Team Neuroprotectors

Drug discovery to protect Soldiers from biothreats and neurodegenerative diseases

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Relevant Challenges to the Warfighter

Fig. 1. Soldier being dragged away from a chem/bio threat, *Nature (*Sydnes, 2013).

Biological threat agents:

- Many biotoxins are toxic to brain cells through mechanisms which involve their interaction with the surface of cells.
- The pathological mechanisms of such acute toxins could be weaponized by our adversaries or used in terrorist attacks.
- Developing countermeasures against these toxins aims to attenuate the impact of such devastating assaults.
- We examined the model toxin melittin. It is a pore-forming peptide that is toxic to brain cells, but safe to work with in a Biosafety Level II lab.

Fig. 2. IED Explosion, *Business Insider* (Ingersoll, 2013)

Neurodegenerative diseases and traumatic brain injuries (TBIs):

- Soldiers experience increased risks of TBIs during training and combat. IEDs and artillery can induce severe brain trauma.
- Depending on the frequency and severity of TBIs, Soldiers are placed at a higher risk of developing Chronic Traumatic Encephalopathy (CTE) and Alzheimer’s disease (AD).
- We investigate the aggregation of the tau protein that causes AD and CTE (Figures 3 and 4).

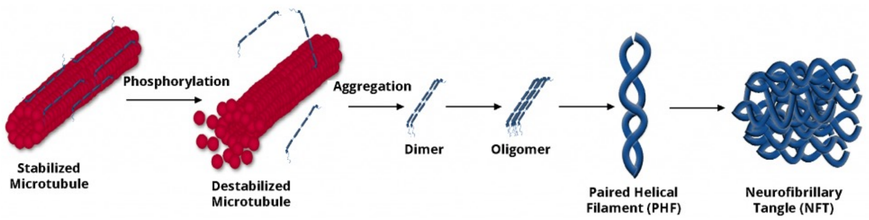

Fig. 3. Tau dissociates from microtubules, leading to their destabilization. It then aggregates into oligomers, paired helical filaments, and ultimately neurofibrillary tangles that are toxic to neurons. (Thomson, StressMarq Biosciences, 2018)

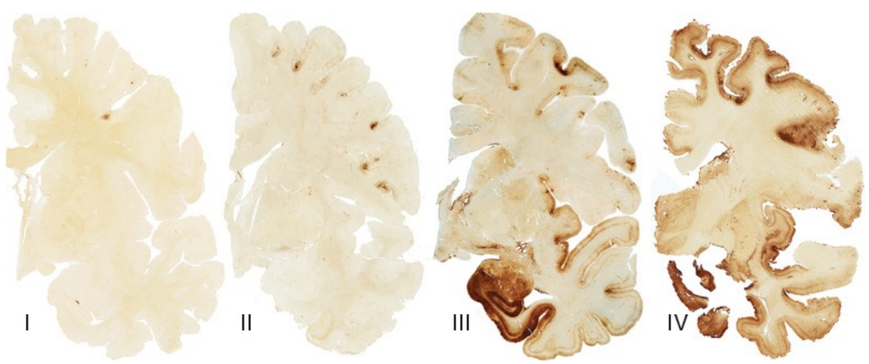

Fig. 4. The four stages of chronic traumatic encephalopathy (CTE). By stage IV CTE, p-tau pathology is densely distributed throughout the cerebrum, medial temporal lobe, brainstem, and cerebellum. (Kriegel et al, *Perspect. Med.,* 2018.)

Claramine Counteracts Cell Damage from Biothreats

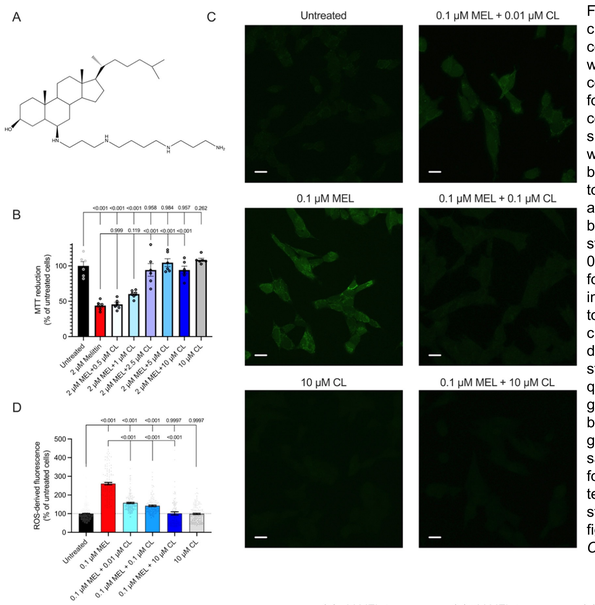

Fig. 5. **(A)** Chemical structure of claramine. **(B)** MTT viability assays after cells were treated with 2 μM melittin (MEL) without (red bar) or with increasing concentrations of claramine (CL, blue bars) for 20 h. *n* = 60,000 cells per condition corresponding to the six technical replicates shown. Conditions were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett’s multiple comparison test relative to untreated cells or cells treated with melittin, as indicated. Data are representative of *n* = 3 biologically independent experiments. **(C)** To study the effects of acute melittin treatment, 0.1 μM of the toxin was incubated with cells for 5 min in the absence or presence of increasing concentrations of claramine (0.01 to 10 μM). The fluorescence of the 6-chloromethyl-2′-7′-dichlorodihydrofluorescein diacetate (CM-H_2DCFDA) general oxidative stress indicator was used to measure the quantity of reactive oxygen species (ROS) generation in the various conditions. Scale bars, 10 μm. **(D)** Corresponding values of green fluorescence from the ROS signal. All samples were analyzed by one-way ANOVA followed by Dunnett’s multiple comparison test, as indicated. Bars indicate mean ± standard error of the mean. Legend and figure adapted from Kreiser et al., *ACS Chem. Neurosci.*, 2022.

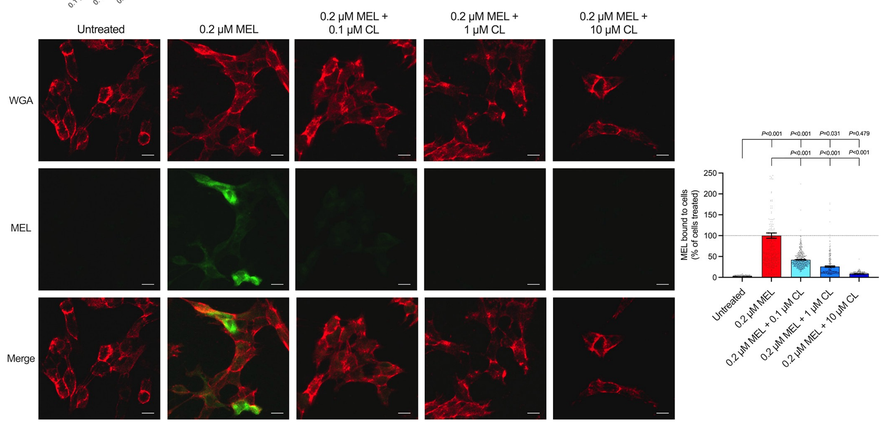

Fig. 6. Claramine reduces the binding of melittin to cell membranes. SH-SY5Y neuroblastoma cells were treated for 5 min with 0.2 μM melittin (MEL) without (red bar) or with 0.1, 1.0, or 10 μM claramine (CL, blue bars). Untreated cells are shown for comparison (black bar). Red and green fluorescence correspond to the cell membrane labeled with wheat germ agglutinin (WGA) and the Alexa 488-labeled melittin, respectively. Scale bars, 10 μm. All samples were analyzed by one-way ANOVA followed by Dunnett’s multiple comparison test. Bars indicate mean ± standard error of the mean. Data shown are representative of *n* = 3 biologically independent experiments. Legend and figure adapted from Kreiser et al., *ACS Chem. Neurosci.*, 2022.

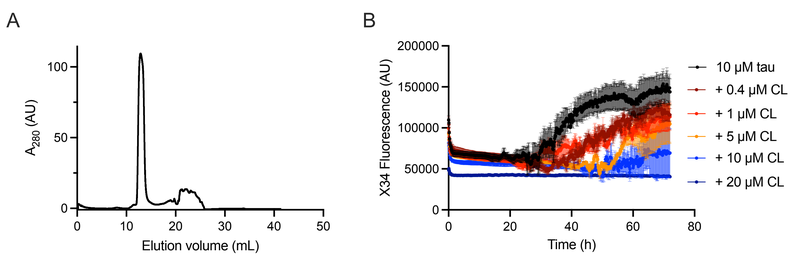

Fig. 7. **(A)** Purification of the fragment of the tau protein involved in AD and CTE. The first sharp peak shows the extraction of the relatively small tau protein in its monomeric form. The broad peak around 25 mL is indicative of the degradation products of tau, which flow less readily through the pores within the FPLC column due to their smaller size. **(B)** Effects of claramine on inhibiting tau aggregation. Data are representative of n=3 independent experiments.

Conclusions and Future Directions

Claramine protects the cell from biological threat agents through a generic, cell-membrane dependent mechanism.

- Similar protective effects were observed with α-hemolysin, a virulence factor involved in staph infections that also disrupts cell membranes.
- Biophysical measurements confirmed that increasing concentrations of claramine did not affect the structure or characteristics of the toxins.
- Claramine acts to protect the cell membrane by interacting with the membrane directly (Figure 8). These data suggest that claramine can protect the cell from the cytotoxic effects of a broad range of biothreats.

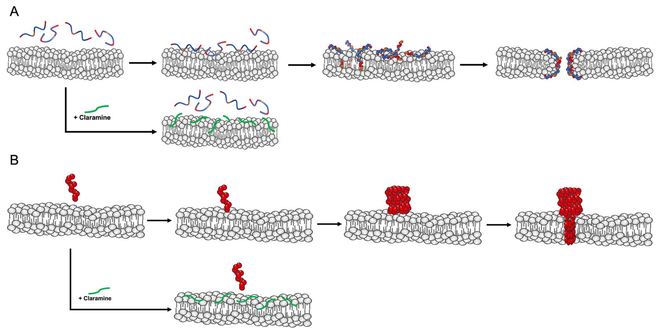

Fig. 8. Schematic for the mechanism by which the toxins disrupt cell membrane integrity. In the illustration, claramine (green) protects the cell by preventing the docking of melittin (**A**) and alpha-hemolysin (**B**). Legend and figure adapted from Kreiser et al., *ACS Chem. Neurosci.*, 2022.

Claramine prevents the formation of toxic protein aggregates that are central to AD and CTE.

By isolating an AD/CTE-associated protein isoform in its monomeric form (Figure 7A) and measuring its aggregation rate in the absence and presence of increasing concentrations of claramine (Figure 7B), we reveal the ability of this molecule to reduce toxic aggregate formation. Such results indicate the potential of claramine to be used to treat the detrimental effects associated with TBIs in the warfighter.

Future work:

- Characterize additional biological and chemical threats in vitro, including with claramine and other novel molecules that are under development.
- Study validated biological threat agents in vivo (external to West Point).
- Develop next generation models for TBI-induced protein aggregation.
- Test the effects of claramine in preventing TBI-induced aggregation.

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Key references for further reading:

- Kreiser, Wright, Sasser et al., *ACS Chemical Neuroscience*, 2022 (biothreats paper)
- Limbocker et al., *Natural Product Reports*, 2022 (cover article, neurodegeneration paper)
- Kreiser and Wright et al., *International Journal of Molecular Sciences*, 2020 (neurodegeneration review)
- Limbocker et al., *Nature Communication*, 2019 (neurodegeneration paper)