

AChE Reactivation Assay in 96-well Microplate

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The inhibition of acetylcholinesterase (AChE) by organophosphorous (OP) nerve agents and insecticides poses a continuous threat due to the possible use in battlefield, terrorist attack or in agriculture. Antidotes containing oxime compounds to reactivate the inhibited enzyme are highly valued for treatment against OP poisoning. 2-PAM has been used to treat OP intoxicated patients, and HI-6 is under evaluation. However, the reactivation efficacy of OP-inhibited AChE by 2-PAM, HI-6, and other oxime compounds remarkably varies among OP types and AChE sources. Therefore, it is needed to develop a new AChE reactivator that can reactivate against various types of OP with minimal side effect and prompt action. For this purpose, we examined the reactivation capability of 2-PAM and HI-6 against housefly head AChE (HF AChE) and bovine red blood cell AChE (RBC AChE) inhibited with diisopropylphosphorofluoridate (DFP), paraoxon, and dichlorvos in 96-well microplate.

The inhibition of HF and RBC AChE with DFP, paraoxon, and dichlorvos were performed, and DFP and paraoxon were used to inhibit HF and RBC AChE with concentration enough to inhibit 99% of AChE activity for 10 minutes at room temperature. After that, the inhibited AChE solution were partitioned with two-fold volume of hexane to remove excess inhibitor. In this process, RBC AChE was prepared with the buffer containing 2.5 % Triton X-100 to preserve enzyme activity. Each of various concentration of 2-PAM or HI-6 was incubated with the aqueous phase for different period at room temperature, and each solution was eluted from the Bio-spin column packed with Sephadex G-50 to remove excess reactivator and other small molecules. The AChE activity of each elute was analyzed with Elman' assay in microplate reader.

The reactivation rate of AChE was differ among enzyme sources, inhibitors, and reactivators. The highest reactivation rate was observed from the combination with HF-AChE, DFP, and 2-PAM with 75.1 % reactivation. The lowest was observed from the combination of HF-AChE, DFP, and HI-6.

In conclusion, *in vitro* AChE reactivation assay in 96-well microplate is useful for the preliminary selection of active reactivators.