PIBB 生物化学与生物物理进展
Progress in Biochemistry and Biophysics
2010, 37(11): 1232~1239
www.pibb.ac.cn

Subchronic Toxicity Organophosphate Insecticide-induced Damages on Endothelial Function of Vessels in Rabbits by Inhibiting Antioxidases*

XIONG Xiao-Ming**, DAI Wen**, LI Peng, WU Shu-Jin, HU Min, LIU Li-Ying***
(*Department of Pharmacology, Pharmaceutical College, Central South University, Changsha*, 410078, China)

Abstract Organophosphates are some the widely used pesticides in the world, their mechanism of acute toxicity is associated with inhibiting acetylcholinesterase (AChE). Recently, increasing attention has been put to paraoxonase (PON1) which hydrolyzes OPs and prevents atherosclerosis by its anti-oxidation effects on low density lipoproteins (LDLs), resulted in decreased pro-inflammatory lipid peroxides formation. Since vascular endothelial dysfunction is known as the primary step in atherogenesis, the influence of OPs on the vascular endothelial function was assessed. Results showed that trichlorfon (18 mg•kg^{-1}) intragastrically daily for 70 days in rabbits resulted in a significant inhibition of endothelium-dependent relaxation (EDR), a decrease of endothelium nitric oxide synthase (eNOS) activity in isolated aorta and the changes of biochemical parameters in plasma, including a decrease of superoxide dismutase (SOD), PON1 and AChE activity and nitric oxide level and an increase of malondialdehyde (MDA) level. In addition, a direct exposure of rabbit aortic rings to paraoxon also inhibited EDR. These findings suggest that subchronic toxicity dose intragastrically trichlorfon or vessels to be exposed directly to paraoxon *in vitro* could induce dysfunction of vascular endothelium. The mechanisms may involve an inhibition of antioxidases and oxidative stress induced by OPs.

Key words trichlorfon, rabbit , paraoxonase, nitric oxide synthase, oxidative stress
DOI: 10.3724/SP.J.1206.2010.00332

Organophosphate pesticides (OPs) are used extensively as agricultural pesticides and household insecticides throughout the world, which can lead to high potential for causing environmental pollution and risk to human health due to long-term inhibition of acetylcholinesterase (AChE) and consumption of paraoxonase (PON1).

PON1, an arylesterase，is synthesized primarily in the liver and is secreted into the plasma, where it is exclusively bound to high-density lipoprotein (HDL) particles containing apoA-I and apoA-j [1]. Besides detoxificating OPs such as chlorpyrifos and trichlorfon, PON1 displays at least two other very important functions including the prevention of unsaturated lipid oxidation and decomposition of lipid peroxidation products of oxidized LDL and HDL in the plasma [2-3]. PON1 may also confer protection against damages of vessel wall by antioxidation and destroying oxidative productions. Despite a lot of literature reports on acute poisoning effects of OPs pesticides as inhibitors of AChE in laboratory animals [4-5], relatively few report on the harm of long-term and low subthreshold poisoning dose exposure to OPs through consumption of PON1.

This study evaluated the endothelium functional damage induced by both chronic daily low subthreshold poisoning of trichlorfon by oral gavage in rabbits and a direct incubation of vessels with paraoxon.

The results showed that both low-dose of trichlorfon by oral gavage for 70 days and direct

*This work was supported by a grant from The National Natural Science Foundation of China (30570754).

**These authors contributed equally to this work.
***Corresponding author.
Tel: 86-731-82355077, E-mail: Liyingliu2004@yahoo.com.cn
Received: June 24, 2010 Accepted: September 6, 2010

incubation of vessels with paraoxon resulted in vascular endothelial dysfunction and development of oxidation stress. Considering that the endothelial dysfunction triggered by oxidative stress characterized by lipid and protein oxidation in the vessel wall is a pivotal event and fundamental step in the pathogenesis of atherosclerosis[6], the results of this study may have important significances for prevention of atherosclerosis.

1 Materials and methods

1.1 Animals

This study was approved by the Institutional Animal Care and Use Committee, the Xiangya Medical College, Central South University, China, and performed according to the guidelines of animal ethical committee for use of experimental animals in China. The animals were individually housed in cages, given free access to water and normal feed.

1.2 Reagents

Acetylcholine, sodium nitroprusside (SNP), phenylephrine (Ph), ρ-nitrophenyl acetate (ρNPA) (substrates for the measurements of PON1 activities) was purchased from Sigma Chemical Co (St. Louis, USA). Cholesterol was purchased from Beijing Abxing Biological Technology Co (Beijing, China), trichlorfon and paraoxon were purchased from Institute of Agricultural Science (Changsha, China). Kits for measurement of MDA and nitric oxide (NO), eNOS, SOD and AChE , HDL-C， LDL-C, and total cholesterol (TC), triglyceride (TG) were purchased from Nanjing Jiancheng Bioengineering Institute, (Nanjing, China).

1.3 The experiment of isolated rabbit aortic ring *in vitro*

The chamber experiments were performed as described previously[7]. Briefly, the ring (3～4 mm in length) of thoracic aorta from normal rabbits, free of fat and connective tissue, was mounted in 6 ml modified Krebs bicarbonate buffer solution at 37℃ gassed with carbogen under tension of 6 *g*. After initial equilibration period of 90 min, ordinary Krebs solution was replaced with fresh Krebs solution containing 60 mmol/L KCl. After washing and another 30 min equilibration, contractile response was evoked by phenylephrine(1 μmol/L) to elicit reproducible responses. At the plateau phase of contraction, accumulative ACh (0.03 μmol/L to 3 mmol/L) or SNP (0.01 μmol/L to 1 mmol/L) was added into bath to induce the endothelium-dependent/non-dependent relaxation, following the rings were incubated with paraoxon at various concentration (3.63×10^{-4}～36.3 μmol/L) for 30 min respectively, the endothelium-dependent/non-dependent relaxation of rings were tested again. Each ring was connected to a force displacement transducer for the measurement of isometric force, which was recorded on-line on a computer *via* a multiple-channel transducer data acquisition system.

1.4 Experiment protocol *in vivo*

Thirty-two male New Zealand rabbits(2.5±0.5) kg were randomly assigned to one of the following 4 groups(with 8 rabbits in each group): animals in group 1 were fed regular rabbit chow (Control); eight in group 2 (trichlorfon treatment group) daily received trichlorfon capsules with a single dose of 18 mg/kg by gavage in the morning, the dosage of trichlorfon was determined as the preliminary results, which to equal 3.8%～4% LD_{50}.[8]; eight in group 3 (HCHL diet) received a high-cholesterol with high-lipid diet (consisted of 1% cholesterol, 7.5% yolk powder, 8% lard) at dosage of 50 g/d; eight in group 4 (trichlorfon plus HCHL diet) received an isodose trichlorfon plus HCHL diet. All animals were complemented with standard rabbit chow and had free access to water.

The rabbits were weighted and peripheral blood samples were collected from ear margin vein in the morning after a 12-h fast on the 35th day and on 70th day of the experiment, respectively. The end (on 70th day) of the experiment, the rabbits were anesthetized and killed by exsanguinations, the aorta was removed immediately for both organ-chamber experiment and analysis of biochemical parameters in vascular tissue. The aortas for biochemical tests were prepared 10% homogenates in an ice-cold buffer solution at –4℃ by a glass homogenizer (DY 89-1 model, Ningbo instruments, China). The tissue homogenization and blood samples were centrifuged at –4℃ , respectively, the supernatant of samples were frozen at –80℃ until being analyzed.

1.5 Measurement of serum lipid profile in rabbits

TC, LDL-C, HDL-C, TG concentrations in sera were measured as described by assay kit from company and using an automatic analysis instrument (Type 7170A, Hitachi).

1.6 Thiobarbituric acid reactive species(TBARS) measurement

MDA levels, as an index of lipid peroxidation, were measured. MDA reacts with thiobarbituric acid

(TBA) as a thiobarbituric acid reactive substance (TBARS) to produce a red colored complex which has peak absorbance at 532 nm.

In brief, 0.1 ml serum was mixed with 1 ml 1% TBA and 0.5 ml of 20% tricloroacetic acid. The mixture was incubated at 100 ℃ for 20 min. After cooling, the mixture was centrifuged at 12 000 *g* for 5 min and the absorbance was measured by spectrophotometrically at 532 nm. TBARS results were expressed as MDA equivalents using tetraethoxypropane as standard and expressed as g/L[9].

1.7 Assay of SOD activity

Assay of SOD activity was performed as previously described [9] and instruction of kit from company and using the inhibition of pyrogallol auto-oxidation. In brief, 1.2 ml of ethanol-chloroform (5∶3, *v*/*v*) was added to 750 μl of serum supernatant. After centrifugation, 25 μl of Tris-HCl was added to several volumes of the supernatant. Finally, 20 μl of pyrogallol was added to start the reaction. Results are expressed as U/L (one unit induces an inhibition of 50% pyrogallol auto-oxidation).

1.8 Assay of serum paraoxonase activity

Paraoxonase activity towards phenylacetate (arylesterase activity) was measured as previously described[10]. In brief, 10 μl serum supernatant was in cuvette in duplicate, then the substrate ρ-nitrophenyl acetate was added to several serum to yield a final concentration of 1 mmol/L in PBS (pH 8.0) buffer containing calcium and magnesium in 20 mmol/L Tris-HCl, at 25℃. After mixing, the cuvettes were read by a continuous spectrophotomeyric method at 270 nm. Non-enzymatic hydrolysis of nitrophenyl acetate was subtracted from the total rate of hydrolysis and 1 U of arylesterase activity is equal to 1 μmol/L of nitrophenyl acetate hydrolyzed per milliliter serum per minute.

1.9 Assay of acetylcholinesterase

The activity of AChE in serum was measured according to manufacturer's instruction from assay kit and Ellman's method [11]. In brief, the supernatant of serum was used as an enzyme source. All extraction steps were carried out at 4℃. Each sample was mixed with the reaction solution contained 5, 5'-dithiobis-2-nitrobenzooic acid (DTNB) and substrate-acetylthiocholine and incubated for 15 min at 37℃. The rate of hydrolysis of acetylcholine in serum by AChE was spectrophotometrically monitored. The absorbance was read at 520 nm. The activity of AChE was calculated as pointed out by assay kit and expressed in per units per milliliter of serum per minute($U \cdot min^{-1} \cdot ml^{-1}$).

1.10 Assay of nitric oxide synthase activity

Total NOS activity in aortic tissue was quantified by measuring conversion of [^{3}H]-L-arginine to [^{3}H]-L-citrulline as we have previously reported [8] using a Nitric Oxide Synthase Assay Kit according to the manufacturer's instructions.

1.11 NO measurement in sera

The content of NO was measured as described previously [12] using the Griess reagent, following the manufacturer's instructions. Serum levels of nitrate and nitrite were measured spectrophotometrically at 540 nm. The amount of total nitrate and nitrate in each sample was calculated by linear regression using the absorbance of the sodium nitrate standards. Total nitrite levels were represented the degree of NO production and was defined as μmol/L in each serum sample.

1.12 Statistical analysis

All values are expressed as $\bar{x} \pm s$. Data were analyzed using a one-way or two-way ANOVA followed by Newman-Student's *t*-test. $P < 0.05$ was considered significant.

2 Results

2.1 Endothelium-dependent relaxation

To examine whether OPs insecticide could induce impairment of vascular function, the EDR to ACh and the endothelium-independent relaxation to sodium nitroprusside (SNP) in aortas of rabbits were examined *in vitro*. A direct incubation of thoracic aorta rings of rabbits with various concentration of paraoxon *in vitro* resulted in a significant impairment of the EDR of aortas in a concentration (Figure 1a) and time-dependent manner (Figure 1b). The rabbits were administrated chronic subtoxic trichlorfon by gavage for 70 days resulted similarly in the impairment of vascular EDR (Figure 2a). It was seen that oral gavage of trichlorfon aggravated significantly injury of EDR induced by high-cholesterol plus high-lipid(HCHL) diet(Figure 1c). There was no substantial difference in endothelium-independent relaxation by SNP among these groups which received oral gavage of trichlorfon, HCHL diet and so on (Figure 2b).

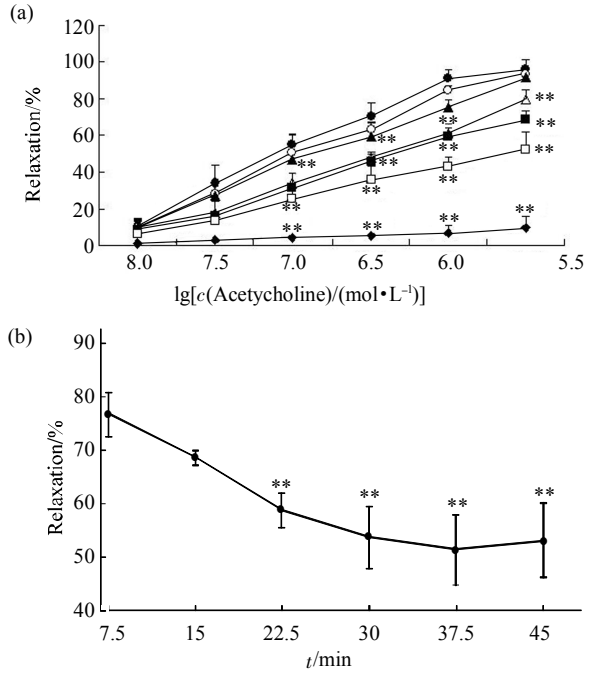

Fig. 1　Concentration- and time-dependent effect of paraoxon on ACh- induced endothelium-dependent relaxation

(a) Exposure of rabbit thorax aortic rings to paraoxon at various concentrations ($3.63 \times 10^{-4} \sim 36.3$ μmol/L) for 30 min *in vitro*.●—●: Control; ○—○: 3.63×10^{-4} μmol/L paraoxon; ▼—▼: 3.63×10^{-3} μmol/L paraoxon; △—△: 3.63×10^{-2} μmol/L paraoxon; ■—■: 0.363 μmol/L paraoxon; □—□: 3.63 μmol/L paraoxon; ◆—◆: 36.3 μmol/L paraoxon; (b) Exposure of rabbit thorax aortic rings to paraoxon at concentration of 3.63 μmol/L for different times. Data are expressed as $\bar{x} \pm s$, n=6 (rings from different rabbits) * $P < 0.05$, ** $P < 0.01$ *vs* control.

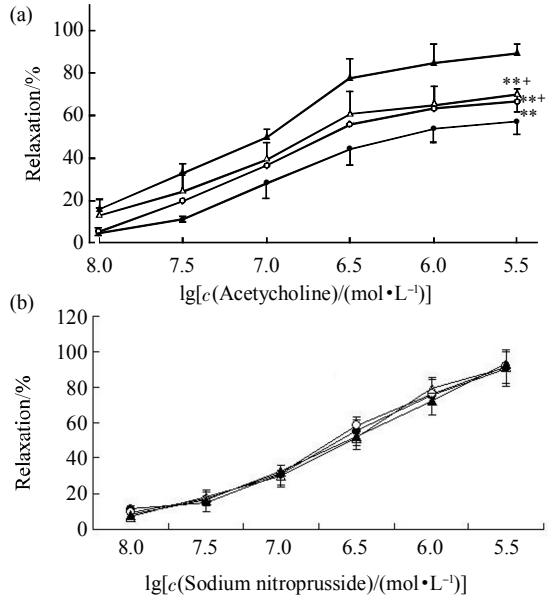

Fig. 2　Changes of ACh-induced endothelium-dependent relaxation (a) and changes of SNP- induced endothelium-independent relaxation (b) in rabbit isolating thorax aortic rings (*in vitro*)

The rabbits were exposed to trichlorfon alone or HCHL diet alone or trichlorfon plus HCHL diet for 70 days. Data are expressed as $\bar{x} \pm s$, n=8 (rings from different rabbits). *P<0.05, **P<0.01 *vs* control, ^{+}P<0.05 *vs* trichlorfon plus HCHL diet group. (a)▲—▲: Control; △—△: Trichlorfon; ●—●: Trichlorfon +HCHL; ○—○: HCHL. (b) ■—■: Control; ○—○: Trichlorfon; ▼—▼: Trichlorfon+HCHL; △—△: HCHL.

2.2　Effects of trichlorfon or HCHL on activity of nitric oxide synthases and nitric oxide production

As shown in Figure 3, the rabbits were received subpoisoning trichlorfon by gavage or HCHL diet alone and HCHL diet plus daily oral gavage of trichlorfon decreased significantly eNOS activity in aortic tissues and NO production in sera. But the decreases of both eNOS activity and NO production were more significant in HCHL diet plus daily oral gavage of trichlorfon than that of HCHL diet or daily oral gavage of trichlorfon alone.

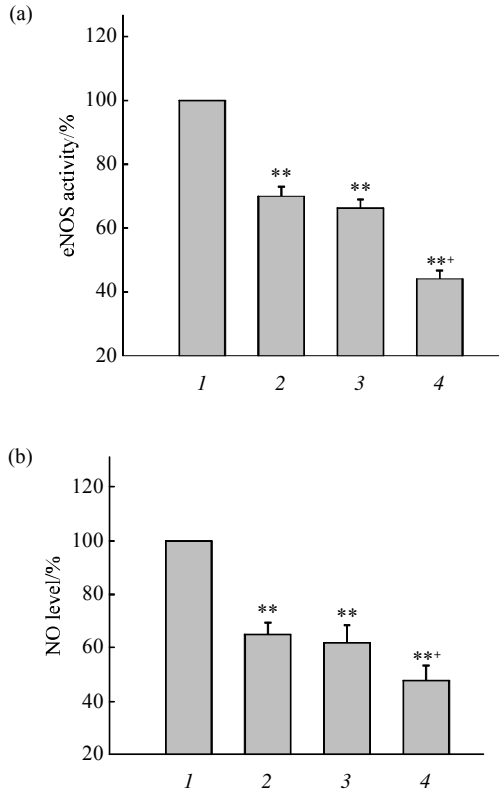

Fig. 3　Changes of nitric oxide synthase in aortic tissues(a) and level of nitrite/nitrate in sera(b)

The rabbits were exposed to trichlorfon alone or HCHL diet alone or trichlorfon plus HCHL diet for 70 days. Data are expressed as $\bar{x} \pm s$, n=8, ** $P < 0.01$ *vs* control group, $^{+}P < 0.05$ *vs*. trichlorfon alone or HCHL alone group. *1*: Control; *2*: Trichlorfon; *3*: HCHL; *4*: HCHL + Trichlorfon.

2.3　Activity of acetylcholinesterase (AChE) in serum

As shown in Figure 4a, daily oral gavage of trichlorfon for 5～10 weeks resulted in a significantly reduction of activity of AChE in sera as compared with control group and before treatment. But HCHL diet increased significantly activity of AChE in sera. The

activity of AChE was higher in HCHL plus trichlorfon than trichlorfon alone. HCHL diet could counteract inhibiting effect of trichlorfon on AChE.

2.4 Activity of serum paraoxonase

The serum PON1 activity was not different among the four groups at the base line. HCHL diet alone or daily oral gavage of trichlorfon alone or HCHL diet plus daily oral gavage of trichlorfon for 70 days resulted in a significant and a time-dependent reduction of PON1 activity (Figure 4b) as compared with control group. The decrease of the PON1 activity was more significant in HCHL diet plus trichlorfon than in HCHL diet or trichlorfon alone group(Figure 4b).

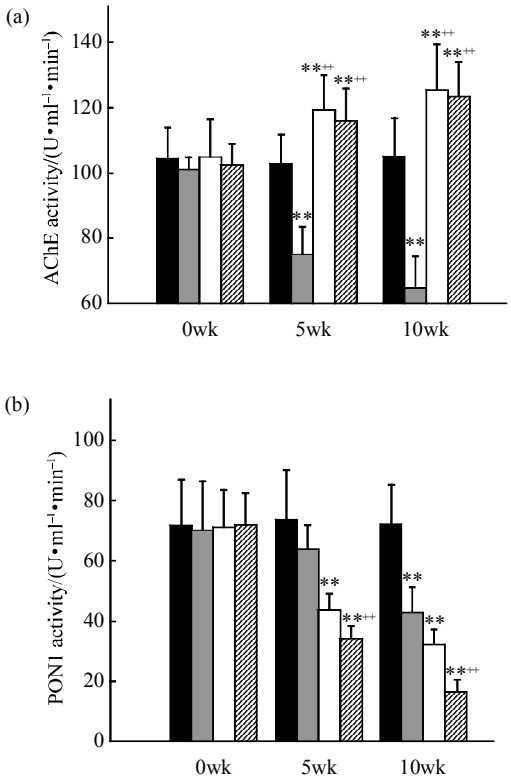

Fig. 4 Activity of AChE (a) and PON1 (b) in rabbit sera at different time points of the experiments

The rabbits were exposed to trichlorfon alone or HCHL diet alone or trichlorfon plus HCHL diet for 70 days. Data are expressed as $\bar{x} \pm s$, n=8, $^{**}P < 0.01$ *vs* control group, $^{++}P < 0.01$ *vs* trichlorfon alone or HCHL diet alone groups. ■: Control; ▒: Trichlorfon; □: HCHL; ▨: HCHL+ Trichlorfon.

2.5 The content of serum malonyldialdehyde and activity of superoxide dismutase

In order to explore whether trichlorfon induces injuries of endothelial function relate to trigger of internal oxidation stress, both serum MDA content and SOD activity were measured. The results as shown in Figure 5, HCHL diet, and trichlorfon alone and HCHL diet plus trichlorfon resulted significantly in an increase of MDA content and a decrease of SOD activity as compared with control group or before treatment. HCHL diet plus oral gavage of trichlorfon aggravated the changes of both MDA and SOD induced by HCHL or trichlorfon alone.

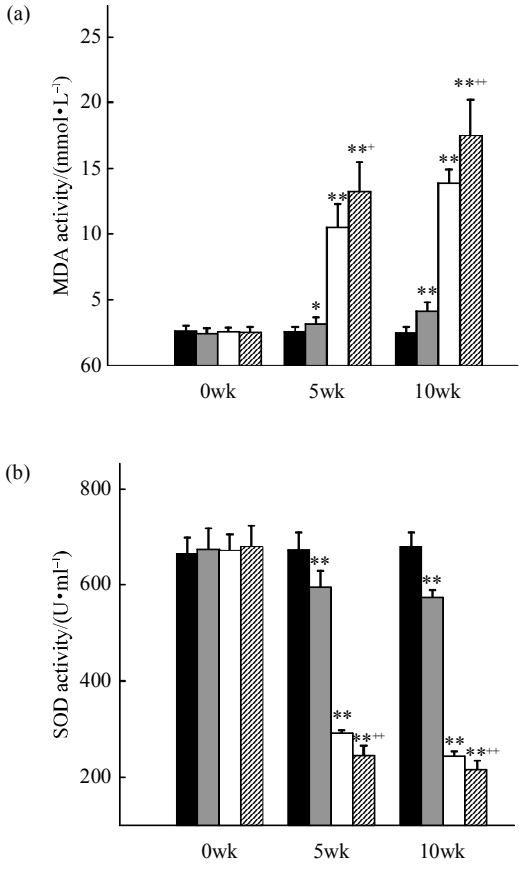

Fig. 5 Changes of MDA level (a) and SOD activity (b) in sera at different time points of the experiments

The rabbits were exposed to trichlorfon alone or HCHL diet alone or trichlorfon plus HCHL diet for 70 days. Data are expressed as $\bar{x} \pm s$, n=8, $^{**}P < 0.01$ *vs* control, $^{++}P < 0.01$ *vs* trichlorfon alone or HCHL alone groups. ■: Control; ▒: Trichlorfon; □: HCHL; ▨: HCHL+Trichlorfon.

2.6 Changes of serum lipid profile and weight

The serum lipid profile including level of TC, LDL-C and TG in both HCHL or HCHL plus trichlorfon group were significantly increased as compared with those in control group and before treatment, but these lipoprotein and lipid did not significantly change in control and trichlorfon alone group at time points of 5th or 10th week after

beginning treatment (data did not show).

The weight changes in the various groups were similar to those changes of serum lipid profile. The body weight of rabbits was significantly decreased in oral gavage of trichlorfon group alone but was increased in HCHL diet alone and HCHL diet plus oral gavage of trichlorfon group as compared to control group ($P < 0.05$, data did not show).

4 Discussion

The organophosphate pesticides (OPs) are widely used in agriculture as the pest control of crops in worldwide.Trichlorfon is one of the OPs mainly used in China. Organophosphate poisons by at least two mechanisms: first, plasma AChE binds these poisons but does not destroy them; second, OPs are bioactivated to highly toxic oxon forms by the cytochrome P450 systems, followed by that paraoxonase (PON1) destroys them by hydrolysis to harmless products that are excreted[13–14]. It was found that Low PON1 activity is associated with cardiovascular disease [15] and OPs sensitivity [16–17]. There are numerous evidences from both epidemiological and experimental laboratory studies that sublethal impact of short term exposure to the OPs due to inhibiting AChE induced the acute poisoning events. But no much experimental study is available on long-term, chronic subintoxicol dose exposure to OP pesticides due to consumption of PON1 whether or not causes direct vascular damages. The results of current study demonstrated that rabbits received an oral gavage of trichlorfon (18 mg/kg body weight for 70 days) alone or oral gavage of trichlorfon plus HCHL diet besides inhibiting both activity of AChE and PON1, also resulted in a significant inhibition of acetylcholine-induced EDR of aorta. It was found that the inhibition of EDR was associated with the decrease of both plasma nitric oxide level and NOS activity in aortic tissues. The changes of these parameters in HCHL diet alone were similar to those induced by trichlorfon, but the changes in oral gavage of trichlorfon plus the HCHL diet group were more pronounced than those in anyone alone groups, which illuminate either chronic oral gavage of trichlorfon or HCHL diet to aggravate each other endothelial damages. In addition, in current study, we also observed that a direct incubation of paraoxon (an active component of OPs) with the thoracic aortic rings of rabbits *in vitro* resulted in damages of EDR of the aortic artery rings. The present study is one of the few studies performed so far addressing damages in vascular endothelial function to relate to consumption of PON1 in animal experiment study by subchronic exposure to OP pesticides. To our best knowledge, so far no study has observed directly injuring action of OPs on vascular endothelial function in animal experimental model *in vivo* and *in vitro*. The present study thus firstly provide evidence that the chronic, long-term (for 70 days), subpoisoning dose (round about 3.8%～4% of LD_{50})[18] expose to trichlorfon or a direct incubation of artery rings with paraoxon could cause a significant vascular endothelial dysfunction, which was associated with the decreases of PON1 activity.

Acetylcholine-induced EDR is mediated by the stimulation of endothelial NOS activity which leads to the synthesis of NO. The release of NO by endothelial cells is important in the mediation of EDR. It also interferes with key events in the development of atherosclerosis, inducing, for example, oxidative damage to the endothelium, monocyte adhesion and migration to vessel walls, platelet aggregation, and vascular smooth muscle proliferation. Although the precise mechanisms underlying the trichlorfon (and paraoxon)-induced impairment of EDR are not fully understood, increasing the degradation of NO and reducing endothelium-derived NO synthesis could be implicated in this dysfunction. The present study demonstrates that OPs (trichlorfon and paraoxon) impaired the EDR of the rabbit aortic artery *in vivo* and *in vitro*, and associated with the decrease of NOS activity and levels of nitrite/nitrate, stable end products of NO that reflect NO synthesis. These results suggested that OP pesticide contributes to endothelial dysfunction by increasing NO degradation and reducing endothelium-derived NO synthesis by decreasing activity of eNOS.

Our study results demonstrated that subchronic exposure to trichlorfon on one hand increased MDA formation, on the other hand, decreased in the SOD activity. This is supported by reporter of literature [19] that the agriculture workers acute exposing to various OPs resulted in the decreases of anti-oxidative enzyme activities. These data suggested that vascular endothelial dysfunction induced by subchronic exposure to trichlorfon was linked to formation lipid peroxidation and reduction of oxygen free radical scavenging enzymes. The results of this study also are in accordance with other studies [20-21]. The lipid

peroxidation has been reported as a major contributor to the loss of cell function under oxidative stress conditions [22]. MDA is an indicator of the lipid peroxidation, and its level increases in tissues when they are exposed to oxidative stress. In the present study, we observed that MDA levels increased in three groups of received treatments but trichlorfon plus the HCHL diet group was more significant than either trichlorfon or the HCHL diet alone group. The SOD catalyses the destruction of the superoxide radical and protects oxygen-metabolizing cells against harmful effects of superoxide free radicals. This study demonstrated that the subchronic exposure of rabbits to trichlorfon resulted in the decrease of SOD activity. This observation is in accordance with other animal studies [23] and may result from the oxidative stress induced by OPs exposure. A lower SOD activity favours the accumulation of oxygen free radicals in blood and other tissue cells, leading to tissue damage. Oxidative stress occurs when the critical balance between oxidants and antioxidants is disrupted due to the depletion of antioxidants or excessive accumulation of the reactive oxygen species (ROS), or both. It has been shown that PON1 contains three cysteine [24] residues with a disulfide bond and can metabolize active oxidized phospholipids and to destroy lipid hydroperoxides. Pesticides are recently known to be able to induce *in vitro* and *in vivo* generation of ROS [25]. It is speculated that trichlorfon induced-damages of EDR not only relate to decreasing PON1 activity, but increasing generation of ROS and decreasing SOD activity.

In conclusion, based on these effects *in vitro* and *in vivo* findings, it was concluded that subchronic and sub-poisoning dose exposure to organophosphate pesticides trichlorfon may induce vascular endothelial dysfunction, decrease of NOS activity, NO production and PON1 activity. The oxidative stress induced by organophosphate pesticides may be attributed to a rather insufficient antioxidant capability owing to a long consumption of PON1 and may be a molecular mechanism involved in organophosphate pesticides-induced endothelium dysfunction.

Acknowledgment We thank professor Chen Xiu for modify on the language of our paper.

References

[1] Kujiraoka T, Hattori H, Miwa Y, *et al*. Serum apolipoprotein j in health, coronary heart disease and type 2 diabetes mellitus. J Atheroscler Thromb, 2006, **13**(6): 314−322

[2] Mackness B, Mackness M I, Arrol S, *et al*. Effect of the human serum paraoxonase 55 and 192 genetic polymorphisms on the protection by high density lipoprotein against low density lipoprotein oxidative modification. FEBS Lett, 1998, **423**(1): 57−60

[3] Sözmen E Y, Mackness B, Sözmen B, *et al*. Effect of organophosphate intoxication on human serum paraoxonase. Hum Exp Toxicol, 2002, **21**(5): 247−252

[4] Rice P J, Rice P J, Arthur E L, *et al*. Advances in pesticide environmental fate and exposure assessments. J Agric Food Chem, 2007, **55**(14): 5367−5376

[5] Kujiraoka T, Hattori H, Miwa Y, *et al*. Organophosphate pesticides: biochemistry and clinical toxicology. Ther Drug Monit, 2002, **24**(1): 144−149

[6] Roquer J, Segura T, Serena J, *et al*. Endothelial dysfunction, vascular disease and stroke: the ARTICO study. Cerebrovasc Dis, 2009, **27**(1): 25−37

[7] Wang S, Peng Q, Zhang J, *et al*. Na^+/H^+ exchanger is required for hyperglycaemia-induced endothelial dysfunction *via* calcium-dependent calpain. Cardiovasc Res, 2008, **80**(2): 255−262

[8] Karademir Catalgol B, Ozden S, Alpertunga B. Effects of trichlorfon on malondialdehyde and antioxidant system in human erythrocytes. Toxicol In Vitro, 2007, **21**(8): 1538−1544

[9] Wang S X, Liu L Y, Hu M, *et al*. Na^+/H^+ exchanger inhibitor prevented endothelial dysfunction induced by high glucose. J Cardiovasc Pharmacol, 2005, **45**(6): 586−590

[10] Mackness B, Durrington P, Povey A, *et al*. Paraoxonase and susceptibility to organophosphorus poisoning in farmers dipping sheep. Pharmacogenetics, 2003, **13**(2): 81−88

[11] Ellman G L, Courteny K D, Andres V J, *et al*. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol, 1961, **7**: 88−95

[12] Liu Y H, Liu L Y, Wu J X, *et al*. Comparison of captopril and enalapril to study the role of the sulfhydryl-group in improvement of endothelial dysfunction with ACE inhibitors in high dieted methionine mice. J Cardiovasc Pharmacol, 2006, **47**(1): 82−81

[13] Davies H G, Richter R J, Keifer M, *et al*. The effect of the human serum paraoxonase polymorphism is reversed with diazoxon, soman and sarin. Nat Genet, 1996, **14**(3): 334−336

[14] Furlong C. PON1 status and neurologic symptom complexes in Gulf War veterans. Genome Res, 2000, **10**(2): 153−155

[15] Mackness M I, Durrington P N, Mackness B. The role of paraoxonase 1 activity in cardiovascular disease: potential for therapeutic intervention. Am J Cardiovasc Drugs, 2004, **4**(4): 211−217

[16] Furlong C E, Cole T B, Jarvik G P, *et al*. Role of paraoxonase (PON1) status in pesticide sensitivity: genetic and temporal determinants. Neurotoxicology, 2005, **26**(4): 651−659

[17]Costa L G, Cole T B, Jarvik G P, *et al*. Functional genomics of the paraoxonase (PON1) polymorphisms: effects on pesticide sensitivity, cardiovascular disease and drug metabolism. Annual

Review of Medicine, 2003, **54** (1): 371−392

[18] Karademir Catalgol B, Ozden S, Alpertunga B. Effects of trichlorfon on malondialdehyde and antioxidant system in human erythrocytes. Toxicol *In Vitro*, 2007, **21**(8): 1538−1544

[19] López O, Hernández A F, Rodrigo L, *et al*. Changes in antioxidant enzymes in humans with long-term exposure to pesticides. Toxicol Lett, 2007, **171**(3): 146−153

[20] Poovala V S, Kanji V K, Tachikawa H, *et al*. Role of oxidant stress and antioxidant protection in acephate-induced renal tubular cytotoxicity. Toxicol Sci, 1998, **42**(2): 403−409

[21] Banerjee B D, Seth V, Bhattacharya A, *et al*. Biochemical effects of some pesticides on lipid peroxidation and free-radical scavengers. Toxicol Lett, 1999, **107**(1−3): 33−47

[22] Monteiro D A, de Almeida J A, Rantin F T, *et al*. Oxidative stress biomarkers in the freshwater characid fish, Brycon cephalus, exposed to organophosphorus insecticide Folisuper 600 (methyl parathion). Comp Biochem Physiol C Toxicol Pharmacol, 2006, **143**(2): 141−149

[23] Panemangalore M, Bebe F N. Short- and long-term exposure to low levels of pesticide and flavonoid mixtures modify endogenous antioxidants in tissues of rats. J Environ Sci Health B, 2009, **44**(4): 357−364

[24] Costa L G, Vitalone A, Cole T B, *et al*. Modulation of paraoxonase (PON1) activity. Biochem Pharmacol, 2005, **69**(4): 541−550

[25] Karademir Catalgol B, Ozden S, Alpertunga B. Alpertunga, Effects of trichlorfon on malondialdehyde and antioxidant system in human erythrocytes. Toxicology *in Vitro*, 2007, **21**(8): 1538−1544

亚慢性亚毒性有机磷酯类杀虫剂抑制抗氧化酶而致兔血管内皮功能损伤 *

熊小明 ** 戴 雯 ** 李 鹏 吴树金 胡 敏 刘立英 ***

(中南大学药学院药理教研室，长沙 410078)

摘要 有机磷酸酯类(OPs)是全球最广泛使用的杀虫剂之一．其除了抑制胆碱酯酶(AChE)活性外， 也抑制对氧磷酶(PON1)的活性．其急性中毒主要与抑制 AChE 有关．最近，OPs 对 PON1 的影响已引起学术界广泛关注．因为 PON1 除了有水解 OPs 的功能外，也有抗低密度脂蛋白(LDL)氧化和降解 LDL 中的脂质过氧化物的作用．因为血管内皮功能损伤是动脉粥样硬化形成的起始步骤．将探讨 OPs 对血管内皮功能的影响作为研究目的．研究结果表明，连续每天给兔灌胃敌百虫(18 mg/kg)70 天，可导致其离体血管内皮依赖性舒张(EDR)反应和 eNOS 活性显著性降低，血浆超氧化物岐化酶活性、一氧化氮水平、PON1 和 AChE 活性降低，脂质过氧化代谢产物丙二醛水平增加．OPs 的活性成分——对氧磷在体外与兔胸主动脉环直接孵育也能浓度和时间依赖性地显著抑制 EDR．研究结果提示，亚慢性 - 亚毒性剂量的敌百虫灌胃或对氧磷与离体血管环直接孵育，均可导致兔血管内皮功能损伤，其机制可能与 OPs 抑制氧化酶和诱发氧化应激反应有关．

关键词 敌百虫，兔，对氧磷酶，一氧化氮合酶， 氧化应激

学科分类号 R363 **DOI**: 10.3724/SP.J.1206.2010.00332

* 国家自然科学基金资助项目(30570754).

** 共同第一作者.

*** 通讯联系人.

Tel: 0731-82355077, E-mail: liyingliu2004@yahoo.com.cn

收稿日期：2010-06-24，接受日期：2010-09-06