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## A Toxidromic Approach for Chemical Medical Countermeasure Development

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### Organophosphate Inhibition of Human Cathepsin A Carboxypeptidase

Patricia Legler, Naval Research Laboratory

Ilija Khavrutski, Uniformed Services University, Armed Forces Radiobiology Research Institute

Jaimee Compton, Naval Research Laboratory

Kayla Jurkouch, Case Western Reserve University, Dept. of Biomedical Engineering

Esterases play an important role in xenobiotic detoxification. Human Cathepsin A (CatA; CTSA) is a lysosomal serine carboxypeptidase that can catalyze the hydrolysis of C-terminal amino acids from endothelin I, angiotensin I, Substance P, oxytocin, and bradykinin. At neutral pH it is a deamidase and can deamidate neurokinin A. Bradykinin is important for blood pressure regulation (vasodilator) and plays a role in inflammation and vascular permeability. Bradykinin is released from damaged tissues and is inactivated by carboxypeptidases. CatA is structurally similar to acetylcholinesterase (AChE). Proteomic studies identified CatA as a potential target of G- and V-type organophosphates (OP). Here we show that CatA is stably inhibited by low  $\mu\text{M}$  to high nM concentrations of sarin (GB), soman (GD), cyclosarin (GF), VX, and VR within minutes to hours at pH 7. Cyclosarin was the most potent with a kinetically measured dissociation constant ( $K_i$ ) of  $2 \mu\text{M}$  followed by VR ( $K_i = 2.8 \mu\text{M}$ ). Bimolecular rate constants for inhibition by cyclosarin and VR were  $1.5 \times 10^3 \text{ 1/M}^*\text{sec}$  and  $1.2 \times 10^3 \text{ 1/M}^*\text{sec}$ , respectively, and were approximately 3-orders of magnitude lower than those of human AChE indicating slower reactivity. Notably, both AChE and CatA bound diisopropylfluorophosphate (DFP) comparably and had  $K_{i\text{DFP}} = 13 \mu\text{M}$ . CatA is highly expressed in the kidney, adrenal gland, digestive tract, liver, and brain. It is currently a drug target for heart failure. CatA can be found on the cell surface or can be secreted from activated platelets. CatA is also present in the urinary proteome. At low pH greater than 85% of this lysosomal enzyme spontaneously reactivated after OP inhibition, conditions under which most OP adducts irreversibly age. A di-glutamate (Glu-69/Glu-149) motif occupies the region equivalent to the choline binding pocket in AChE. AChE binds positively charged choline esters, whereas CatA binds negatively charged  $\text{COO}^-$  groups on the C-terminal amino acids of its peptide substrates. At neutral pH the enzyme remained stably inhibited ( $t_{1/2} \sim 83 \text{ days}$ ), and was partially reactivated by 2-PAM. Our crystal structure of DFP-inhibited CatA is consistent with an aged or partially aged adduct. Serine carboxypeptidases have not been previously shown to be stably inhibited by both G- and V-type agents, or shown to age. CatA and several other OP-inhibited enzymes are part of the renin-angiotensin pathway; their inhibition may contribute to CWA-induced inflammation.