



To date, no finding from this research effort indicates that PB should be employed differently than prescribed in current military doctrine for protection of servicemembers in a chemical environment. Nor does any finding provide an explanation for persistent unexplained symptoms reported by GW veterans.

As part of the DoD Gulf War Illnesses (GWI) research program, 24 peer-reviewed studies totaling \$20 million dollars specifically address health consequences of PB use for nerve agent pretreatment. The studies clarify some effects of PB and its interaction with operational stressors and other chemicals. Information about anti-cholinesterase inhibitory action will advance the development of more effective cholinesterase inhibitors. Other information on previously unexplored cholinesterase actions may advance treatment of head injuries and neurodegenerative diseases.

Background

When given prior to nerve agent exposure, PB binds reversibly to acetylcholinesterase (AChE) and shields some AChE from irreversible binding by nerve agent. Subsequent escape of AChE from its PB bond makes AChE available for continued, controlled modulation of nerves, organs and muscle.

To counter the threat of nerve agent use, PB was used by a large number of servicemembers during the Gulf War. Deployed troops usually reported having taken PB from one to seven days, 30 mg every 8 hours. Reports of ill health attributed to service in the Persian Gulf led the DoD to initiate studies into additional possible side effects of PB.

Current Studies

Exposure Epidemiology: One study correlates medical out-comes recorded in GW veteran medical databases to projected exposures and potential risk factors including PB use.

Genetic Susceptibility: There is a wide variation in whether a person shows side effects from a given dose of PB as well as the specific side effects seen. Some of this is due to individual absorption of the drug, but genetic variability may also play a role. Four studies test the theory that there are subpopulations that are genetically super-sensitive to cholinergic stimulation and that these individuals respond adversely to PB. One study examines genetic variations in subpopulations for anti-cholinesterase agent responses, organophosphate toxicity, and protective variants of AChE. It identified genetic alterations in human subpopulations which make them super-sensitive to cholinesterase inhibitors. Another study investigates genetic

alterations in cholinesterase activity as a cause of GW veterans' symptoms. It asks whether the presence of specific GWI symptoms correlate with chemical damage to nerves due to genetically abnormal or low levels of plasma cholinesterase (BChE) which acts as a scavenger of anticholinesterases (e.g. organophosphates and PB). Reductions in BChE activity or levels would permit more anti-AChE agents to bind to AChE: that is, would result in a higher effective dose of any pretreatment PB. However, study results from self-reporting ill veterans indicate that the GW veterans studied had a lower frequency of abnormal BChE genes compared to frequencies in other studies.

| Effects of Pyridostigmine Pretreatment* | |
|--|------------------------------|
| Effect | Range of Incidence, % |
| Gastrointestinal Symptoms | > 50 |
| Urinary Urgency and Frequency | 5 - 30 |
| Headaches, Runny Nose, Skin Redness, Extremity Tingling | < 5 |
| Need for Medical Visit | 1 |
| Bad Dreams | < 0.1 |
| * Based on reports from medical personnel providing care to 41,650 soldiers (6.5% women) who took pyridostigmine bromide orally at 30 mg every 8 hours for periods of 1 to 7 days. Drug administration resulted in 483 clinic visits, and use of the drug was discontinued in 28 soldiers. | |

Keeler JR, Hurst CG, Dunn MA. Pyridostigmine Used As a Nerve Agent Pretreatment Under Wartime Conditions. JAMA (Aug 7) 1991, 266(5):693-695.

Gender Differences: Results from two studies found only minor adverse effects from PB use, primarily gastrointestinal side effects and headaches. No differences in drug tolerance were found with variation in body weight or gender. However, one study found PB dose and biological actions (pharmacokinetics) were dependent on both gender and body weight, and that the lowest blood levels from the doctrinal dose (trough) did not provide red blood cell AChE inhibition greater than 10%. (Doctrinal dosage is based on 20% to 40% red blood cells AChE inhibition, which provides protection while limiting side effects). A third study reported dose-dependent decreases in motor activity in novel environments, without other signs of toxicity, and this effect was more severe in female animals. Extrapolated to humans, military women may be vulnerable to more side effects from the doctrinal dose than men.

Neurological Effects: PB may stimulate intracellular reactive oxygen generation resulting in nerve cell death. Results of one study show damage in specific brain regions at high doses. Degeneration appears to continue up to 30 days post-dosage. Pretreatment with antioxidant did not protect against PB-induced damage but atropine did block the cell damage.

Associated Psychological Effects: Two studies examine low-level, subchronic exposure to an organophosphorus cholinesterase inhibitor on normal cognitive function. Results indicate that exposure causes abnormalities in central nervous system acetylcholine (ACh) function that may result in impaired cognitive function even after cessation of the exposure. Spatial learning impairments were halted when PB was added to the dosage regimen. The second study will investigate the optimal use of PB by examining individual differences in response to cholinesterase inhibition.

Interactions with Other Chemicals: Two studies hypothesize central nervous system deficits in GW veterans from chemical exposure. One study will develop neurobehavioral-screening tests for neurological damage. Having previously correlated specific GW exposures and three distinct symptom groupings, the other study will enlarge the original sample population to verify previous findings and provide a stronger basis to support identification of anatomic damage and pathophysiologic mechanisms. Seven studies investigate the effect of concurrent exposure to PB, DEET (the primary military insect repellent) and other chemicals or physiological variables (heat, stress, and exercise). One study exam-

ines PB use with permethrin (an insecticide commonly used by the military) and DEET. Results show all compounds decreased behavioral responses in test animals but demonstrated no additive interactions. In laboratory tests of white blood cell proliferation all compounds produced dose dependent immunosuppression: PB showed "minimal" immunosuppression. In tests of interactions, permethrin and DEET altered immune activity more than



permethrin/PB and DEET/PB. A second study will clarify interaction mechanisms and health consequences of exposure to these chemicals and study PB induced blood-brain-barrier changes (a previous study showed that the dose of PB needed to inhibit brain AChE in mice stressed by a forced swim was reduced by 50%; this suggested that PB, in stressed animals, crosses the blood-brain-barrier). The other five studies examine effects from PB, DEET or jet fuel on neurotoxicity and immune function and the effect of PB, DEET and physical stress on the interaction of the nervous and immune systems, but each will look at slightly different variables or test results. Another six studies examine health consequences of PB in combination with low-level sarin (levels with no clinical signs). Results from one of these studies indicate that, in rats given PB and exercise, plasma and muscle cholinesterase activity significantly decreased. Muscle damage enzymes increased 122%, and oxidative stress increased. No electron microscopic changes were seen in nerve tissues (spinal cord and sciatic nerve). One conclusion was that interactive and delayed effects occurred in skeletal muscle and that physical stress amplifies the delayed muscular effects of PB in mice. A new study is designed to test skin absorption and toxicity of chemical mixtures to which GW veterans may have been exposed and determine biomarkers for use in future research and diagnostic applications.

Interaction with
Environmental
Exposure



Detailed information on the status of individual projects may be obtained from the **Annual Report to Congress on Federally Sponsored Research on Gulf War Veterans' Illnesses** at the web site below.

<http://www.va.gov/resdev/report3.htm>

