Effects of Acute Malathion Exposure on Renal Oxidant and Antioxidant Balance in Rats

Akut Malation Maruziyetinin Rat Böbrek Oksidan ve Antioksidan Dengesi Üzerine Etkileri

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ABSTRACT

Objective: Pesticides have been used in agriculture to enhance food production by eradicating undesired insects and controlling disease vectors. Malathion is one of the most widely used organophosphate pesticides. However, it has been postulated that organophosphate pesticides lead to oxidative stress through formation of excessive reactive oxygen species and disrupting the antioxidant defense systems.
Methods: Four groups (n=6) were designed to evaluate the effects of different doses of acute malathion exposure on rat kidney. Control group was given only corn oil. Malathion dissolved in corn oil was administered to Group 2 (100 mg/kg), Group 3 (200 mg/kg) and Group 4 (400 mg/kg) via oral gavage. The rats were sacrificed after 24 hours and renal tissue supernatants are analyzed to determine cholinesterase (ChE) activity, total oxidant status (TOS), TNF-α, advanced glycation end products (AGEs), advanced oxidation protein products (AOPP), superoxide dismutase (SOD) activity, malondialdehyde (MDA), and paraoxonase 1-arylesterase activity (PON1-ARE).
Results: ChE activities decreased, TOS levels and TNF- α levels increased in all experimental groups compared with control group (p'<0.05). AGEs levels of the high dose group elevated significantly compared with control and group 3 (p'<0.05). SOD levels of group 3 and 4, and MDA levels of group 3 showed an increase compared with control group, however this did not reach statistical significance (p'=0.054). PON1-ARE levels diminished in group 3 and 4 compared with control (p'<0.05). AOPP levels did not change significantly (p'=0.735).
Conclusion: Acute exposure to malathion disrupts the oxidant-antioxidant balance and causes oxidative stress in kidneys.

Key Words: Malathion, organophosphate, pesticide, oxidant-antioxidant balance, kidney, oxidative stress

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ÖZET

Amaç: Tarımda pestisitler, istenmeyen böcekleri ortadan kaldırmak ve hastalık vektörlerini kontol etmek yoluyla besin üretimini artırmak amacıyla kullanılmaktadır. Malathion, en sık kullanılan organofosfat pestisitlerden birisidir. Organofosfat pestisitlerin, aşırı reaktif oksijen türü oluşturarak ve antioksidan savunma sistemlerine zarar vererek oksidatif strese yol açtığı öne sürülmektedir.
Yöntem: Farklı dozlarda akut malathion maruziyetinin rat böbreği üzerindeki etkilerini değerlendirmek amacıyla dört grup (n=6) oluşturuldu. Kontrol grubuna sadece mısırözü yağı verildi. Mısırözü yağında çözülen malathion, Grup 2 (100 mg/kg), Grup 3 (200 mg/kg) ve Grup 4 (400 mg/kg)'e oral gavaj yoluyla uygulandı. Ratlar 24 saat sonra feda edildi ve renal doku süpernatantları, kolinesteraz (ChE) aktivitesi, total oksidan durum (TOS), TNF-α, ileri glikozilasyon son ürünleri (AGEs), ileri oksidasyon protein ürünleri (AOPP), süperoksit dismutaz (SOD) aktivitesi, malondialdehit (MDA) ve paraoksanaz 1-arilesteraz aktivitesi (PON1-ARE) tayinlerinde kullanıldı.
Bulgular: Kontrol grubuyla karşılaştırıldığında, tüm deneysel gruplarda ChE aktivitesi azalırken, TOS düzeyleri ve TNF-α düzeyleri arttı (p'<0.05). Yüksek doz grubunun AGEs düzeyleri kontrol grubuna ve grup 3'e göre anlamlı artış gösterdi (p'<0.05). Grup 3 ve grup 4'ün SOD düzeyleri ve grup 3'ün MDA düzeyleri, kontrol grubuyla karşılaştırıldığında bir artış gösterdi; ancak bu artış, istatistiksel anlamlılığa ulaşmadı (p'=0.054). PON1-ARE düzeyleri grup 3 ve 4'de kontrol grubuna göre düşük bulundu (p'<0.05). AOPP düzeyleri anlamlı bir değişim göstermedi (p'=0.735).
Sonuç: Malathiona akut maruziyet, böbreklerde oksidan-antioksidan dengesini bozmakta ve oksidatif strese yol açmaktadır.

Anahtar Sözcükler: Malathion, organofosfat, pestisit, oksidan-antioksidan dengesi, böbrek, oksidatif stres

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INTRODUCTION

Organophosphate pesticides are commonly used chemicals in agriculture as an insecticide for eradication of agricultural products from unwanted insects and for control of vector diseases. Although approximately 70% pesticide use is reported in the United States, it is the most commonly used pesticide due to the high toxicity, persistence and accumulation on insects. Organophosphates were first produced as phosphorus-containing insecticides in the 1940s. This group of chemicals includes malathion, chlorpyrifos, diazinon, parathion and methyl parathion (1). Malathion exposure of humans occurs in various ways such as consuming food with pesticides and drinking contaminated water. Malathion, like other organophosphates, causes the death of insects with the accumulation of acetylcholine by inhibiting the cholinesterase enzyme in synapses in the nervous system and has a similar effect in all living organisms. It also has harmful effects on endocrine and immune systems, as well as on the liver, muscle, heart, kidney and other systems (2, 3).

Malathion plays a role in the production of free radicals, possibly in a different way than cholinesterase inhibition (4). Various studies have shown that malathion can induce oxidative stress in rats. Poovala et al. (5) found that malathion was involved in tubular necrosis as a result of increased reactive oxygen species (ROS) and lipid peroxidation. Fortuno et al. (6) observed in the brains of rats that free radicals and oxidative stress in the brain were increased by malathion. In another study, the degree of lipid peroxidation of organophosphates was associated with cardiotoxicity (7). Mise Yonar et al. (8) studied the effects of malathion on fish and showed that malathion increased superoxide dismutase (SOD), glutathione peroxidase, catalase (CAT) and glutathione S-transferase activities. Al-Othman et al. (9) revealed increase in malondialdehyde (MDA) levels and decrease in total antioxidant activity and glutathione levels in malathion-exposed rats. Normally there is a balance between antioxidants and oxidants. When this balance is disrupted in favor of oxidants, increased ROS and free radicals leads to lipid peroxidation, inactivation of certain proteins and fatty acids, changes in enzymatic activities, loss of cell membrane and DNA damage and ultimately to cell death (10). Due to oxidative stress and negative alterations on antioxidant enzyme activities, the toxic effects of malathion on liver, erythrocytes, reproductive system and kidneys have been investigated in experimental studies (11-14). In some of these studies, it has been revealed that Malathion shows its harmful effects on kidneys by reduced total renal cell numbers (15), glomerular inflammation (16), histological and functional impairment of renal tubules (17, 18), proteinuria (19) and nephrotic syndrome (20). Whether this toxic effect of malathion on the kidneys is caused by the negative effect on the oxidant and antioxidant system has not yet been sufficiently revealed in the literature. Therefore, the aim of this study was to investigate the acute effect of malathion at different doses on the oxidant-antioxidant system of renal tissue.

MATERIAL and METHODS

Chemicals

Malathion (O,O-dimethyl-S-1,2-bis ethoxy carbonyl ethyl phosphorodithionate) and all other chemicals were obtained from Sigma. The required dose of malathion was dissolved in corn oil prior to administration by gavage.

Experimental Protocol

This study was carried out in accordance with the regulations of Animal Experimentation Ethics Committee of Gazi University (Approval Code: G.Ü. ET. 14.015). Twenty four female Wistar albino rats with an average weight of 230 ± 14 g was obtained from Gazi University Experimental Animals Research and Application Center. The animals were housed under standard care conditions at constant temperature and humidity on a 12-h in light-dark cycle. Tap water and standard rat chow were available ad libitum. Rats were randomly divided into four groups of six animals. Groups and administrations by gavage were as follow:
Group 1 (Control group): Corn oil without malathion in proportion to body weight
Group 2 (Malathion 100 mg/kg): 100 mg/kg/day malathion dissolved in corn oil
Group 3 (Malathion 200 mg/kg): 200 mg/kg/day malathion dissolved in corn oil
Group 4 (Malathion 400 mg/kg): 400 mg/kg/day malathion dissolved in corn oil

As stated by Karami-Mohajeri et al. (21), 100 mg/kg dose of malathion which is known as toxic dose was defined as low dose, 400 mg/kg dose which inhibits 70% of cholinesterase activity was defined as plateau level and 200 mg/kg dose was defined as intermediate dose.

The experiment was carried out for 24 hours and at the end of the experimental period, rats were sacrificed by exsanguination under ketamine-xylazine anesthesia. Renal tissues of rats were removed, washed in physiological solution, frozen in liquid nitrogen and stored at -80°C until homogenization.

Preparation of Tissue Extract

Renal tissues of animals were homogenized (1:10 ratio) on ice in 50 mM Tris-HCl buffer. Homogenate were centrifuged at 3500 rpm for 1 hour, supernatants were collected and stored at -80°C until the experiments.

Biochemical Parameters

Renal tissue supernatants were analyzed to determine cholinesterase (ChE) activity using Roche Cobas E411 model autoanalyzer, total oxidant status (TOS) using Rel Assay Diagnostics Kit and TNF-α level using YH Bio search brand ELISA kit. Advanced glycation end products (AGEs) (22), advanced oxidation protein products (AOPP) levels (23), superoxide dismutase (SOD) activity (24), malondialdehyde (MDA) level by thiobarbituric acid reaction (25), and paraoxonase 1-arylesterase activity (PON1-ARE) (26) parameters were determined using previously described methods. Protein contents of the samples were measured by Lowry method (27).

Statistical analysis

Statistical analysis was performed using SPSS Statistics 20 software. P value <0.05 was considered as statistically significant. Kruskal-Wallis test was used to analyze the significance differences among all groups. The Mann-Whitney U test was used to determine statistical differences between two groups. Holm-Bonferroni correction was used and corrected p values (p') were calculated to evaluate significance (28, 29). Correlation analysis was performed using Spearman's correlation test.

RESULTS

Results of biochemical parameters of all groups are shown in Table 1. Kruskal-Wallis test showed significant differences between the groups in all parameters except AOPP (all p'<0.05, p'=0.735 for AOPP). A significant decrease in ChE activity was found in all groups compared with the control group (p'<0.05). A significant increase in TOS levels was also observed in all groups compared with the control group (p'<0.05). Kidney TOS levels decreased significantly in group 3 and group 4 compared with group 2 (p'<0.05). Kidney AGEs levels in group 4 increased significantly compared with group 1 and group 3 (p'<0.05). A trend toward significant increase in SOD levels was observed in group 3 and 4 compared with the control group (p'=0.054) and in MDA levels in group 3 in comparison with the control group (p'=0.054). Kidney TNF-α levels showed a significant increase in group 2, 3 and 4 compared with the control group (p'<0.05). Kidney PON1-ARE levels decreased significantly in group 3 and 4 compared with control (p'<0.05).

The results of correlation analysis of the biochemical parameters were depicted in Table 2. Strong and significant positive correlations were found between ChE activity and AOPP, ChE activity and PON1, TOS level and MDA level, TOS level and TNF-α level, AGEs level and TNF-α as well as SOD and TNF-α level. Negative correlations were observed between ChE activity and AGEs levels, ChE activity and TNF-α level, ChE activity and TOS level, PON1-ARE activity and SOD levels as well as PON1-ARE activity and TNF-α level of the kidneys.

Table 1: Results and significant differences of renal parameters

	Group 1 (Control group)		**Group 2 (Malathion 100 mg/kg)**		**Group 3 (Malathion 200 mg/kg)**		**Group 4 (Malathion 400 mg/kg)**		
	Mean ±SD	Median (Max. - Min.)	Mean ±SD	Median (Max. - Min.)	Mean ±SD	Median (Max. - Min.)	Mean ±SD	Median (Max. - Min.)	*P*
ChE (mU/mg protein)	317.42 ±56.02	321.67 (382.04 - 239.07)	194.44 ±59.46	195.40 [a] (275.57 - 122.32)	187.82 ±72.86	167.77 [b] (305.89 - 106.76)	178.19 ±37.49	177.03 [c] (224.29 - 118.50)	**0.011**
TOS (µmol/g tissue)	0.23 ±0.04	0.24 (0.27 - 0.15)	0.36 ±0.02	0.36 [a] (0.38 - 0.34)	0.33 ±0.02	0.33 [b, d] (0.38 - 0.31)	0.31 ±0.03	0.31 [c, e] (0.35 - 0.28)	**0.000**
AGEs (ng/g tissue)	255.11 ±11.72	256.82 (269.54 - 235.62)	273.78 ±25.77	276.43 (299.93 - 227.14)	261.59 ±21.79	253.46 (288.62 - 243.04)	305.47 ±34.30	291.80 [c, f] (368.13 - 270.95)	**0.013**
AOPP (µmol/mg protein)	2.03 ±0.40	2.05 (2.45 - 1.30)	2.21 ±1.22	1.79 (4.44 - 1.17)	2.40 ±0.71	2.34 (3.52 - 1.58)	2.13 ±0.57	2.07 (3.10 - 1.50)	0.735
SOD (U/mg protein)	1.01 ±0.26	0.94 (1.49 - 0.76)	1.34 ±0.23	1.29 (1.78 - 1.17)	1.76 ±0.34	1.69 [b] (2.29 - 1.46)	1.71 ±0.36	1.74 (2.21 - 1.30)	**0.005**
MDA (nmol/g tissue)	169.17 ±20.10	170.00 (200.00 - 145.00)	200.00 ±22.36	200.00 (225.00 - 175.00)	213.33 ±31.41	202.50 [b] (275.00 - 190.00)	185.00 ±12.65	185.00 (205.00 - 170.00)	**0.019**
TNF-α (ng/g tissue)	3.08 ±0.38	3.10 (3.48 - 2.48)	3.94 ±0.29	3.96 [a] (4.28 - 3.55)	3.91 ±0.28	3.89 [b] (4.36 - 3.49)	4.25 ±0.32	4.26 [c] (4.60 - 3.83)	**0.002**
PON1-ARE (U/mg protein)	21.45 ±11.06	17.30 (42.74 - 13.95)	16.02 ±5.71	14.81 (23.68 - 9.57)	9.19 ±2.97	9.38 [b] (12.58 - 5.73)	10.57 ±2.79	11.38 [c] (12.53 - 5.07)	**0.006**

a= Significance difference between group 1 and group 2, b= Significance difference between group 1 and group 3, c= Significance difference between group 1 and group 4, d= Significance difference between group 2 and group 3, e= Significance difference between group 2 and group 4, f= Significance difference group 3 and group 4, ChE: Cholinesterase, TOS: Total Oxidant Capacity, AGEs: Advanced Glycation End Products, AOPP: Advanced Oxidation Protein Products, SOD: Superoxide Dismutase, MDA: Malondialdehyde, TNF-α: Tumor Necrosis Factor- α, PON1-ARE: Paraoxonase 1-arylesterase activity

Table 2: Correlation analysis among the parameters of the kidney

		ChE	TOS	AGEs	AOPP	SOD	MDA	TNF-α	PON1-ARE
ChE	r	1	-0.551**	-0.460*	0.472*	-0.38	-0.314	-0.660**	0.692**
	p	.	**0.005**	**0.024**	**0.02**	0.067	0.135	**0.000**	**0.000**
TOS	r		1	0.271	-0.013	0.306	0.499*	0.512*	-0.249
	p		.	0.2	0.952	0.146	**0.013**	**0.011**	0.241
AGEs	r			1	-0.231	0.159	0.171	0.728**	-0.368
	p			.	0.276	0.458	0.425	**0.000**	0.077
AOPP	r				1	0.295	0.156	-0.034	0.27
	p				.	0.162	0.467	0.875	0.201
SOD	r					1	0.302	0.492*	-0.428*
	p					.	0.151	**0.014**	**0.037**
MDA	r						1	0.258	-0.403
	p						.	0.223	0.051
TNF-α	r							1	-0.556**
	p							.	**0.005**
PON1-ARE	r								1
	p								.

** Correlation is significant at the 0.01 level, * Correlation is significant at the 0.05 level, r= correlation coefficient, p= significance, ChE: Cholinesterase, TOS: Total Oxidant Capacity, AGEs: Advanced Glycation End Products, AOPP: Advanced Oxidation Protein Products, SOD: Superoxide Dismutase, MDA: Malondialdehyde, TNF-α: Tumor Necrosis Factor- α, PON1-ARE: Paraoxonase 1-arylesterase activity

DISCUSSION

In this study, the acute toxic effect of malathion on kidney oxidants and antioxidants was investigated. For this reason, 100 mg/kg dose of malathion was selected as acute toxicity, 400 mg/kg dose as plateau level (70% inhibition of ChE) and 200 mg/kg dose as medium dose and rats were grouped according to these doses.

Malathion is an organophosphate used as pesticide that shows its effect by inhibiting the ChE enzyme. ChE activity is used as a biomarker to determine organophosphate poisoning (3). In our study, a significant decrease was observed in kidney ChE activity compared with the control group in all groups (all p' <0.05). The effect of malathion on the ChE enzyme is dose and time dependent. While the subchronic or chronic effects of malathion were investigated in most of the studies, the acute effect of malathion was investigated in our study and in the study conducted by Aksoy and Alper (30). They reported a significant decrease in ChE enzyme activity compared with the control group in serum samples taken 24 hours after malathion administration at a dose of 800 mg/kg (decrease from 165.59 U/L to 94.54 U/L). Coban et al. (31) revealed a significant decrease in serum and brain tissue in ChE activity compared with the control group in rats exposed to 100 mg/kg of malathion for 28 days. Wankhade et al. (32) also observed that malathion inhibits ChE activity in the liver in parallel with our findings. In our study, renal ChE activity decreased from 317.42 mU/mg in the control group to 194.44 mU/mg after administration of 100 mg/kg malathion, to 187.82 mU/mg after administration of 200 mg/kg malathion, to 178.19 mU/mg after administration of 400 mg/kg malathion. In addition, the fact that ChE activity correlates negatively with TOS, AGEs and TNF-α, and positively with PON1-ARE, indicates the strict coexistence of oxidant system and enzyme activities resulting from acute exposure of malathion.

Lipids are biomolecules that can be destroyed by reactive oxygen species. Lipid peroxidation is one of the most harmful reactions in metabolism, leading to irreversible cell death. The final product of lipid peroxidation is MDA and it is often used as a measure of oxidative stress (33). Malathion can pass into the membrane and initiate lipid peroxidation due to its lipophilic structure. In the study conducted by Aksoy and Alper (30), liver, erythrocyte, kidney and brain MDA levels in groups exposed to acute malathion increased significantly compared with the control group. Possamai et al. (4) emphasized that MDA is an appropriate marker for the demonstration of lipoperoxidation in patients with acute and subchronic malathion exposure in different tissues. In a study of rats chronically exposed to malathion, it was found that MDA levels in blood, liver, testis, brain and kidney showed a significant increase compared with the control group (34). In our study, it was found that there was an increase in MDA levels in all group but this increase was not significant in groups exposed to 100 mg/kg and 400 mg/kg malathion. In correlation analysis, MDA levels were found to correlate positively with TOS.

Superoxide dismutase enzyme is an anti-oxidant metalloenzyme that protects the organism against the ROS by catalyzing superoxide into hydrogen peroxide and molecular oxygen (35). In this study, as a result of malathion administration, an increase in renal SOD levels in all groups was observed but it was not significant in groups 2 and 4. The depletion of catalase and SOD enzyme activities which are the antioxidant enzymes were observed in various tissues of rats exposed to malathion in other studies in the literature (30, 31, 36). In our study, the reason for the increase in SOD levels in contrast to these studies may be due to the fact we investigated the effects of acute toxicity of malathion, while other studies investigated chronic or sub-chronic effects. An increase in antioxidant enzyme levels may have been observed to neutralize increased oxidant activity in the early period of the toxicity.

Sharma et al (37) found an increase in liver cytochrome P450 activity as a result of acute exposure of rats to organophosphates. Cytochrome P450 triggers oxidation of oxygen molecules and ROS production. In a study where NO levels included in ROS were investigated in malathion toxicity, NO levels in erythrocyte and liver tissues were significantly higher in the malathion group than in the control group (30). In another study nitride oxide activities in lung, liver and kidney tissues significantly increased as a result of acute malathion toxicity (38). In our study, kidney tissue TOS levels were significantly higher in all groups compared with the control group. The positive correlation of TOS levels with MDA and TNF-α, which are the determinants of oxidative stress, indicates that they act together in the oxidative stress by malathion.

AGEs and AOPP are biological markers of oxidative stress and are involved in the pathogenesis of various diseases and their complications (39). Since proteins play crucial roles in the organism, oxidation of proteins as a result of oxidative stress (the formation of AOPP) leads to the loss or accumulation of the proteins, the death of the cell and the progression or onset of the diseases. AGEs which form resulting from non-enzymatic glycation of macromolecules are capable of cross-reacting with proteins and interacting with AGE-specific receptor RAGE (40). The formation of AGEs increases especially in hyperglycemic situations and plays a role in the pathogenesis of diabetic complications. However, in recent years, the number of publications investigating the role of AGEs in oxidative stress and inflammation has increased (40, 41). In our study, when investigating AGEs and AOPP in oxidative stress in a malathion toxicity model, AGEs were found to be significantly increased only in the group that was administered high dose (400 mg / kg) of malathion compared with the control group and correlated with TNF-α in the correlation analysis. There was no significant difference between the groups regarding to AOPP levels. Thus it can be concluded that the levels of AGEs and AOPPs were not affected in this study except high dose malathion exposure. As the formation and accumulation of AGEs and AOPP is a relatively longer term process, the study period might not be sufficient for these parameters to show a difference. Studies involving both acute and chronic malathion exposure should be conducted to clarify this.

Oxidative stress leads to inflammation and is involved in the pathophysiology of some chronic diseases. This situation causes the activation of some transcriptional factors. Organophosphate insecticides have been shown to be responsible for the increase of proinflammatory cytokines, including TNF-α (42). Mostafalou et al. (43) investigated the effects of malathion on the inflammatory response in rats and found that administration of 50 mg/kg malathion for 32 days significantly increased TNF-α levels. Yonguc et al. (44) found that organophosphate exposure increased TNF-α gene expression in the brains of rats. Similarly, in the study of Ince et al. (34), TNF-α gene expression increased in rats. In our study, in accordance with these studies, acute exposure of malathion increased significantly TNF-α levels in all groups. Also, in the correlation analysis, the positive correlation of TNF-α with TOS, AGEs and SOD shows that TNF-α and other molecules act together in oxidative stress.

Lukaszewicz and Hussain (45) revealed a significant reduction in paraoxonase activity with an increase in lipid peroxidation as a result of subchronic exposure of chlorpyrifos, an organophosphate. In our study, it was found that groups 3 and 4 had a significant decrease in PON1-ARE activity in kidneys compared with the control group. This decrease was also correlated with ChE enzyme activity.

This study gives new insights into the acute effects of malathion on the oxidant and antioxidant system in the kidneys. The results we found in the study revealed that malathion functions in the kidneys by reducing ChE and PON1-ARE enzyme activities independently of the dose and that oxidation plays an important role in kidney toxicity via proteins such as MDA, TNF-α, TOS and AGEs, which are also markers of oxidation.

Conflict of interest

No conflict of interest was declared by the authors.

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