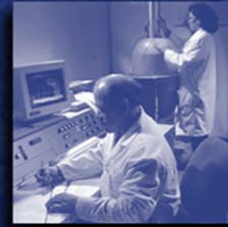


USAMRICD



"Research for the Warfighter"

United States Army Medical Research
Institute of Chemical Defense

Report Documentation Page

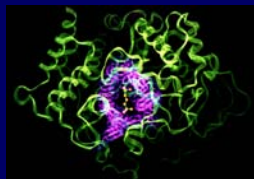
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USAMRICD Product Development

Milestone 0



Chemical Agent Prophylaxis (00)

- Reactive Topical Skin Protectant (00)
- Advanced Anticonvulsants (97)
- Methemoglobin Monitor (97)

Development



Multichambered Auto Injector



TSP - Topical Skin Protectant



M291 - skin decontamination kit

Fielded



MARK I Kit -
antidote for nerve agents



Pyridostigmine bromide -
pretreatment tablets



CANA - antidote for nerve agent

Therefore, based on what we've already fielded and what will be available in the future, we define four research priorities:

- (a) Simplify the approach to nerve agent medical protection by scavenging enzymes
- (b) Develop a specific countermeasure to HD
- (c) Develop and advanced or active TSP
- (d) In the short term, build a better mousetrap to anticonvulsants.

Let us look at each of these strategies in turn>

Biological Scavengers for Nerve Agents

Stoichiometric Scavengers (1:1)

Acetylcholinesterase (AChE)
Butyrylcholinesterase (BChE)
Carboxylesterase (CaE)

Catalytic Scavengers (1: Many)

Organophosphorus hydrolase
Modified AChE
Modified BChE
Modified CaE
Catalytic antibodies

Studies with equine or human BuChE or fetal bovine serum AChE showed that none of these scavengers exhibited behavioral side effects when given alone to rats or monkeys. Furthermore, each was capable of providing protection against 2 to 16 LD50s of GD, GB or VX depending on the scavenger and the test species.

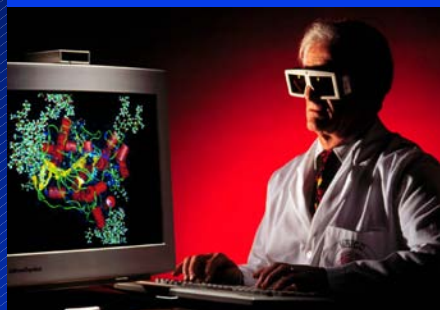
Candidate bioscavenger proteins, in general, function either by stoichiometrically binding and sequestering the anticholinesterase or by catalytically cleaving the OP substrate into biologically inert products. Enzymes such as cholinesterases (ChEs) and carboxylesterases (CaEs), as well as antibodies specific for nerve agent haptens are stoichiometric. Each of these stoichiometric scavengers has the capacity to bind one or two molecules of nerve agent per molecule of protein scavenger. While this approach has been proven to be effective in laboratory animals, it has the disadvantage that the extent of protection is directly proportional to the concentration of unexposed, active scavenger in the bloodstream at the time of nerve agent exposure.

Candidate enzymes with *bona fide* catalytic activity against nerve agents include the human organophosphorus acid anhydride hydrolases (OPAHs), such as paraoxonase (hu-Pon). Additionally, the ability to generate catalytic antibodies in response to appropriate transition state analogs (16, 17) suggests that nerve agent-specific antibodies that catalyze hydrolysis of their ligands could be effective bioscavengers. Finally, the ability to engineer site-specific amino acid mutations into naturally occurring scavenger enzymes can allow investigators to alter the binding and/or catalytic activities of these enzymes. In general, the use of scavengers with catalytic activity would be advantageous because small amounts of enzyme, meaning lower concentrations in circulation, would be sufficient to detoxify both large amounts of nerve agent.

**Defense Technology
Objective (DTO)**

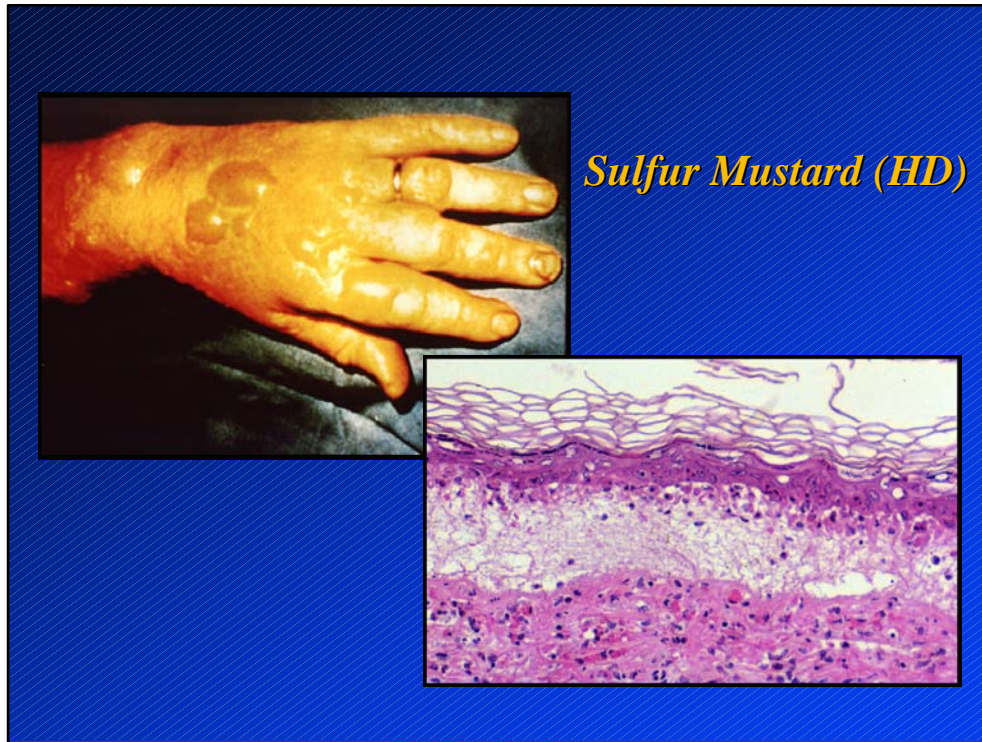
Chemical Agent Prophylaxes

Develop countermeasures that provide protection against CW agents without operationally significant side effects.



Accomplishments:

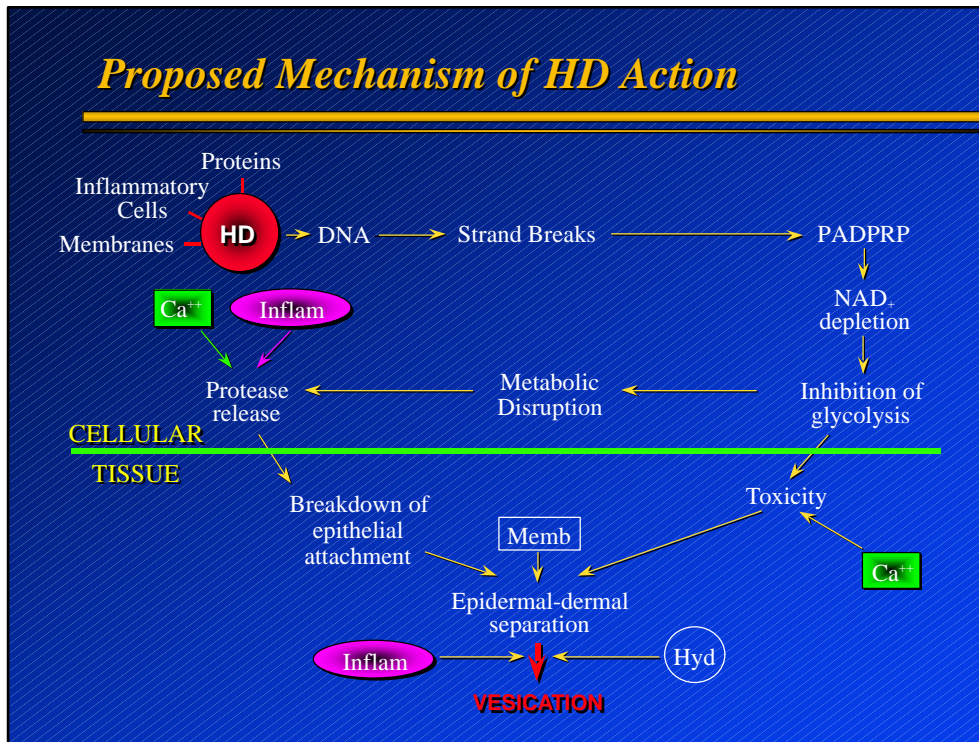
- AChE and BuChE
- Organophosphorus anhydride hydrolase
- Human BuChE
- Human carboxylesterase
- Met Milestone 0 (Feb 00)



Several challenges present themselves here:

- (1) No new human data
- (2) Understanding of the mechanism of injury
- (3) “high-fidelity” animal or other model

Proposed Mechanism of HD Action



The cellular and tissue alterations induced by HD that are proposed to result in blister formation. HD can have many direct effects such as alkylation of proteins and membrane components (Memb) as well as activation of inflammatory cells. One of the main macromolecular targets is DNA with subsequent activation of PADP ribose. Activation of PARP can initiate a series of metabolic changes culminating in protease activation. Within the tissue, the penultimate event is the epidermal-dermal separation that occurs in the lamina lucida of the basement membrane zone. Accompanied by a major inflammatory response and changes in the tissue hydrodynamics(Hyd), fluid fills the cavity formed at this cleavage plane and presents as a blister.

The first breakthrough in HD by USAMRICD and its extramural collaborators was the development of a model. From this research, we were able to construct a schema of the major events of the pathological processes documented in cells and tissues exposed to HD. This schema was presented at numerous DoD and professional scientific forums, including the 20th Army Science Conference. The research findings of this program served as the core of a NATO sponsored monograph on HD research.

Strategies for Pharmacological Intervention

<i>Biochemistry</i>	<i>Strategy</i>	<i>Example</i>
DNA Alkylation	Intracellular Scavengers	N-acetyl Cysteine
DNA Strand Breaks	Cell Cycle Inhibitors	Mimosine
PARP Activation	PARP Inhibitors	Niacinamide
Disruption of Calcium	Calcium Modulators	BAPTA, Dimercaprol
Proteolytic Activation	Protease Inhibitors	Sulfonyl fluorides
Inflammation	Anti-inflammatories	Indomethacin

USAMRICD

Over 500 candidate prophylactic compounds have been evaluated through the antivesicant DTN. Sixty-two compounds have demonstrated an ability to provide significant modulation of edema and/or histopathology caused by HD *in vivo*. Of these 62 compounds, nineteen have demonstrated at least 50% protection against the pathological indicators of mustard injury (Table 2). All of these 19 successful candidates fall into four of our six original proposed strategies: anti-inflammatories (7), antiproteases (3), scavengers (6), or PARP inhibitors (3).

An intermural partner often provided major injectors in each of these areas, e.g.

Prtease Inhibitors	Georgia Tech	Povers
Information	P&G, Dupont	Several PIs
PARP	Parke-Davis & others, Georgetown	Smulson
Scavenger	Porton, UTA	Ternay

*Examples of Protection Against Injury in the
MEVM at 24 hr Post HD Exposure*

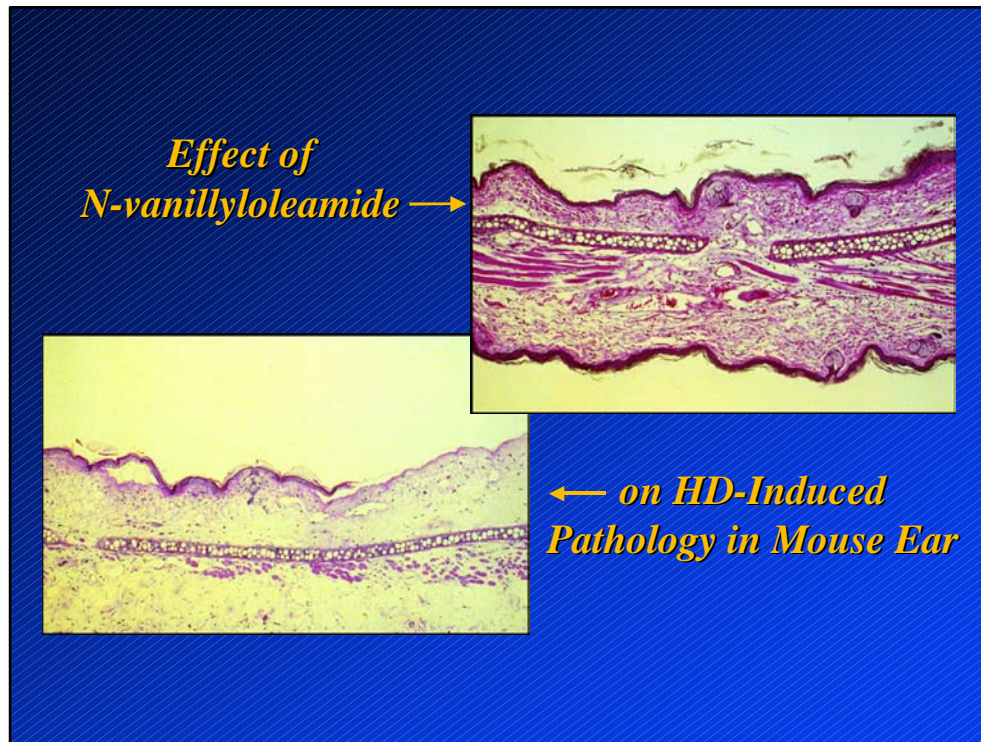


**Mustard
Control**



**Olvanil +
Indomethacin**

To show you how well our leading candidate antivesicant compounds work, we show here mice exposed to sulfur mustard as well as exposed mice treated with antivesicants. It is easy to see that the ears of treated mice look relatively normal compared to untreated mice.



The findings of wet weight changes in mouse ear are corroborated histopathologically.

Mouse Ear Vesicant Model
72 hours



HD Exposed Ear

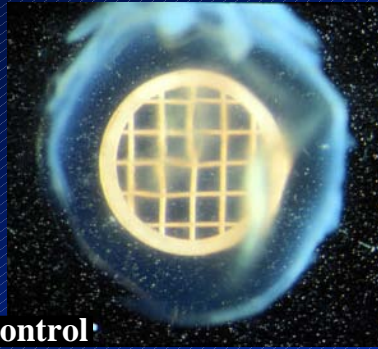


**HD Exposed Ear
+
Protease Inhibitor**

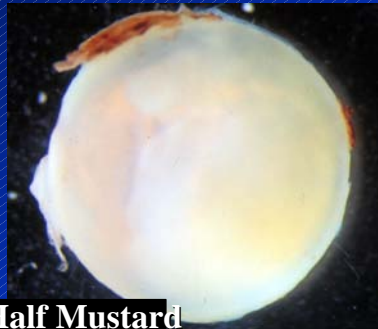
Protease inhibitors

1-(40-aminophenyl)-3-(4-chlorophenyl) urea	1883	54%
N-(O-P)-L-Ala-L-Ala-benzy ester hydrate	2780	62%
Ethyl p-Guanidino Benzoate Hydrochloride	1578	62%

*Effects of Half Mustard
on Rat Cornea*



Control



Half Mustard



Half Mustard+Varma Mixture



In this study we explored the use of two FDA approved medications (triamcinolone and cefazolin) as treatments for sulfur mustard injury. Female NewZealand White rabbits were divided into 5 groups: naïve controls (n=6), HD positive controls (n=14) and 3 treatment groups. Treatment groups received subtenons injection(s) of a combination of 20 mg triamcinolone and 15 mg cefazolin. Treatment group 1 (n=5) received a single injection 10 min after HD exposure. Treatment group 2 (n=6) received an injection 10 min after HD exposure plus a second injection 7 days later. Treatment group 3 (n=6) received an injection 10 min after HD exposure, a second injection 7 days later and a third injection 7 days after the second. Rabbits were observed for a total of 19 weeks after HD exposure. Pachymetry (cornea thickness) data and the presence or absence of neovascularization were recorded weekly for 6 consecutive weeks and then on weeks 15 and 19 post-exposure. Results show that a triamcinolone /cefazolin combination treatment is extremely effective in treating corneal injury from liquid HD exposure.



IMPACT - Vesicant Agent

Science and Technology
Objective (STO)

Advanced Anticonvulsant

Demonstrate safety and efficacy
for an advanced anticonvulsant

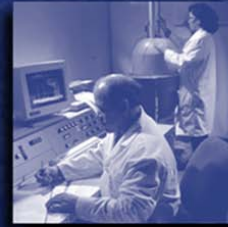


Accomplishments:

- Determined diazepam dose in NHP
- Identified compounds superior to diazepam
- Identified optimal treatment
- Milestone I
- Currently evaluating in NHP



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