NEUROPROTECTION FOR NERVE AGENT-INDUCED BRAIN DAMAGE

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SUMMARY

This presentation will explain the rationale behind the neuroprotection Science Plan which has been established at the US Army Medical Research Institute of Chemical Defense as part of the medical chemical defense program. This program attempts to address a need that has not been specifically addressed before in any country, which is specifically to save vulnerable neurons that have been damaged due to seizures secondary to exposure to nerve agents. Preliminary work in this laboratory has demonstrated proof of concept using a compound not yet approved for clinical use by the US Food and Drug Administration. We will continue work with neuropathological correlation and add a neurobehavioral component to the testing program to be able to exploit preparations developed by industry, particularly in neuroprotection for stroke. If successful we will be able to give field physicians a new treatment with an excellent chance of minimizing or preventing neurobehavioral dysfunction after nerve agent poisoning, should primary protection against exposure and acute therapy of exposure both fail and status epilepticus ensue. We intend by presenting this program at this forum to stimulate Canadian and European colleagues to consider work in this area.

PRÉCIS: Cette présentation veut expliquer la rationale fondamentale pour le nouveau Plan Scientifique de la Recherche (STP en anglais) que nous avons commencés a l'Institut de la Recherche Médicale de la Défense Chimique de l'Armée des États-Unis. Ce plan adresse un besoin que, en notre opinion, aucun pays n'a adresse avant que maintenant. Ce besoin est a sauver des neurones qui ont été blesses a cause des convulsions, qui sont, elles-mêmes, a cause de l'exposure aux agents nerveux. Le travail préliminaire complète dans notre laboratoire a montre la preuve de cette idée, mais seulement en employant des agents qui n'ont pas l'approbation de l'Administration des Aliments et des Drogues des États-Unis pour les humains. Nous continuons a travailler avec les agents approbés sur la relation neuropathologique avec l'effet de la traitement. Nous ajoutons du travail sur la relation avec le comportement en une modèle exemplaire animale. Nous voulons employer les préparations (les drogues) qui sont développés par l'industrie, en particulière, pour protéger les neurones du cerveau en cas de l'apoplexie. Si nous succédons, nous donnerions aux médecins militaires un nouveau traitement avec une grande probabilité de minimiser ou prévenir la perte de la fonction nerveuse après l'exposure toxique avec les agents nerveux, en le cas quand la prévention a l'origine et le traitement rapide ont tous les deux échoués, et le sujet est entré en status epilepticus ou les convulsions continuées. En présentant notre programme a cette conférence, nous intentons a stimuler les collègues canadiennes et européennes a considérer du travail en "neuroprotection" elles-mêmes.

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Protection against brain damage produced by exposure to chemical nerve agents is of significant military concern. The currently fielded antidotal therapy for nerve agent poisoning on the battlefield--pretreatment with pyridostigmine bromide, atropine, oxime, and acute anticonvulsant therapy with diazepam--addresses only the acute, life-threatening consequences of nerve agent toxicity. Soldiers surviving initial life-threatening effects of nerve agents are likely to develop electrical seizure activity. Anticonvulsants such as diazepam can arrest chemical agent-induced seizures when administered shortly after seizure onset, but their effectiveness wanes after approximately twenty minutes, allowing seizures to recur. According to the McDonough-Shih hypothesis, this may reflect recruitment of other neurotransmitter systems beyond the original cholinergic crisis. Unless seizures are arrested, the currently fielded therapy does not afford protection against brain damage, specifically, apoptotic change and death of cortical neurons.

It may be practically impossible to distinguish on the battlefield between a casualty who is undergoing nonconvulsive status epilepticus, either because of the distribution of the seizure focus or, more likely, because ATP stores are depleted and thus no movement can be generated, and a casualty who is post-ictal, particularly if the casualty is wearing chemical protective gear. Since neither of these possibilities is associated with the usual movements seen with typical seizures, these victims are not likely to receive anticonvulsant treatment. Left untreated or refractory to anticonvulsants, nerve agent–induced seizures progress to status epilepticus, resulting in extensive neuronal death, particularly in cholinergically rich areas of cortex, and subsequent permanent neurological disability. Experience from the Tokyo subway attack demonstrated that cerebral hypoxia may well complicate this situation, which will worsen any damage to already vulnerable brain neurons. Thus, there is a clear need for a neuroprotective compound that is capable of preventing further neuronal damage, even when seizures have progressed to status epilepticus. Such a compound would greatly increase the window of opportunity to prevent or minimize neurologic dysfunction resulting from chemical agent-induced seizures and would augment the beneficial effects of currently fielded anticonvulsants.

A 1999 report prepared for the US Assistant Secretary of Defense for Health Affairs at the request of Congress concluded that brain damage from exposure to chemical warfare agents is one of the two major neurological threats on the modern battlefield. The other was neurologic damage due to head trauma. To address this problem, a new Science and Technology Plan (STP) for neuroprotection against seizure-related neuronal cell death has been established at the Medical Research Institute of Chemical Defense. The objectives of this STP are to develop an improved medical protection capability against neuronal loss and the resultant decrease in neurologic function resulting from prolonged seizures, status epilepticus and hypoxia caused by organophosphate nerve agents and to demonstrate that in the absence of other therapies, medical protection can be initiated to save neurons that have been subject to the excitotoxic effects of seizure activity. To ensure optimal neurological outcome, a neuroprotectant should have efficacy when administered one or more hours after seizure onset and have minimal detrimental side effects.

In terms of the echelons of medical care in the US military, the requirement for a candidate treatment to be efficacious when treatment is delayed as many as 4 hours is of great importance. By 3-4 hours after exposure and discovery on the battlefield, it is quite likely that a casualty will have been evacuated to what the US military calls Echelon II or III, at which level of care a physician or at least a physician assistant can manage his or her care. Although in the US a non-physician medic cannot use medications independently, a physician can do so. A drug approved for another use by the US Food and Drug Administration could be used "off label" by a physician.

The STP intends to exploit work already ongoing in the pharmaceutical industry for stroke. Unlike nerve agent poisoning, stroke has an enormous economic impact in all advanced countries. In the US it is the third leading cause of death and kills 960,000 people yearly. The

American Stroke Association estimated in 1999 that the direct and indirect cost of stroke care in the US is $45.3 billion. Stroke results in neuronal death in a small area of brain, but also creates a much larger ischaemic penumbra in which neurons are potentially salvageable. The only US Food and Drug Administration-approved acute therapy for stroke today is recombinant tissue plasminogen activator, which must be administered within 3 hours of stroke ictus. If delayed beyond that, outcome actually is worse. As a result, less than 5% of acute strokes in the US are receiving this treatment. Thus, many pharmaceutical companies are investigating neuroprotective compounds for stroke that might have a longer therapeutic window.

Potential stroke neuroprotectants come from many, unrelated drug classes. Classes including at least one product that has reached clinical trials in humans include calcium channel blockers (nimodipine, flunarizine, dextrorphan, selfotel, magnesium sulphate, eliprodil, GV150526); presynaptic glutamate release inhibitors (lubeluzole, fosphenytoin), antioxidants and free radical scavengers (trilazad, ebselen, nitrones), other ion channel inhibitors (clomethiazole, MBQX, GM1 ganglioside, Bay 3702), and some agents directed against delayed injury (BFGF, citicholine, enlimomab, piracetam). Unfortunately, several of these have proven disappointing in recent clinical Phase III trials. It is very likely that the reasons these drugs have not shown clinical effectiveness is that stroke itself are highly heterogeneous, both in terms of pathophysiology and also in terms of neuroanatomy. For example, a drug that acts at any receptor site is not likely to be useful in a white-matter or lacunar infarction, if only because white matter tracts contain few if any synapses. Bogousslavsky and others (Brott T and Bogousslavsky J. Treatment of acute ischemic stroke. New England Journal of Medicine 343:710-722, 2000) point out that, as stroke trials become more successful in isolating subpopulations of stroke by anatomy and physiology, it is much more likely that a putative neuroprotectant agent will prove efficacious than in the heterogeneous stroke populations so far studied. One or more of these compounds may well reach FDA approval in the next few years.

An approved neuroprotectant may prove useful in nerve agent toxicity as well, since in animals who have survived nerve agent challenge many of the neurons undergo apoptosis. Neurons that can be prevented from going into the apoptotic cascade in stroke models may well represent neurons that can be saved after nerve agent-induced status epilepticus, since the mechanism of cell death is identical.

Initial approaches in development of neuroprotectants include poly ADP-ribose polymerase (PARP) inhibitors such as benzamide and 3-amino benzamide. Benzamide showed neuroprotectant efficacy against soman when administered after seizure onset (Meier HL, Ballough GPH, Forster JS and Filbert MG. The poly ADP ribose polymerase inhibitor (PARPI) is neuroprotective against soman-induced seizure related brain damage. In: Trembly B and Slikker W, eds.: Fourth International Conference on Neuroprotective Agents. Annals of the New York Academy of Sciences 890:330-335, 1999). Other potential approaches are the use of scavengers of reactive oxygen species (ROS), such as the naturally occurring alpha lipoic acid, its reduced form dihydrolipoic acid, free radical traps such as nitrones and inhibitors of N-acetylaspartylglutamate (NAAG) peptidase to reduce the formation of glutamate from NAAG.

Neuroprotectant efficacy was demonstrated against soman-induced seizure-related brain damage with a nonpsychotropic cannabinoid, dexanabinol, or HU-211 (Filbert MG, Forster JS, and Ballough GPH. Neuroprotective effects of HU-211 on brain damage resulting from soman-induced seizures. In: Trembly B and Slikker W: Fourth International Conference on Neuroprotective Agents. Annals of the New York Academy of Sciences 890:505-514, 1999). When administered 40 min after onset of seizures and despite having no effect on the severity or duration of the seizure activity HU-211 reduced the lesion volume 70% (Ballough GPH, Cann FJ, Smith CD, Forster JS, Kling CE, and Filbert MG. GM1 monosialoganglioside pretreatment protects against soman-induced seizure-related brain damage. Molecular and Chemical Neuropathology 34: 1-23, 1998).

An interesting finding that has come out of these studies with dexanabinol is that lesion volume as measured at autopsy correlates very highly ($p<0.001$) with relative beta-2 activity on electrocorticography at 60 minutes after seizure onset. This time point coincides with the irreversible effects of intracellular calcium overload and may reflect that absolute window for preventing seizure-related neuronal death. This finding allows one to predict with certainty whether a putative neuroprotectant has indeed protected neurons long before subjecting the animal to sacrifice and full neuropathological examination (Ballough GPH, Jaworski MJ, Filbert MG and Newmark J. Electrocorticographic correlates of dexanabinol-induced neuroprotection following soman-induced status epilepticus. Neurology (suppl. 1), in press, 2001)

These preliminary studies demonstrate proof of concept that medical chemical defense against the delayed effects of nerve agents offers hope of decreased disability to victims of exposure to nerve agents.

We plan this year to begin both neuropathological and behavioral testing of a clinically approved agent, magnesium sulphate solution, which has the further advantage of being absurdly cheap ($0.08/dose). We will use the previous protocol shown above in the case of HU-211 to test the hypothesis that post-exposure treatment of a nerve agent-challenged, seizing animal can reduce the loss of neurons. At the same time, we also will test the ability of this agent to improve the mental functioning of an animal survivor of nerve agent challenge in a behavioral task. Although the first agent tested may not prove useful, this work will, if nothing else, establish these testing protocols as baseline in our laboratory. If either magnesium sulphate solution does prove to be neuroprotective in this situation, we may recommend it to field medical staff immediately for treatment of human nerve agent poisoning survivors with good basis for saying that it will reduce neurologic dysfunction.

How far into the business of research into neuroprotective agents should the military research establishment penetrate? In my opinion, most of the basic science and all of the safety work in new agents will be done by industry. The military research establishment should do that part of the work that industry will not fund, which, in this case, is the actual work with live nerve agents. Further, the military probably will do well to restrict itself to therapeutic agents already approved by the regulatory agency charged in a particular country with approving human drugs; in the US, this is the Food and Drug Administration. It will be extremely difficult to license a new neuroprotectant agent with no other clinical use, but it is not necessary, at least in the US, to get the FDA to approve the use of an already approved agent for a new indication. Although the US military cannot recommend it as a matter of doctrine, physicians are legally entitled to use any licensed product in any fashion "off-label" if there is good data to support its clinical use.

This area has not previously been considered a part of the military research effort into countermeasures for nerve agent exposure. NATO can contribute to this effort since many clinical trials of new neuroprotectants are being carried out in Canada or Europe before they are repeated in the US. One purpose of this presentation is to stimulate consideration of work in this area by those in countries where this has not yet been considered.

NOTE: The opinions expressed herein are solely those of the authors and not necessarily those of the United States Army, the United States Department of Defense, or the United States Army Medical Research Institute of Chemical Defense.