For example, cycle 1 refers to data from phase 3, cycle 2 refers to data from phase 5, etc. In the experiments where the nerve remained continuously oxygenated, the data from each cycle refer to the same time periods referred to above. The normalized values of the five measurements—amplitude, velocity, duration, conditioned stimulus response, and AUC—are described by the variable TYPE. Although the peak-to-peak amplitude of the NAP and the AUC are defined in all conditions, the other variables cannot be measured when there is no NAP and so are coded as missing data in these cases. The variable DUST is 1 if the nerve was exposed to the WTC dust and 0 if not. On the basis of the graphs of the data, a separate analysis of any NAP parameters that seemed significant was undertaken.

Analyses were performed separately for each of the three experiments. Although the number is relatively small, in previous publications, only small numbers were needed to find significant effects.6–8

RESULTS

Stability—No Methanol

There is no statistically significant effect of DUST or any significant interactions of DUST with TYPE or CYCLE or TYPE*CYCLE. Figure 1 shows that there may be an effect of the dust to lower peak-to-peak amplitudes and to lower velocities over time during the experiment. This effect is, however, not statistically significant because of the small number of nerves studied (Table 1).

Anoxia—No Methanol

Figure 2 and Table 2 again show no effect of DUST or interaction with other variables, but this is limited by the small number of nerves studied. There is a suggestion of lower velocities in the DUST nerves.

Stability—With Methanol

Figure 3 shows that only for the velocity does there seem to be an effect of the dust with lower velocities in the nerves exposed to the WTC dust. Table 3 suggests that this difference, however, is not statistically significant when combining all the measurement types. Nevertheless, if a repeated-measures analysis of variance is performed using only the normalized velocity measurements, there

FIGURE 1. Changes over time in five NAP parameters in continuously oxygenated peripheral nerves that were and were not exposed to the WTC dust. No significant differences are noted. AUC, area under the curve; CSR, conditioned stimulus response; WTC, World Trade Center.
is a statistically significant effect of the DUST (Table 4), CYCLE, and DUST*CYCLE.

**DISCUSSION**

These preliminary results have shown that, with limited numbers of tests, there is no statistically significant effect of the WTC dust on nerve conduction in the in vitro model when the dust was dissolved in the artificial cerebrospinal fluid solution when the nerves were either continuously oxygenated or subjected to intermittent anoxia. Nevertheless, the NAP velocity was significantly lower in continuously oxygenated nerves in which a high concentration of dust was extracted with methanol compared with nerves exposed to the same amount of methanol without dust. No significance was seen when all of the parameters were mixed but in that case the variation in each parameter reduces the power of the test. In the limited number of nerves studied, this effect is especially significant.

**TABLE 1. Summary of Analysis of Variance Results for Continuously Oxygenated Nerves—No Methanol Extraction**

<table>
<thead>
<tr>
<th>Variable</th>
<th>df</th>
<th>F</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>DUST</td>
<td>1,8</td>
<td>0.264</td>
<td>0.621</td>
</tr>
<tr>
<td>TYPE</td>
<td>4,32</td>
<td>5.117</td>
<td>0.003</td>
</tr>
<tr>
<td>CYCLE</td>
<td>4,32</td>
<td>15.355</td>
<td>&lt;0.001</td>
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<tr>
<td>TYPE*DUST</td>
<td>4,32</td>
<td>0.360</td>
<td>0.835</td>
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<tr>
<td>CYCLE*DUST</td>
<td>4,32</td>
<td>0.582</td>
<td>0.678</td>
</tr>
<tr>
<td>TYPE*CYCLE</td>
<td>16,128</td>
<td>10.386</td>
<td>0.000000</td>
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<tr>
<td>TYPE<em>CYCLE</em>DUST</td>
<td>16,128</td>
<td>1.085</td>
<td>0.376</td>
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</table>

**TABLE 2. Summary of Analysis of Variance Results for Intermittently Anoxic Nerves—No Methanol Extraction**

<table>
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<th>Variable</th>
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<tr>
<td>DUST</td>
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<td>0.5407</td>
<td>0.470</td>
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<tr>
<td>TYPE</td>
<td>4,88</td>
<td>30.3603</td>
<td>&lt;0.001</td>
</tr>
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<td>CYCLE</td>
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<td>13.4176</td>
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<tr>
<td>TYPE*DUST</td>
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<td>0.8211</td>
<td>0.515</td>
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<tr>
<td>CYCLE*DUST</td>
<td>4,88</td>
<td>0.1368</td>
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<td>TYPE*CYCLE</td>
<td>16,352</td>
<td>26.7574</td>
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</tr>
<tr>
<td>TYPE<em>CYCLE</em>DUST</td>
<td>16,352</td>
<td>0.3180</td>
<td>0.995</td>
</tr>
</tbody>
</table>

The fact that only when the dust was extracted with methanol was the velocity lowered indicates that the toxin may be a hydrocarbon as suggested by the dust analysis performed by Lioy, although in these experiments a significantly higher concentration of the dust was used as well. A number of hydrocarbons have been shown to be neurotoxic, especially n-hexane, 2,5-hexanedione, and methyl n-butyl ketone. In these cases, the main electrophysiologic finding involved reduced conduction velocity as shown in this study. Neuropathy can also be induced by other hydrocarbons such as toluene and xylene among others, and as in the case of the n-hexane neuropathies are electrophysiologically characterized by reduced conduction velocity. Of course, in these experiments, the nerves were exposed to a much higher concentration of dust than would be encountered in real situations. Nevertheless, the model studied here is a very acute model in which testing is performed over roughly 16 hours, whereas in real life there is exposure to lower concentrations.

**FIGURE 2.** Changes over time in five NAP parameters in peripheral nerves subjected to intermittent anoxia that were and were not exposed to the WTC dust. No significant differences are noted. AUC, area under the curve; CSR, conditioned stimulus response; WTC, World Trade Center.
FIGURE 3. Changes over time in five NAP parameters in continuously oxygenated peripheral nerves that were and were not exposed to the methanol extracted WTC dust. There seems to be a reduction in the NAP velocity in the nerves exposed to the dust. AUC, area under the curve; CSR, conditioned stimulus response; WTC, World Trade Center.

TABLE 3. Analysis of Variance Results for Continuously Oxygenated Nerves—With Methanol Extraction

<table>
<thead>
<tr>
<th>Variable</th>
<th>df</th>
<th>F</th>
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<tbody>
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<td>CYCLE</td>
<td>4, 16</td>
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<tr>
<td>TYPE*DUST</td>
<td>4, 16</td>
<td>0.967</td>
<td>0.452613</td>
</tr>
<tr>
<td>CYCLE*DUST</td>
<td>4, 16</td>
<td>0.110</td>
<td>0.977282</td>
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<tr>
<td>TYPE*CYCLE</td>
<td>16, 64</td>
<td>4.122</td>
<td>0.000024</td>
</tr>
<tr>
<td>TYPE<em>CYCLE</em>DUST</td>
<td>16, 64</td>
<td>0.263</td>
<td>0.997702</td>
</tr>
</tbody>
</table>

for a very much longer period of time. The higher concentrations would be necessary to see a change over such a short experiment.

Currently, 67,788 responders and survivors of the 9/11 attacks are enrolled in the WTC Health.13 The program was created by Congress and signed into law in 2011 by President Obama to address the numerous health issues that survivors and responders were experiencing.14 The WTC Health Program covers certain diseases, which the National Institute for Occupational Safety and Health has placed on a list of covered conditions.15 The conditions on the list include asthma, chronic cough syndrome, chronic laryngitis, chronic nasopharyngitis, chronic respiratory disorder,16 chronic rhinosinusitis, gastroesophageal reflux disease, interstitial lung diseases, Reactive Airways Dysfunction Syndrome, sleep apnea, upper airway hyperreactivity, and WTC-exacerbated chronic obstructive pulmonary disease.17,18 In addition to these, certain mental health conditions are covered.17 Following the submission and review of additional scientific evidence, a number of cancers were added to the original list of covered conditions.19–21 We believe that the neuropathy observed in a number of responders is related to their exposure post-9/11, and these preliminary in vitro tests suggest that this effect may be due to complex hydrocarbons in the dust. On the basis of these results, further studies are needed to document the short- and long-term effects of the dust on nerve exposure. In addition, epidemiologic studies are needed to document the incidence of neuropathy in responders and determine whether the incidence is higher than observed in the general population.

CONCLUSIONS

The limited data from this acute, in vitro pilot study suggest that poorly soluble hydrocarbons in the WTC dust may have significant effect on nerve conduction velocities. Additional in vitro and epidemiologic studies would be important in confirming these results and determining the nature of the toxin(s) responsible for these effects.

ACKNOWLEDGMENT

The authors thank Dr Paul Lioy, PhD, for providing the WTC dust samples used in this study.

REFERENCES

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