



Neurological Effects after Chemical Nerve Agent Exposures
National Institute of Neurological Disorders and Stroke
National Institutes of Health Bethesda, Maryland
Feb 26-27, 2014

Workshop Summary

Overview

The National Institutes of Health (NIH) Countermeasures Against Chemical Threats (CounterACT; www.ninds.nih.gov/counteract) Program is currently supporting research on the neurological effects of organophosphate (OP) nerve agent and pesticide exposures. On February 26-27, 2014, the NIH held a workshop to explore the availability of human data on the nonlethal effects of OP poisoning, and how these data can be used to develop animal models for testing promising therapeutics. The workshop was attended by clinicians with direct experience in treating victims of OP poisonings, as well as many of the world's top scientific experts on OP poisoning and treatment in animal models. There was a lively and extremely informative discussion of how to develop a meaningful translational research agenda that addresses the difficult problem of studying the nonlethal effects of OP exposure. The meeting was also attended by federal officials in key leadership positions to provide perspective on requirements and activities within the Public Health Emergency Medical Countermeasures Enterprise (PHEMCE). Highlights include breakout sessions on the types of human and animal data known to be available, a qualitative comparison of the human versus animal data, working groups on animal model optimization and research gaps, and highly informative presentations and videos on OP poisonings in humans in Japan and Sri Lanka.



Day 1

General Session I

This session included presentations by leadership from the NIH, U.S. Department of Health and Human Services Office of the Assistant Secretary for Preparedness and Response (ASPR), and the U.S. Food and Drug Administration (FDA). The need for understanding and defining the neurological and other endpoints for nonlethal effects in humans was emphasized, so that appropriate animal models could be

developed based on human data. Currently, data gaps in human effects impede the regulatory process, and part of building a research agenda should include possible prospective clinical studies in humans after an incident or accident involving chemical agents. In addition, the context of use of a potential therapeutic in humans must be simulated to the greatest extent possible in animal models. In this session, the FDA's Medical Countermeasures initiative (MCMi) was also described and a very informative presentation on the FDA's Animal Rule was offered to researchers and managers, which included an introduction to the Animal Model Qualification Program.



The session ended with two overviews. The first was on the effects of OPs on humans, including what has been observed in humans poisoned with OP pesticides or nerve agents. It was noted that for pesticides, the solvents used may influence toxicological outcomes. Also it appears that seizures are more likely to be observed in humans after exposure to nerve agents like sarin, than for OP pesticides. Overall, OPs can cause a variety of effects including deficits in cardiac, immune, and reproductive functions. However,

the most consistent nonlethal effects that should be targeted for animal models are neurologic and if possible, psychiatric. The second session described the wide variety of animal models currently being used to study OP poisoning and potential therapeutics. These range from nematodes to non-human primates, and there is variability among the different species, age, gender, and even strain. There are also differences between the effects observed for OP pesticides and OP nerve agents. The correlation between OP-induced seizures and brain pathology is not as strong in pesticide models as it is with nerve agents. It is clear from the discussion that the availability of such a large and diverse body of literature on animal models is promising; however, selecting from these models the characteristics that best simulate the human condition may be challenging.

Breakout Session Highlights

Evidence for Neurological Effects in Humans

This session was held concurrently with a similar session on animal data. It was designed as a roundtable discussion guided by an information-gathering exercise on human effects and data sources that took place weeks in advance of the meeting. Specific questions and topics prepared in advance of the workshop also facilitated the discussion. Delineating the temporal component of the effects of OPs in humans was identified as a major requirement for understanding which endpoints were most important for animal model development. Another highlight of the discussion was a call for diagnostic techniques capable of discriminating between OP effects and those caused by stress as seen in Post-Traumatic Stress Disorder (PTSD). Genetic studies may also offer a method of verifying whether the effects of OPs are pathologic or psychogenic. Establishing a link between OP exposure and long-term effects, currently a significant data gap in the field, would be facilitated significantly by the development of point-of-care biomarkers of exposure and effects to verify chemical exposures during or soon after a chemical

incident. Useful biomarkers should include not only measures of exposure, but also a signature of the pathology associated with that exposure. Imaging techniques may be beneficial in this regard and may help identify early markers of pathology and inflammation. The potential for collecting valuable data on OP effects in humans from the patients in Japan and Syria was discussed, but this will require fiscal and human resources. Other sources of human data were discussed and these will be investigated.

Evidence for Neurological Effects in Animals

The discussion in this session was facilitated by extensive pre-workshop activities and preparation by participants with expertise in animal models of OP poisoning. During the workshop, many common effects were outlined, some transient when nonlethal, some permanent and observed well after the initial exposure. Examples of some important observations include weight loss as a predictor of lethality, even more so than seizures, and the impact of environmental conditions in the laboratory on the toxicity of OPs. There are only transient effects in non-human primates if they survive a nerve agent exposure; however, they usually die if they have seizures. Interestingly, rodents can show pathology and behavioral changes even in the absence of seizures. A link between anxiety and changes in the amygdala has been observed in OP-exposed rats, thus anxiety may be an endpoint that can be linked to specific neuropathology caused by OPs. In terms of general research needs, there is ample knowledge of toxicity in the acute phase, but little is known about the intermediate phases, and even less on the long-term effects. Indeed, it was observed that the validity of the animal model seems to decrease with increasing time between exposure and outcome measurement.



Comparison of Human and Animal Data

Participants in the human and animal data breakout sessions reconvened and jointly reviewed the discussions from each session to identify points of convergence and divergence. The general audience was invited to participate in this discussion. Each member was given an electronic voting device that was linked to the presentation, such that audience response to specific questions could be recorded in real time. There were several toxicity endpoints that were common to both humans and laboratory animals including: death, seizures, acetylcholinesterase (AChE) inhibition, anxiety, abnormal electroencephalograms (EEGs), and learning and fine motor impairment. However, more than one animal model may be required for exposure paradigms using different types of OPs. Based on the audience voting, it was suggested that one of the most important considerations for bridging the gap between human and animal data is the confirmation of exposure and exposure levels in humans. In terms of extrapolating animal endpoints to human endpoints: anxiety, learning, neuropathology, and *in vivo* brain imaging were seen as promising. But these endpoints will need to be studied carefully before they can be adopted as best practices.

Scientific Seminar and Informal Dinner

A scientific seminar presented by Dr. Byron Ford (Morehouse School of Medicine) concluded day 1 of the workshop. This seminar described studies with the compound neuregulin which is being tested in a rat model for its potential neuroprotective therapeutic activity after exposure to a seizurogenic dose of the OP diisopropyl fluorophosphate. The challenges of developing an animal model and some of the results of the study were discussed. An informal dinner followed at a local restaurant that was attended by over 40 workshop participants where lively discussion continued.

Day 2

General Session II

Welcome from the Assistant Secretary for Preparedness and Response

Dr. Nicole Lurie, HHS Assistant Secretary for Preparedness and Response, provided some important remarks about the overall mission of her office and why the work being pursued in medical countermeasures research is important to the nation. Dr. Lurie stressed the importance of human data and the ability to mount a scientific response after a chemical attack or disaster in order to learn from the incident.

Video and Discussion of Human OP poisonings in Tokyo and Sri Lanka



Dr. Tetsu Okumura (Tokyo Metropolitan Police Agency) presented film and commentary on the terrorist attack using sarin in the Tokyo subway system in 1995. Important observations from this incident were the lack of personal protective equipment worn by many first responders, and the occurrence of secondary exposures after trapped sarin gas was released from the clothing of exposed victims. Reports from Syria indicate that more convulsions and agitated mental status was observed than in Japan; also they did not observe as much running nose and vomiting in Syrian victims. Victims of the Tokyo incident were followed for several years and exhibited anatomical and behavioral changes long after the incident. These patients are no longer being followed due to lack of funding. Dr. Nicholas Buckley (University of New South Wales) then presented statistics and a video of his work in Sri Lanka treating patients for OP pesticide poisonings. These poisonings are a major problem in Sri Lanka and in the Western Pacific and Southeast Asia – there may be as many as 3 million cases per year, of which 10% are fatal. Many are suicide attempts and remain unreported since suicide is illegal in India. These patients exhibit profound salivation, muscle fasciculation and twitching, weakness, and often need intubation. Some of the muscle weakness is characteristic of the OP-induced Delayed Neuropathy (OPIDN) syndrome. Patients in Dr. Buckley's cohort that are being followed but the studies are limited by the number of clinical personnel available for follow-up studies.

Working Groups

Working Group on Animal Model Best Practices

In this session a discussion was led on the possibility of developing a set of optimal characteristics for animal models of nonlethal effects of OPs. There are many different models of lethal effects in use today, but few specifically addressing nonlethal effects. There are many different variables to consider in these models, but it was suggested that a “Common Data Elements” approach, similar to that for other neurological diseases, could be used to standardize some of the models. This does not necessitate the development of a single animal model, but it will facilitate the comparison of data across studies and laboratories. For example, all studies with OPs should determine the time course of AChE inhibition, with a subset of animals used to assess OP levels in the serum. The pros and cons of using specific co-treatments like atropine methyl nitrate were discussed, as well as the best methodology for determining neuropathology. Another important discussion clarified that the animal models developed now will be primarily used for screening studies, and that models for the more pivotal studies needed for regulatory approval may need to be improved as we learn more about the human condition.



Working Group on Research Gaps



This session was driven by audience participation and real-time voting. Specific research gaps in each of six general areas (e.g. Establishing Natural History of Disease, Mechanisms of Toxicity) were provided and discussed briefly before voting. Examples of research gaps that received the highest votes include: 1) understanding the mechanisms underlying the neurological and psychiatric alterations observed after agent exposure; 2) collecting data of indicators of exposure in humans, short and long term; 3) utilization of non-human primate animal models of agent exposure; 4) identification of biomarkers including metabolites after acute agent exposure; 5) identification and characterization of clinically relevant non-cholinergic targets; and 6) the identification in animal models of techniques and/or outcomes that can be translated to humans. A common theme was the paucity of rigorous human data. One suggestion was to develop a prospective registry to collect and follow any new cases of OP pesticide or nerve agent exposures, and to develop a rapid response toolkit that can be mobilized to an incident in the immediate aftermath to collect essential exposure data. A clear case definition for these and other epidemiological studies in humans is needed.

The Way Ahead

In summary, this workshop is the initiation of a concerted effort to document and understand the nonlethal effects of OP nerve agents and pesticides. It is hoped that through these efforts and further research to fill gaps in our knowledge, animal models can be developed that are more predictive of the human condition, and thus, can be used to test promising therapeutics in lieu of unethical and prohibited efficacy testing in humans. The way ahead includes a compilation and analysis of the data provided by workshop participants and others, followed by data mining activities using some of the data sources identified, and comprehensive literature reviews culminating in peer-reviewed articles to be shared with the scientific community.