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## The experimental study of protective effect of the recombinant human serum paraoxonase1 subtype isozyme against organophosphate poisoning

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**Objective:** Organophosphorus insecticide is a kind of common agriculture capital widely used around the world, especially in the developing countries. But in the process of production, transportation and application of organophosphorus chemicals, acute and chronic poisoning cases occurs occasionally. The statistics show that millions of people subjected to organophosphorus poisoning every year. And the organophosphorus poisoning cases are even among the top one of suicide cases in some developing countries. We can also find the contents about organophosphorus neurotoxic agents such as sarin, soman, tabun and VX in the news report related to the Gulf War and terrorism attack. Even so, the use of organophosphorus insecticide is still extensive for its lower price and availability. Therefore, to meet the need of security of society and defense, the research of detoxification of organophosphorus poisoning is very urgent. PON 1 is a non-specific esterase exists in the mammalian's liver, blood, kidney and brain so on, which can hydrolyze a broad spectrum of substrates such as organophosphate, carbamate and aromatic carboxylate, which is named for its toxicological significance. The molecule of PON1 is combined with HDL in the blood, protecting the cell from attack of toxin and peroxide. Paraoxonase 1 (PON 1) has attracted wide attention because it has some efficient and non-toxic hydrolysis effect on several kind organophosphorus chemicals, even including G type neurotoxic agents such as sarin, soman and tabun, in vivo and vitro studies, which make it the focus of research on detoxication of organophosphorus poisoning. Although PON1 exist in everyone body, the difference of the PON1 activity is very great, ranging from 10 to 40 times, among the individuals. Some researchers have shown that the difference is determined by its genetic polymorphism. The polymorphism sites are dozens in the sequence of PON1 DNA, but most of them are introns which can not be translated. The phenotypes of the PON1 gene are determined by the exons primarily. There are two exons, having polymorphism in 55 and 192 locus of the PON1 DNA sequence, which are reported to affect the concentration and activity of PON1 in vivo respectively. The polymorphism of the 55 site is Leu/Met (L/M) mutation and the polymorphism of the 192 site is Gln/Arg (Q/R) mutation. The polymorphism of the 192 site is reported to affect people's susceptibility to the different organophosphorus chemicals, which make the people having Q subtype PON1 susceptible to chlorpyrifos, dichlorvos and paraoxon, but the others having R subtype PON1 susceptible to diazinon and sarin. In the earlier experiment, we used the Escherichia coli to express the recombinant PON1 gene. But we found that the prokaryotic expression system lack of post-translation modification, which may affect the activity of expressed eukaryotic protein. And the expressed protein aggregated and formed inclusion bodies, which may undermine the output. Bac-to-Bac system is a kind of insect cell-baculovirus protein expression system, which can express eukaryotic genes well for its post-translational modification such as phosphorylation and glycosylation, making the expressed protein closer to the natural one in functions. In this study, we plan to use the Bac-to-Bac system to express the Q/R subtype PON1 of polymorphism in the 192 site and explore the difference of the two recombinant subtype isozymes as antidote against organophosphorus poisoning. At the same time, we investigate the function of glycosylation in keeping the activity of PON1.

**Methods:** 1 Identification and preparation of subtype isozyme of PON1 by gene engineering. We get the subtype DNA sequence of PON1 from the GeneBank. Then we use the gene synthesizer to produce the subtype gene sequence with a bit modulation artificially. The target gene was carried to the receptive IPLD-SF 21 cells by the vector pBacPAK8. We get the Bacmid DNA containing the subtype

gene sequence of PON1 after the transposition. We used the bacmid virus to infect the SF21 cells to acquire the strains with higher level of target DNA after cultures. Then the virus strains of higher titer were transfected to the SF21 cells in order to express the subtype isozyme of PON1 by suspension culture. We could acquire the purified expressed PON1 by the cobalt agarose affinity chromatography and test its activity by spectrophotometric method. 2 Investigate the effect of glycosylation on the PON1 activity. The recombinant PON1 subtype and mixed human PON1 were glycosylated by endoglycosidase Endo F2 to test the function of post-transcription glycosylation. We use paraoxon, chlorpyrifos, diazinon and trithion as the substrate to test and compare the activity of the purified recombinant subtype isozyme before and after deglycosylation. 3 Explore the protective effect of rhPON1 subtype isozyme on the central nervous system of the rats exposed to organophosphorus chemicals. 81 wistar rats were chosen and divided into three groups randomly, named exposed group, treatment group A and B. Then each group was divided into three subgroups according to the exposed poison including chlorpyrifos, diazinon and trithion. All the rats were exposed to organophosphorus insecticides at a dose of two times LD50 by intragastric administration. The rhPON1<sub>R192</sub> and rhPON1<sub>Q192</sub> were administered by injection vena caudalis as antidote at a dose of 10U/kg to the group A and B respectively within 1 minute after the exposure. The onset time of signs and symptoms such as salivation, muscle fibrillation, dyspnea and muscle strength above 3 grades were recorded. The experiment was terminated after continuous observation for 12 hours. All the rats were decapitated to collect the serum and brain tissue at the termination point. The cholinesterase activity of the serum and brain tissue was detected by spectrophotometry among the groups. Use the optical microscope and transmission electron microscope to observe and compare the ultrastructure change of the hippocampal region in the brain among the groups. The change of cholinesterase was compared among the groups by means of Western Blot and immunohistochemistry. Investigate the substrate specialty of the subtype isozyme of PON1 through these comparisons to find the acute antidote of efficient hydrolysis to the different organophosphorus poisoning.

**Results:** 1 The bac-to-bac system can be used to express the subtype isozyme of PON1 with great efficiency and output. The positive vectors containing the DNA of target subtype isozymes with polymorphism in 192 loci were expressed in the SF21 cells successfully, with a molecular weight about 43 KD determined by Western Blot. And a great amount of enzyme was acquired after suspension culture and purified by cobalt agarose affinity chromatography. The specific activity of purified rhPON1<sub>R192</sub> was increased 435 fold over the crude extract, up to 152U/mL, with the yield of 8.5%. And the specific activity of the purified rhPON1<sub>Q192</sub> was increased 597 fold over the crude extract, up to 74.1U/L, with the yield of 12.5%. The purified rhPON1<sub>R192</sub> and rhPON1<sub>Q192</sub> were tested to have the phosphatase activity. 2. Deglycosylation of the rhPON1 will affect its phosphatase activity. (1) We found that rhPON1<sub>R/Q192</sub> and hPON1<sub>mix</sub> of the same amount have different hydrolysis activity toward different organophosphorus insecticides such as chlorpyrifos, diazinon and trithion. The hydrolysis efficiency on chlorpyrifos of the different PON1 were showed in such sequence that rhPON1<sub>R192</sub> > hPON1<sub>mix</sub> > rhPON1<sub>Q192</sub>; while the hydrolysis efficiency on diazinon was shown in opposite sequence that rhPON1<sub>R192</sub> < hPON1<sub>mix</sub> < rhPON1<sub>Q192</sub>; whether rhPON1 or hPON1 had shown lower hydrolysis efficiency on the trithion. (2) We found the hydrolysis activity of rhPON1 and hPON1 toward organophosphorus poisoning and the substrate speciality decreased obviously. (3) The molecular weight of the rhPON1<sub>R192</sub>, rhPON1<sub>Q192</sub> and hPON1<sub>mix</sub> decreased to the same extent. 3 The protective effects of rhPON1 on the nerve system exposed to organophosphorus insecticides are different. In the treatment groups, the results of observation about onset time of poisoning signs and symptoms are better than that of exposed group. The cholinesterase activity in the brain tissue of the treatment group are higher than that of the exposed group as well. All the results of chlorpyrifos subgroup are better than that of the diazinon and trithion subgroup among the group A in these comparisons, while the results of diazinon are better than that of the chlorpyrifos and trithion subgroup among the group B. The results of

the trithion subgroup showed the worst among the three subgroups. The differences are of statistical significance. The ultrastructural changes of rat's brain hippocampus cells were observed by light and electron microscope, which shows that the injury cause by the organophosphorus poisoning in the exposed group led to neuron cell necrosis and cavitation, emergence of heterocromatin, the breaking or disappearance of the cell zone under light microscope. The neurons become deformity, pyknotic nucleolus disappeared, the quantity of ribosome declined, the nuclear structure unclear and nucleus disaggregated under the electron microscope observation. In the treatment group, the damaged to the tissue and neurons shows slight. The injury to the brain cell in the chlorpyrifos subgroup is lest among the treatment group A, while the injury in the diazinon subgroup is lest among the group B. The damage to the neurons is the worst among the three subgroups, approximate to the exposed group.

**Conclusion:** 1 rhPON1 subtype isozymes with higher activity and yield can be expressed by the bac-to-bac system, which has the similar biological characteristics as natural hPON1 that the rhPON1<sub>R192</sub> with higher activity than the rhPON1<sub>Q192</sub>. The difference can be related to the molecular structure of the isozyme and similar to the human susceptibility to organophosphorus pesticides. 2 Glycosylation play an important role in keep the activity of PON1. The rhPON1 and natural hPON1 show the same substrate specificity before deglycosylation, while the molecular weight decreased to the same extent after deglycosylation and the specificity decreased. 3 Because the P-S bond is stronger than the P-O bond, PON1 can hydrolyze the P-O bond better than P-S, which make the organophosphorus chemicals with P-S bond harder to be hydrolyzed by the PON1. 4 The rhPON1 R/Q<sub>192</sub> can protect the rat's never system exposed to the organophosphorus pesticides, because it can debate the inhibition on the cholinesterase caused by the organophosphorus poisoning. The effects of rhPON1<sub>R192</sub> and rhPON1<sub>Q192</sub> as antidote against different organophosphorus pesticides are different