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Protective efficacy of catalytic bioscavenger, paraoxonase 1 against sarin and soman exposure in guinea pigs☆

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ABSTRACT

Human paraoxonase 1 (PON1) has been portrayed as a catalytic bioscavenger which can hydrolyze large amounts of chemical warfare nerve agents (CWNAs) and organophosphate (OP) pesticides compared to the stoichiometric bioscavengers such as butyrylcholinesterase. We evaluated the protective efficacy of purified human and rabbit serum PON1 against nerve agents sarin and soman in guinea pigs. Catalytically active PON1 purified from human and rabbit serum was intravenously injected to guinea pigs, which were 30 min later exposed to 1.2 × LCt_{50} sarin or soman using a microinstillation inhalation exposure technology. Pre-treatment with 5 units of purified human and rabbit serum PON1 showed mild to moderate increase in the activity of blood PON1, but significantly increased the survival rate with reduced symptoms of CWNA exposure. Although PON1 is expected to be catalytic, sarin and soman exposure resulted in a significant reduction in blood PON1 activity. However, the blood levels of PON1 in pre-treated animals after exposure to nerve agent were higher than that of untreated control animals. The activity of blood acetylcholinesterase and butyrylcholinesterase and brain acetylcholinesterase was significantly higher in PON1 pre-treated animals and were highly correlated with the survival rate. Blood O_2 saturation, pulse rate and respiratory dynamics were normalized in animals treated with PON1 compared to controls. These results demonstrate that purified human and rabbit serum PON1 significantly protect against sarin and soman exposure in guinea pigs and support the development of PON1 as a catalytic bioscavenger for protection against lethal exposure to CWNAs.

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1. Introduction

Chemical warfare nerve agents (CWNAs) and organophosphate (OP) pesticides exert their biological effects mainly by the irreversible inhibition of acetylcholinesterase (AChE), an enzyme involved in the catabolism of neurotransmitter acetylcholine (ACh) [1–3]. The inhibition of AChE results in accumulation of ACh in the peripheral and central nervous system leading to acute cholinergic crisis. The existing pretreatments against CWNA toxicity are centered on the development of reversible cholinesterase inhibiting compounds such as pyridostigmine bromide [4]. Post-exposure treatments include AChE re-activator 2-pralidoxime, anti-cholinergic drug atropine sulfate and anticonvulsant diazepam [5–7]. But current treatments do not completely protect against CWNA induced neuropathology and behavioral deficits.

Prophylactic pre-treatment with bioscavenger enzymes such as human butyrylcholinesterase (BChE) has emerged as a promising strategy for protection against lethal doses of CWNA exposure [8–10]. BChE is able to scavenge the toxic CWNA and OP in the blood before they reach the target organs. The stoichiometric 1:1 binding of BChE and nerve agent diminishes its protective efficacy with lower doses of the enzyme. Researchers worldwide are now more focused on the discovery and development of catalytic bioscavenger enzymes, which are expected to hydrolyze large amounts of CWNAs and OPs without the loss of activity. Development of

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Abbreviations: PON1, paraoxonase 1; HPON1, human PON1; RPON1, rabbit PON1; OP, organophosphate; CWNA, chemical warfare nerve agent; DFP, diisopropyl-fluorophosphate; CPO, chlorpyrifos oxon; AChE, acetylcholinesterase; ACh, acetylcholine; BChE, butyrylcholinesterase; p-NPA, p-nitrophenyl acetate; DTNB, dithionitrobenzoic acid; DTP, 44′-dipyridyl disulfide, 4,4′-dithiodipyridine; Iso-OMPA, tetra monoisopropyl pyrophosphor-tetramide; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; Sarin, 2-(fluoro-methylphosphoryl)oxypropane; Soman, 2-(fluoromethylphosphoryl) oxy-3,3-dimethylbutane.

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catalytic bioscavenger will reduce the amount of purified enzyme required for treatment, and lesser demands on future logistics and resources for military operational medicine.

A promising catalytic bioscavenger against CWNAs and OPs is human serum paraoxonase 1 (PON1) (EC 3.1.8.1) [11–13]. PON1 is synthesized mainly in the liver and secreted to blood to associate with high density lipoproteins, although mRNA analysis showed its presence in many other tissues [14,15]. The secreted human blood PON1 enzyme is 355 amino acids long with a molecular mass in the range of 43–45 kDa [16,17]. On the other hand, rabbit serum PON1 is a 359 amino acid long protein with 85% identity to human serum PON1 [18–20]. Both human and rabbit serum PON1 exhibit multiple types of polymorphisms which affect the catalytic activity of the enzyme [21–23].

Purified human and rabbit serum PON1 was reported to hydrolyze various CWNAs and OPs *in vitro* [11–13,17]. Human recombinant PON1 expressed in *Escherichia coli* and *Trichoplusia ni* larvae also showed similar hydrolysis of substrates [11,24,25]. We have recently shown that purified human and rabbit serum and recombinant human PON1 efficiently hydrolyze nerve agents tabun, sarin and soman under *in vitro* conditions [26]. Furthermore, we demonstrated that the CWNA hydrolysis activity in the purified enzyme is intrinsic to PON1 activity and PON1 acts as a catalytic bioscavenger.

Evaluation of the *in vivo* efficacy of purified PON1 pre- or post-exposure treatment in mice showed significant protection against chlorpyrifos oxon (CPO) [27]. Further investigations using PON1 knock-out mice showed dramatic increase in the sensitivity to CPO toxicity [28]. Endogenous expression of PON1 in mice using adenovirus vector or naked PON1 DNA administration also showed significant expression and protection against chlorpyrifos, diazoxon and soman induced toxicity [29–31]. *In vivo* evaluation of protective efficacy of PON1 has been described in mice using other OPs and injury models [32–35].

The objective of this study was to evaluate the protective efficacy of purified human and rabbit serum PON1 against sarin and soman exposure in a guinea pig inhalation model. Microinstillation inhalation exposure that is well established in our laboratory was used to endotracheally aerosolize the CWNAs [36,37]. The technology involves aerosolization of nerve agents using a microcatheter, which has several peripheral holes that pump air at the tip to aerosolize the agent delivered in the central hole. The microinstillation technology is safe, and requires minimum amount of the agent to produce a meaningful dose response. We demonstrate that pre-treatment with purified PON1 protects against nerve agent exposure.

2. Materials and methods

2.1. Materials

Phenylacetate, p-nitrophenylacetate (p-NPA), acetylthiocholine, butyrylthiocholine, tetra monoisopropyl pyrophosphortetramide (iso-OMPA), huperzine A, dithionitrobenzoic acid (DTNB), 4,4′-dipyridyl disulfide, 4,4′-dithiodipyridine (DTP), heparin, and PON1 polyclonal antibody were purchased from Sigma (St. Louis, MO). Tissue protein extraction reagent and BCA assay kit for protein estimation were purchased from Pierce (Rockford, IL). Microplates (96-well) were purchased from BD Biosciences (San Jose, CA). SDS-PAGE running and transfer buffer, 4–20% Tris–glycine gradient gel, and protein molecular weight marker was purchased from Invitrogen (Carlsbad, CA). Immobilon polyvinylidene difluoride membrane was purchased from Millipore (Billerica, MA). Enhanced chemiluminescence reagent was purchased from GE Healthcare (Piscataway, NJ). Telazol was purchased from Wyeth Pharmaceuticals (Madison, NJ). Meditomidine was obtained from Pfizer Pharmaceuticals (New York, NY). Chemical warfare nerve agents sarin and soman were obtained from US Army Medical Research Institute of Chemical Defense (USAMRICD), Aberdeen Proving Ground, MD.

2.2. Animals

All animal research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adhered to principles stated in the Guide for the Care and Use of Laboratory Animals (NRC Publication 1996 edition). All animal procedures were performed at USAMRICD with an IACUC approved protocol. Male Hartley guinea pigs (250–300 g) from Charles River Laboratories (Wilmington, MA) were used in this study.

2.3. Purification of human and rabbit serum PON1

Large amount of PON1 was purified from human and rabbit serum as described earlier using multiple chromatographies with modifications [38,39]. A brief methodology of the PON1 purification was described recently [26]. The purity of human and rabbit serum PON1 was confirmed by SDS-PAGE and immunoblotting. The catalytic activity of highly purified PON1 preparations (>95% pure) was analyzed by using p-NPA or phenyl acetate as substrates. Enzyme activity in units was calculated from the initial linear rates of hydrolysis of substrates (p-NPA or phenyl acetate) using molar extinction coefficients of p-nitrophenol (18 $mM^{-1}\,cm^{-1}$ at 405 nm) or phenol (1.31 $mM^{-1}\,cm^{-1}$ at 270 nm), respectively.

2.4. Microinstillation inhalation exposure

Guinea pigs were quarantined for 1 week prior to inhalation exposure to sarin and soman. Microinstillation inhalation exposure was performed in these animals as described earlier [36,37]. Briefly, guinea pigs were anesthetized using a combination of telazol (40 mg/kg, im) and medetomidine (0.125 mg/kg, sc) and intubated with a translucent polystyrene tube. The microinstillation catheter (Trudell Medical International, Canada) passed through the intubation tube and placed 2 cm above the bifurcation of the trachea. Sarin and soman diluted in saline was aerosolized with a pulse rate of 40 pulses/min for 2–4 min. The animals were exposed to a final concentration of 846 mg/m^3 of sarin or 841 mg/m^3 of soman ($1.2 \times LCt_{50}$) [40,41]. Terminally ill animals were euthanized and biological samples were collected. The surviving animals were allowed to recover for 24 h and euthanized by exsanguination and blood and tissues were collected for biochemical analysis.

2.5. Administration of purified human and rabbit serum PON1

Animals were randomly divided into three groups – 1, control; 2, human serum PON1 treated; and 3, rabbit serum PON1 treated. Prior to the experiment, ear blood (20–50 μl) was collected from all the animals into heparinized tubes. Purified human (~1 mg protein) or rabbit (~0.5 mg protein) serum PON1 (50–200 μl) was administered intravenously to the experimental group of animals through saphenous vein (5 units of enzyme per animal). After 30 min, ear blood was again collected from these animals. Animals were exposed to sarin or soman by using the microinstillation method as described above. At the end of the experiment or at 24 h, cardiac blood was collected into heparinized tubes followed by necropsy.

2.6. Measurement of pulse rate and blood O_2 saturation

Blood O_2 saturation and pulse rate were recorded using a pulse oximeter (Nonin Medical Instruments, Minneapolis, MN) in