Effects of Organophosphate Insecticides on Mechanical Properties of Rat Aorta

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Summary

The present study was carried out to search whether organophosphate pesticides affect the mechanical properties of the thoracic aorta. Wistar female rats (aged 6-8 weeks) were assigned randomly to a control group and groups treated with either dichlorvos or chlorpyriphos for 90 days at a dose of 5 mg/kg/day. After that period, animals were killed and thoracic aorta strips in longitudinal direction were isolated. The stress, strain and elastic modulus were obtained from the strips. Our results showed that chronic administration of chlorpyriphos and dichlorvos caused downward shift of the stress-strain relations compared to the control curve. The elastic modulus-stress curve revealed distinct characteristics in the low and high stress regions. A power function was used to simulate the low stress region while a line was fit to the high stress region. Curve fitting procedure illustrated that both pesticides influenced mainly the high stress region, but they had diverse effects at the low stress region. The results also imply that chlorpyriphos and dichlorvos decrease the strength of the aorta and therefore might influence the response of the aorta to mechanical loading induced by blood pressure.

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Introduction

The mechanical properties of aorta have important role in regulating left ventricular performance, myocardial perfusion and blood flow in the circulation system (O'Rourke *et al.* 2004, Safar *et al.* 2003). When the aorta becomes stiffer, the systolic pressure increases as a result of the stiffer wall. In addition, the reflected pressure wave returns earlier in a stiffer vessel and augments the systolic pressure (Nichols and O'Rourke 2005, Safar *et al.* 2003). The increased systolic pressure in turn causes an increase in load on the heart. Conversely, a decrease in the stiffness of the aortic wall would lead to a reduced load on the heart by decreasing the impedance of the aorta. Besides to its conduit function, the aorta acts as an elastic chamber. It absorbs part of the hydraulic energy imparted to the blood during systole and releases it later during diastole, thus converting the pulsatile flow from the heart into a more steady flow in the arterial system. Therefore, the mechanical properties of the aorta are crucial for its proper function. Increase in the aortic stiffness associated with aging (Zulliger and Stergiopulos 2007), hyperlipidemia (Tyrell *et al.* 2001), atherosclerosis (Giannattasio 2006), and smoking (Mahmud and Feely 2003) leads to impairment in its conduit and Windkessel functions. Decrease in the stiffness and strength of aorta due to abnormalities in the biosynthesis or structure of aortic wall elements also causes cardiovascular pathologies, because the wall material can not resist high blood pressure (Fisher *et al.* 1991, Wenstrup *et al.* 2006). Substances, which prevent the formation of cross-links in

elastin or collagen, increase the extensibility and reduce the ability of aorta to withstand the force of blood pressure (Brüel *et al.* 1998). Biosynthetic growth hormone intake decreases the elastin content and stiffness at the low strain region in rat aorta (Brüel and Oxlund 1991). Therefore it is important to assess the environmental factors which affect the mechanical properties of aorta.

Organophosphate insecticides are widely used for the control of agricultural, industrial and domestic pests. However, the uncontrolled use of insecticides has diverse effects on ecological system and public health. There are also several studies on the effects of insecticides on the circulation system (Davies *et al.* 2008, Richardson *et al.* 1975, Smith *et al.* 2001, Berberian and Enan 1987, Saldana *et al.* 2009). In addition, a number of studies reported that acute and chronic toxicity of organophosphate insecticides could lead to degeneration of collagenous and elastin fibers of vascular wall (Antov *et al.* 1984, Akimov and Kolesnichenko 1985, Yavuz *et al.* 2005). However, as to our knowledge, there is no study investigating the effects of organophosphate compounds on the mechanical properties of arteries. The aim of this study is to investigate the chronic effects of organophosphate insecticides, namely chlorpyriphos and dichlorvos (2,2-dimethyl-dichlorovinyl phosphate), on the mechanical properties of aorta.

Methods

Twenty-five healthy adult female Wistar rats (6-8 weeks of age, average body weight 150-200 g) were used in this study. Rats were obtained from the Experimental Animal Center, University of Mersin, Turkey. The study was approved by the research and ethical committee of the Mersin University. The rats had free access to standard laboratory diet and water, and were maintained according to the recommendations of the National Instutes of Health's guidelines for the care and use of laboratory animals. The rats were randomly divided into three experimental groups as follows: control group (n=8), chlorpyriphos group (n=8) and dichlorvos group (n=9). Literature survey showed that different doses have been used to observe the chronic effects of chlorpyriphos (2.5-38 mg/kg/day) and dichlorvos (0.2-12 mg/kg/day) in rats (Raheja and Gill 2007, Verma *et al.* 2009, Kobayashi *et al.* 1980, Kaur *et al.* 2007). It is reported that 5 mg/kg-day is the dose of chlorpyrifos which inhibits the AChE activity in the brain when it is

administered repeatedly for ninety days. Therefore, the chlorpyriphos and the dichlorvos groups were treated by intraperitoneal injection of chlorpyriphos and dichlorvos for 90 days at a dose of 5 mg/kg/day, dissolved in 0.5 ml distilled water. Only 0.5 ml distilled water was given in the same way to the control rats. At the end of 90 days period, the animals were sacrificed by a high dose of ether anesthesia. Thoracic aorta was dissected and put into the Krebs solution of the following composition (in mM): NaCl 118; KCl 4.7; $CaCl_2$ 2.5; KH_2PO_4 1.18; $MgSO_4$ 0.57; $NaHCO_3$ 14.2; glucose 5.5 at 4 °C. Then it was cleaned of connective tissues. Longitudinal strip 6 mm in length and 0.5 mm in width was prepared and put horizontally in an organ bath of 25 ml containing Ca^{2+}-free Krebs solution. Ca^{2+}-free solution was prepared as the Krebs solution but by omitting $CaCl_2$ and adding 2 mM EGTA (ethylene glycol tetraacetic acid). One end of the preparation was connected to a vernier system, which was used to apply length perturbations, and the other end to a force transducer (Grass FT03, Grass Instruments, Quincy, USA). The force response from the transducer was amplified (Hugo Sachs 301, Germany) and then digitized by 12 bit A/D converter with a sampling frequency of 50 Hz, and stored on a computer for offline analysis. Throughout the experiments, the perfusing solution was gassed with a mixture of 95 % O_2 and 5 % CO_2. All experiments were conducted at 37 °C.

Experimental procedure

Initially, the preparation was attached to the system loosely and left in the Ca^{2+}-free solution for one hour. After the muscle relaxed completely, the length of the preparation was increased until a deflection was seen in the force. The length of the preparation at this condition was assumed as the length at no load condition and denoted by *Lo*. Length changes of 0.1 mm were applied every 2 min until the length of the preparation was *2Lo*. This procedure was applied three times and the last one was used for the evaluation of the force-length relation. At the end of the experiment, the weight of the muscle strip was measured after blotting the muscle briefly on a filter paper.

Mechanical data analysis

In the present study, the mechanical properties of the aorta were investigated in the longitudinal direction. Thus, the longitudinal strain (ε) was calculated as the ratio of the length change (*L-Lo*) to the length (*Lo*) at no load condition. Stress (σ) was defined

as the ratio of the force to the cross-sectional area. The cross-sectional area was calculated by dividing the weight of the strip to its length (L), assuming that the aorta is incompressible and the density of the aorta equals to 1.06 g/cm³. The elastic moduli were calculated as the slope of the stress-strain relations and plotted against stress. The general course of the stress-strain relationship presented in this study (Fig. 1A) was similar to those given in the literature for arteries (Silver *et al.* 2003, Coulson *et al.* 2002, Cox 1978, Dobrin 1978). Therefore, mathematical models used in the literature were tested in the present study to quantify the elastic properties of the aorta. Models with a single or multiple exponential functions or a combination of a line and exponential functions did not produce a good fit to the stress-strain relationship. However, two distinct regions were identified in the elastic moduli vs stress curves, referred to as the low-stress and high-stress region (Fig. 1B). The method employed by Sokolis *et al.* (2002) was used for the analysis of each region. The elastic modulus-stress curve at the low stress region was a type of nonlinear increasing function. Therefore a power function of the form $Y(\sigma) = A\sigma^B$ was used for simulation of the low stress region (Sokolis *et al.* 2002), where Y is the elastic modulus, A is the coefficient and B is the power of the stress (Fig. 1B, filled circles). On the other hand, the high stress region was a linearly increasing curve. Thus the equation $Y = m\sigma + yo$ was fit to the high stress region where m is the slope of the elastic modulus-stress curve, yo is the intersection of the elastic modulus and the stress axis (Fig. 1B, empty circles). First, starting from the last point, the line fitting procedure was carried out such that the number of data included to the fitting was varied unless the correlation coefficient reached a maximum (Fischer *et al.* 1991, Sokolis *et al.* 2002). Then the remaining points at the low stress region were fit to the power function. Curve fitting procedures were performed by linear and nonlinear least square analysis methods.

Statistical analysis

All measurements and fitted parameters are expressed as means ± S.E.M. After documenting normal distribution (Kolmogorov-Smirnov), the statistical comparisons were performed by using one-way analysis of variance and Tukey *post-hoc* test for curve-fitting parameters. Values of P<0.05 were considered statistically significant.
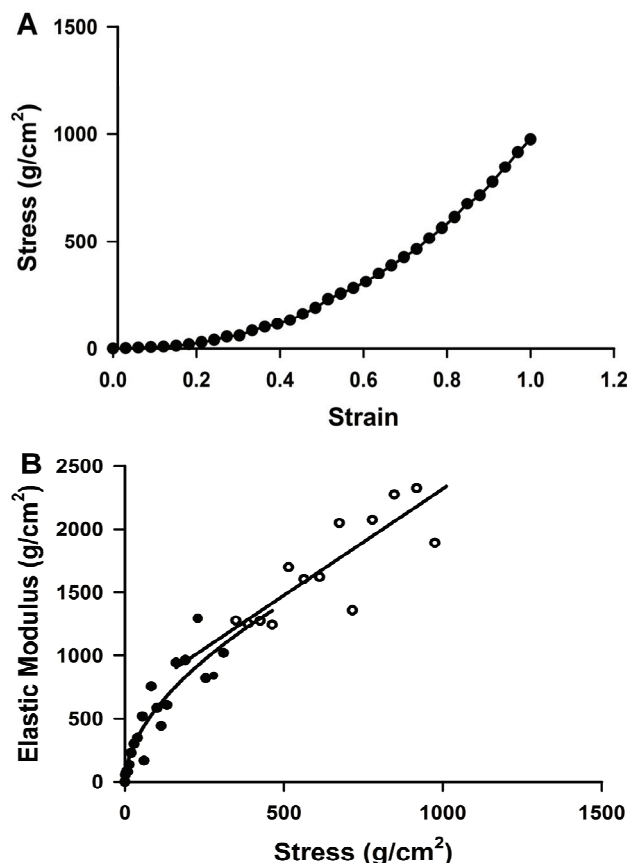

Fig. 1. A typical stress-strain relationship of an aortic strip in the control group (**A**) and the elastic modulus-stress relationship (scattered data) of the same strip (**B**) calculated as the slope of the stress-strain curve in (A). The elastic modulus-stress data at the low stress region (closed circles) were fit to the power function of the form Aσ^B, where A=46.49 g/cm² and B=0.549 (R=0.9519) for this preparation. Data at the high stress region (open circles) were fit to a line whose slope was 1.68 and intersect was 632.91 g/cm² (R=0.9367). The curves of the fitted equations were illustrated in solid lines.

Results

The chronic administration of chlorpyriphos and dichlorvos for 90 days caused a downward shift of the stress-strain relations compared to the control curve (Fig. 2A). Stress values at any level of strain in the dichlorvos group and at strains above 0.5 in the chlorpyriphos group were significantly decreased compared with the values in the control group (P<0.05). The decrease in the stress was greater in the dichlorvos group than in the chlorpyriphos group. The relationship between the elastic modulus and stress revealed a rapid rise initially; then increased steadily (Fig 1B, Fig. 2B). The equations used in the simulation of the elastic modulus-stress relationship provided a good fit to data in all groups at each region (R>0.9327). The fit parameters for the curves are given in Table 1.

Table 1. The mean values of the fit parameters of the control, chlorpyriphos and dichlorvos groups for the low and high stress regions.

	Low Stress Region ($A\sigma^B$)		High Stress Region ($m\,\sigma + y_0$)	
	A (g/cm^2)	B	m	yo (g/cm^2)
Control (n=8)	44.33 ± 2.44	0.563 ± 0.013	1.95 ± 0.15	451.84 ± 69.65
Chlorpyriphos (n=7)	55.61 ± 6.62	0.506 ± 0.015*	1.51 ± 0.07*	488.35 ± 44.30
Dichlorvos (n=7)	52.80 ± 6.75	0.549 ± 0.03	1.53 ± 0.10*	548.61 ± 55.76

* Significantly different from control at p<0.05. The values are mean ± S.E.M.
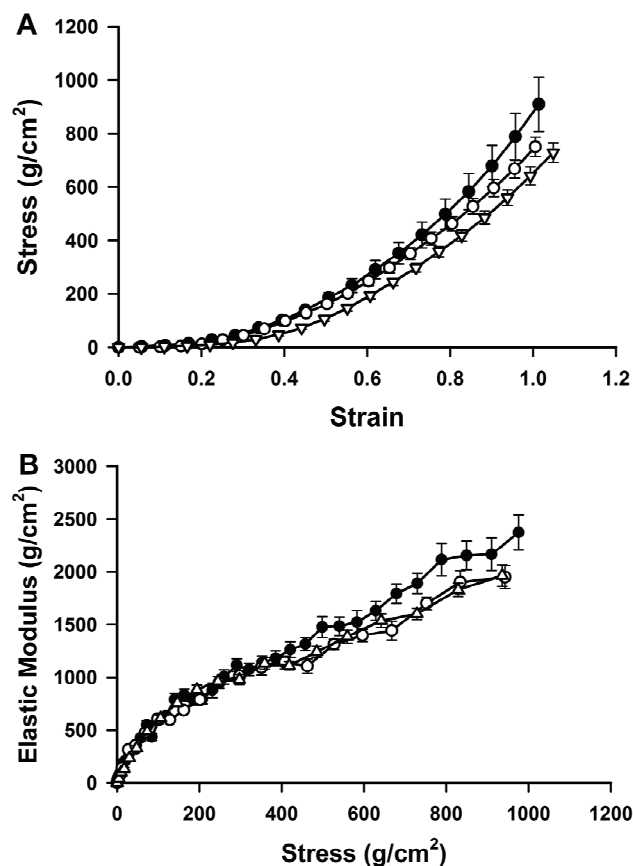

Fig. 2. Mean stress-strain (**A**) and elastic modulus-stress (**B**) relationships of aorta in the control (•, n=8), chlorpyriphos (○, n=8) and dichlorvos groups (∇, n=9). Data are presented as means ± S.E.M.

Accordingly, the coefficients (*A*) of the power function for the chlorpyriphos and dichlorvos groups were not significantly different than for the control group at the low stress region. The power value (*B*) for the dichlorvos group was not significantly different from the value of the control group. On the other hand, the power value decreased significantly from 0.563±0.013 in the control group to 0.506±0.015 in the chlorpyriphos group. At the high stress region, the slope of the elastic modulus-stress curve (*m*) was significantly decreased from 1.95±0.15 in the control group to 1.51±0.07 in the chlorpyriphos and 1.53±0.10 in the dichlorvos groups, while the parameter *yo* (the intersection), was not significantly different in the chlorpyriphos and dichlorvos groups compared to the control group. There were no significant differences between the chlorpyriphos and dichlorvos groups for all parameters at the low and high strain regions.

Discussion

Although aorta has heterogeneous, anisotropic, and nonlinear elastic properties, uniaxial stress-strain relations in the circumferential (Brüel and Oxlund 1991, Brüel *et al.* 1998) and longitudinal directions (Assoul *et al.* 2008, Angouras *et al.* 2000, Sokolis *et al.* 2002, Silver *et al.* 2003) have been extensively used to characterize its mechanics. In additon, models have been developed to assess the mechanics of the aorta in the longitudinal direction (Angouras *et al.* 2000, Sokolis *et al.* 2002, Silver *et al.* 2003, Sokolis *et al.* 2006). In the present study, we followed the analysis method proposed by Sokolis and his colleagues to examine the effect of the pesticides on the aorta and assessed mechanical properties of aorta in the longitudinal direction.

The main result obtained in the present study is that chronic administration of chlorpyriphos and dichlorvos shifted the stress-strain relation downward, which indicated that the aorta has become less stiff. Curve fitting procedure further illustrated that the pesticides mainly influenced the high stress region. In addition, chlorpyriphos decreased the rate constant of the power function, which implies that chlorpyriphos also decreased the stiffness of the aorta at the low stress region. The main elements contributing to the passive elastic properties of the aorta are elastin and collagen. Thus the composition and organization of elastin and collagen in the aortic wall determine its mechanical

properties (Roach and Burton 1957, Cox 1978, Garcia and Kassab 2009). Furthermore, a correlation has been observed between the elastin content and the elastic modulus of the arteries at the low strain region, and the collagen content and the elastic modulus at the high strain region (Wells *et al*. 1999, Roach and Burton 1957, Cox 1978, Garcia and Kassab 2009, Brüel and Oxlund 1991). The stress-strain curve at midrange has been attributed to the transfer of stress from elastin to collagen by progressive recruitment of collagen fibers (Wolinsky and Glagov 1964, Roach and Burton 1957, Armentano *et al*. 1991, Cox 1978, Fonck *et al*. 2007). In addition, decreased or loose cross-links in collagen and elastin are associated with a decreased stiffness (Brüel *et al*. 1998). Mutations decreasing the collagen fiber formation lead to a decrease in the aortic stiffness (Wenstrup *et al*. 2006). These results show that the strength of cross-links between collagen or elastin fibers is important in determining the stiffness of aorta as well as the content of elastin and collagen and their organization on the aortic wall.

When the results obtained in the present study are evaluated in this regard, the decrease in the slope (m) of the elastic modulus-stress curve at the high stress region in the chlorpyriphos and dichlorvos groups indicates that both pesticides affected collagen fibers, and the number of collagen fibers contributing to the same strain level is less in the pesticide groups compared to the collagen number in the normal aorta. There is no study investigating the relationship between arterial stiffness and organophosphate poisoning. Therefore we are unable to compare our biomechanical results. However, several studies reported that acute and chronic toxicity of organophosphate pesticides led to disorganization, breaks and fragmentation in collagen and elastin fibers in aortic wall of rats (Antov *et al*. 1984, Akimov and Kolesnichenko 1985, Yavuz *et al*. 2005). Thus loose connections between collagen fibers due to their break and disorganization might be the reason for the decrease in the elastic modulus of the aorta at the high stress region in the chronically exposed groups.

In addition, the decrease in the rate constant of the power function in the chlorpyriphos group implies that elastin fibers have also been influenced by chlorpyrphos. On the other hand, according to the fit parameters, dichlorvos did not influence the mechanics of the aorta at the low stress region. Thus it seems that dichlorvos and chlorpyriphos have diverse effect on elastin fibers. Although both dichlorvos and chlorpyriphos are organophosphate insecticides their molecular structure is different (Rezg *et al*. 2010). As a result, dose-response curves for dichlorvos and chlorpyriphos exhibit different sensitivity, which indicates that the dose required for a specific effect is different for the two pesticides (Gupta *et al*. 2007). Therefore, the diverse effect of dichlorvos and chlorpyriphos on elastin fibers might be due to the higher sensitivity of elastin to chlorpyriphos compared to dichlorvos.

A number of studies reported that workers exposed chronically to pesticides or people living in the neighborhood of the exposed area had high blood pressure (Berberian and Enan 1987, Saldana *et al*. 2009). Chronic exposure to pesticides also introduced the same effect on blood pressure in rabbits (Anand *et al*. 1990). Under the *in vivo* conditions, arterial stiffness is determined by both the stiffness of passive structural elements and the tone of vascular smooth muscle cells. We found in the present study that chronic administration of pesticides decreased the stiffness of the passive aorta. The changes we observed in the present study in the mechanical properties of the aorta are not in the direction that could lead to an increase in the blood pressure. Then the change in the tone of the vascular muscle might be a reason for the observed hypertension in chronic exposure. The results given in the literature support this suggestion. It was found that the increase in pressure was associated with the amount of the pesticides, adrenaline and acetyl-cholinesterase level in blood (Berberian and Enan 1987, Saldana *et al*. 2009). The animal studies suggest that pesticides affect cholinergic pathways in the brainstem that mediate pressor responses, thus leading to hypertension by increasing total peripheral resistance (Buckley *et al*. 1994, Gordon and Padnos 2000, Smith *et al*. 2001). Thus neurohumoral effects might be the reason for the high pressure in the exposed people. In that case, the lowered stiffness of passive aorta might counteract the effect of the increased pressure due to active processes. A similar discrepancy between the observed high pressure and decreased stiffness was also observed in thyrotoxic patients (Obuobie *et al*. 2002).

Collagen is the tissue element that resists to high pressure and limits the expansion of the aorta and protects it at high pressure levels. The decrease in the elastic modulus of the aortic wall at the high stress region indicates that aortic wall exposed to chronic pesticides can dilate more for a given pressure level and is weak against mechanical forces such as high pressure and thus

is susceptible more easily to tearing compared to a normal aorta. Yavuz *et al*. (2005) also reported that point based on their results from histological examinations. Furthermore, if chronic administration of pesticides would alter the passive properties of smaller arteries such as mesenteric, cerebral and coronary arteries, then the decrease in the stiffness of these arteries might impair the autoregulation of blood flow. These points need to be explored further.

In conclusion, both chlorpyriphos and dichlorvos decreased the elastic modulus of the aorta at the high stress region but they had diverse effects at the low stress region; chlorpyriphos decreased while dichlorvos did not influence the elastic modulus of the aorta at the low stress region. The results imply that chronic exposure to organophosphate pesticides decreases the strength of the aorta and therefore might influence the response of the aorta to mechanical loading induced by blood pressure.

Conflict of Interest
There is no conflict of interest.

Acknowledgements

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