POLYCLONAL ANTIBODIES AGAINST MULTIPLE CHEMICAL THREATS

Efficacy Of A Variety Of Transition State Analogue Haptens In A Prophylactic Vaccine Against Organophosphorus Nerve Agent Exposure

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The only currently fielded prophylactic for protection against exposure to organophosphorus nerve agents (OPNAs) is pyridostigmine bromide. This prophylactic approach suffers from poor pharmacokinetics, thus requiring frequent dosing, and pyridostigmine bromide leads to undesirable inhibition of acetylcholinesterase, potentially exacerbating the symptoms of OPNA exposure. Newer prophylactic approaches in development, such as stoichiometric and catalytic bioscavengers, often suffer from a number of problems including lack of broad-spectrum OPNA protection, repeat dose host immunity, poor pharmacokinetics for in vivo administration, and an inefficient mass imbalance for detoxification of the small molecular weight OPNA. We attempt to overcome these limitations by using a multivalent, active immunization approach to develop catalytic antibodies (abzymes) which are capable of binding and degrading a broad spectrum of OPNAs. Linus Pauling proposed that enzymes are efficient catalysts due to their complementarity towards the transition state of the reaction being catalyzed, thus leading William Jencks to speculate that abzymes could be created for any reaction by developing antibodies towards a transition state analogue. Herein we have developed pentacoordinate phosphorus (phosphorane) compounds to mimic the transition state of OPNA hydrolysis for use as haptens, small molecules linked to an immunogen for the development of antibodies. After down-selecting a linker with the appropriate length and polarity, the best conjugation density of the hapten on the immunogen (the number of haptens on each immunogen), and the most effective adjuvant, we sought to determine which hapten structures would be most effective at protecting against OPNA exposure by testing in vitro protection of acetylcholinesterase by the antibodies as well as in vivo OPNA challenge. We examined nine different pentacoordinate phosphoranes with structural diversity around the phosphorus that included mimics of the thiolate leaving group of VX, while also considering two different attachment points for the linker to the immunogen (CRM197). Each hapten conjugate was used to vaccinate CD1 and C57BL/6 mice through subcutaneous injection, along with two boosters over the course of a month. Antibody responses were obtained by ELISA evaluations for both the CD1 and C57BL/6 mice in order to assess dosing times, species effects, and any variability and specificity for the immune response. For the in vitro protection experiments, the CD1 mice were sacrificed at 42 days and the serum collected for purification and testing of the antibodies. In vivo efficacy was determined by challenging the fully immunized CD1 animals with an LD90 of OPNA and determining survivability compared to a control group.

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