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In vivo Effects of Malathion on Glutathione-*S*-Transferase and Acetylcholinesterase Activities in Various Tissues of Neonatal Rats

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Abstract: Glutathione-*S*-transferase (GST) has been reported to play an important role in the detoxification of several chemical compounds, including pesticides. Organophosphate pesticides are potent inhibitors of acetylcholinesterase (AChE). The present study was conducted to assess the in vivo effects of the organophosphate pesticide malathion on the activity of GST and AChE and the protein levels of different organs in neonatal rats. GST activity increased approximately two-fold in adult female rats fed malathion, whereas AChE activity decreased less than 20% of the control in the liver. Similarly, liver and heart GST activities (0.14 and 0.035 U/mg protein, respectively) were also increased approximately two-fold in neonatal rats, but in the brain a decrease in GST activity (0.074 U/mg protein) was observed. The acetylcholinesterase activity in the liver, brain, kidney and lung (0.002, 0.035, 0.046, and 0.018 U/mg protein, respectively) seemed to be significantly decreased compared with the control. There was no significant change in protein contents. In conclusion, it is suggested that malathion has some biochemical effects on rats.

Key Words: Glutathione-*S*-transferase (GST), Acetylcholinesterase (AChE), Glutathione (GSH), Enzyme induction, Organophosphate pesticide, Malathion.

Yenidoğan Sıçanların Çeşitli Dokularındaki Glutation-*S*-Transferaz ve Asetilkolinesteraz Aktiviteleri Üzerine Malation'un In vivo Etkileri

Özet: Glutation-*S*-transferazın (GST) pestisitleri de içeren bir çok kimyasal bileşiğin detoksifikasyonunda önemli rol oynadığı rapor edilmiştir. Organofosforlu pestisitler asetilkolinesterazın (AChE) kuvvetli inhibitörüdür. Bu çalışma, yenidoğan sıçanların farklı organlarındaki protein seviyesi, GST ve AChE aktiviteleri üzerine organofosforlu bir pestisit olan malationun in vivo etkilerini değerlendirmek için yapılmıştır. Malationla beslenen yetişkin dişi sıçanlarda karaciğer GST aktivitesi yaklaşık 2 kat artarken, AChE aktivitesi kontrole göre % 20'den daha fazla azalmıştır. Benzer şekilde, yeni doğan sıçanlarda karaciğer ve kalp GST aktiviteleri (0.14 ve 0.035 U/mg protein, sırasıyla) yaklaşık 2 kat artmıştır, fakat beyin GST aktivitesinde (0.074 U/mg protein) bir azalma gözlenmiştir. Karaciğer, beyin, böbrek ve akciğer AChE aktivitelerinde de (0.002, 0.035, 0.046, ve 0.018 U/mg protein, sırasıyla) kontrolle karşılaştırıldığında önemli derecede azalma gözlenmiştir. Protein içeriklerinde önemli bir değişiklik bulunmamaktadır. Sonuçta malationun sıçanlar üzerinde bazı biokimyasal etkilere sahip olduğu saptanmıştır.

Anahtar Sözcükler: Glutation-*S*-transferaz (GST), Asetilkolinesteraz (AChE), Glutation (GSH), Enzim indüksiyonu, Organofosforlu pestisit, Malation.

Introduction

Organophosphorus (OP) compounds are widely used in agriculture, medicine and industry. OP pesticides, in addition to their intended effects like the control of insects or other pests, are sometimes found to affect non-target organisms including humans (Chantelli-Forti et al., 1993; Chaudhuri et al., 1999). Exposure to OPs is also a potential cause of longer-term damage to the nervous system, with reports of poor mental health and deficits in memory and concentration (Davis, 1991; Mason, 2000; Nigg and Knaak, 2000). Because of the serious

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environmental problems resulting from the use of pesticides in the agricultural sector, several governments are seeking to employ biological and other nonpolluting methods for combating pests. Several biocides and/or their metabolites are suggested to be prior mutagenic and/or teratogenic compounds (Ridgway et al., 1978; Fishbein, 1982; El-Sharkawy et al., 1994).

Malathion is one of the most widely used organophosphate insecticides throughout the world. It is used to control pests affecting agricultural crops, ornamentals, greenhouses, livestock, stored grain, forests, buildings, households and gardens. Contributing to its popularity is its relatively low acute mammalian toxicity (Brenner, 1992; Hazarika, 2003). However, like other pesticides that have been found to cause irreparable damage to human and environmental health, malathion may pose a greater risk than the product label would lead one to believe. Shown to be mutagenic, a possible carcinogen, implicated in vision loss, causing myriad negative health effects in human and animal studies, damaging nontarget organisms, and containing highly toxic impurities, malathion has a legacy of serious problems (Brenner, 1992).

In an attempt to evaluate the effects of malathion on the detoxification/toxification of xenobiotics, there is a gap in our knowledge regarding the influence of this family of organophosphates on glutathione-*S*-transferases (GST), which conjugate numerous carcinogenic, mutagenic, toxic and pharmacologically active compounds with glutathione (Chasseaud, 1970; Wilce and Parker, 1994). Therefore, the endogenous availability of glutathione at the target site might be a limiting factor for GST-catalyzed conjugation and thereby influence the protective function of the enzyme. Alterations in the activity of these conjugative enzymes can also disturb the activation-detoxification balance operating in different tissues to detoxify potential toxic agents (Guengerich, 1963; Bock et al., 1987; Mannervik and Danielson, 1988). Among the biocides, organophosphates are the cholinesterase-inhibiting pesticides of primary concern. These chemicals bind to cholinesterase and block the hydrolysis of the acetylcholine to choline and acetic acid at the post-synaptic junctions. Without junctioning acetylcholinesterase (AChE), acetylcholine accumulates, producing rapid twitching of voluntary muscles, incoordination, convulsions, paralysis and ultimately death (Korhonen et al., 1990; Lai and Ito, 1997).

The current study was undertaken to investigate the family-mediated effects of malathion on the activity of glutathione-*S*-transferase and acetylcholinesterase in neonatal rats.

Materials and Methods

Chemicals

All fine chemicals used in this study were purchased from Sigma Chemical Co. (St. Louis MO, USA). All other chemicals were analytical grade or better from E. Merck (Darmstadt, Germany). Commercially available malathion (Hekthion 20 EM, Hektaş, Turkey) was used in this study.

Animals and experimental design

The protocols were approved by the Animal Ethical Committee of Ege University, Faculty of Medicine. Adult male (n = 10) and female (n = 20) Wistar albino rats (100-150 g) were obtained from Ege University, Faculty of Medicine, Center for Animal Breeding.

All animals were acclimatized for 10 days before the start of the experimental procedure. The males and females were assigned randomly to either the control or an exposure group and housed individually in labeled cages until mating. The animals were housed in cages (19 x 19 x 10 cm) with solid plastic sides and stainless-steel grid tops and floors in a room designed for control of temperature (approximately 21 ± 1 °C), humidity (45-75%) and light cycle (12 h light, 12 h dark). Animals in the control group (five males and 10 females, n = 15) were orally fed daily a normal diet in standard laboratory pellets (10 g/day per rat), while animals in the treatment group (five males and 10 females, n = 15) were fed laboratory pellet food combined with 500 ppm malathion (10 g chow/day per rat). The treatment dose of the malathion was calculated based on LD_{50} and body weight data (LD_{50}: 1375 mg/kg body weight for male rats and 2800 mg/kg body weight for female rats). Both male and female rats were fed laboratory pellet food combined with malathion for 1 month before mating, in a regimen of six consecutive days per week followed by normal food on the 7^{th} day. Tap water was available ad libitum. Food and water were changed daily (Francis et al., 1990). During mating one male was housed with two females. Females were assigned randomly in pairs to each male for

the required mating period. After mating, males and females were housed individually and then all females were treated with malathion during pregnancy and until the end of the breast-feeding period.

At the end of the experiment, 10 offspring assigned randomly from each group were sacrificed by cervical dislocation and the organs were excised, cleaned, weighed and homogenized in a Potter-Elvehjem homogenizer with four volumes of 50 mM phosphate buffer (pH 7.0) containing 1 mM EDTA. The particle-free supernatant was obtained by centrifugation at 10,000 x g for 20 min at 4 °C and used as the enzyme source.

Enzyme assays

The GST activity was assayed spectrophotometrically (JASCO, UV-VIS) at 340 nm by measuring the rate of 1-chloro-2, 4-dinitro benzene conjugation with reduced glutathione as a function of time according to the established method of Habig et al. (1974). The reaction mixture contained 1 mM glutathione (GSH), 50 mM phosphate buffer, pH 7.0 and 50 µl of an appropriate dilution of the enzyme source in a total volume of 3 ml were preincubated for 2 min at 25 °C and the reaction was started by adding 0.1 ml, 30 mM 1-chloro-2, 4-dinitro benzene (CDNB). The increase in A_{340} was measured for 3 min at 25 °C against a blank containing GSH and CDNB to eliminate non-enzymatic conjugation interferences. One unit (U) of activity was defined as the formation of 1 µmol/min of conjugated product. The extinction coefficient 9.6 mM^{-1} cm^{-1} CDNB was used for the calculations.

The AChE activity was assayed as described by Ellman et al. (1961). The assay mixture contained 0.259 mM 5,5-dithiobis-2-nitrobenzoic acid (DTNB) in 67 mM phosphate buffer, pH 7.4, 0.298 mM acetylthiocholine chloride and 20 µl of an appropriate dilution of the enzyme source in a total volume of 3.02 ml. The reaction was followed at 410 nm for 10 min intervals at 37 °C against a blank containing acetylthiocholine chloride and phosphate buffer. The extinction coefficient of the product of the chemical reaction, 5-thio-2-nitrobenzoate, is $\varepsilon = 13.61$ mM^{-1} cm^{-1}.

The protein content was estimated according to Lowry et al. (1951). Statistical analyses were performed using an unpaired Student's t-test. Probability values less than 0.05 were considered significant (Snedecor and Cochran, 1968).

Results and Discussion

The Figure shows the effect of malathion on the levels of protein, GST and AChE in different organs of adult female rats fed malathion incorporated in standard laboratory chow before mating, during pregnancy and until the end of breast feeding in comparison with the control.

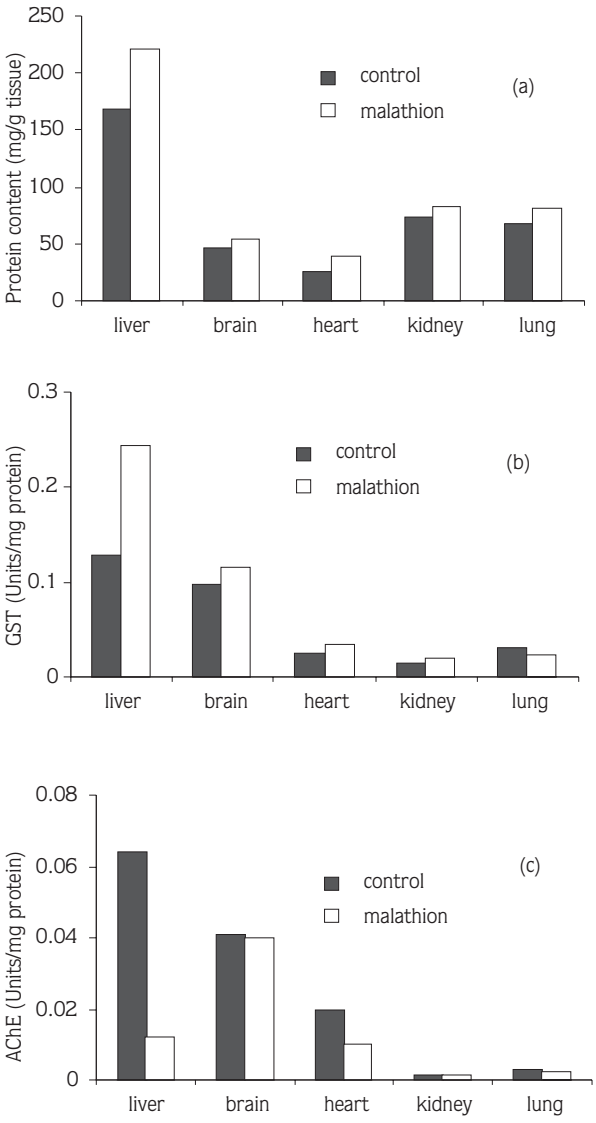

Figure. Effect of malathion on protein (a), glutathione-*S*-transferase (b) and acetylcholinesterase (c) levels of female rats.

The results revealed that the administration of malathion caused insignificant changes in the protein contents of all tissues (Figure a). On the other hand, GST

activity increased approximately two-fold (Figure b), whereas AChE activity significantly decreased to less than 20% of the control in the liver (Figure c). However, activity changes in other tissues seemed not to be significant as the induction can be said to be tissue dependent. The significant decrease in acetylcholinesterase activity after the administration of malathion, especially in the liver, could be explained on the basis of the properties of this enzyme. It is well known that acetylcholinesterase is irreversibly inhibited by organophosphate and organothiophosphate insecticides (after S replacement by O) by phosphorylating the serine –OH group of the enzyme (Mortensen et al., 1998; Dumschat et al., 1991), but the unexpected result is the insignificant change in the enzyme in the brain where AChE inhibition on nerve cells in the brain appears to be particularly important (Nakagawa, 1991; Siddiqui et al., 1990). The toxicity of OP stems mainly from the accumulation of acetylcholine due to the inhibition of AChE.

Three enzyme systems (GST, esterases and monooxygenases) are involved in the detoxification of the organophosphate insecticide class. These enzymes act by rapidly metabolizing the insecticide to nontoxic products, or by rapidly binding and very slowly turning over the insecticide (Guengerich, 1963; Hemingway et al., 1998; Kostaropoulos et al., 2001). In contrast with recent work (Hazarika et al., 2003), an increase in GST activity especially in the liver was observed. This can be understood in view of the fact that organophosphates consume GSH through a GST-catalyzed reaction as a major way of detoxification, and these chemicals are expected to induce the activity of GST as a potent protection mechanism of the organism. Besides this, the amount of GSH inside the cell as the co-substrate for GST appears to be enough to cope with the increased activity of the enzyme (data not shown).

As malathion has been associated with birth defects in domestic laboratory animals, the litters of the exposed females in comparison with the controls were also tested (Brenner, 1992). The effects of malathion on the levels of protein, GST and AChE in neonatal rats are given in the Table.

As is easily seen, similar results for protein contents were observed, where enzyme activities showed significant differences. However, in contrast with the parent, in addition to liver, heart GST activity (0.035 ± 0.002 U/ mg protein) was also increased approximately two-fold, but in the brain a decrease in GST activity (0.074 ± 0.007 U/mg protein) was observed. The acetylcholinesterase activity in the liver, brain, kidney and lung of neonatal rats seemed to be significantly decreased (0.002 ± 0.001, 0.035 ± 0.005, 0.006 ± 0.001, and 0.018 ± 0.004 U/mg protein, respectively).

The effects of OP pesticides are mediated indirectly via the activation of cholinergic receptors by the accumulated acetylcholine subsequent to AChE inhibition.

Table. Protein content, glutathione-*S*-transferase and acetylcholinesterase activity in various tissues of neonatal rats treated with malathion.

Protein content (mg/g tissue)					
Treatment	Liver	Brain	Heart	Kidney	Lung
Control	173 ± 7.1	60 ± 4	62 ± 10	104 ± 24	128 ± 31
Malathion	159 ± 8.7	59 ± 5	59 ± 11	102 ± 11	110 ± 7
GST (Units/mg protein)					
Control	0.06 ± 0.01	0.110 ± 0.080	0.020 ± 0.014	0.035 ± 0.005	0.039 ± 0.001
Malathion	0.14 ± 0.02*	0.074 ± 0.007*	0.035 ± 0.002*	0.032 ± 0.003	0.039 ± 0.004
AChE (Units/mg protein)					
Control	0.060 ± 0.004	0.061 ± 0.005	0.065 ± 0.013	0.046 ± 0.011	0.071 ± 0.010
Malathion	0.002 ± 0.001*	0.035 ± 0.005*	0.040 ± 0.005	0.006 ± 0.001*	0.018 ± 0.004*

Results are expressed as mean ± S.E.
*Significantly different from control ($P < 0.05$) as determined using an unpaired *t*-test.

Recent evidence also suggests that OP compounds may exert direct actions on muscarinic and nicotinic receptors, when their concentration in the circulation exceeds micromolar levels (Savolainen, 2001). AChE levels of adult female rats were lower than those of neonatal rats, because adult female rats were exposed to malathion directly via their diet, whereas neonatal rats received this compound indirectly via their mothers. Because of this, malathion had a greater effect on the parents than on the neonatal rats. Furthermore, malathion is a potent cholinesterase inhibitor, and this could also be responsible for the genotoxic effect. There are several cases known where malathion-induced genetic damage occurs at doses far below acutely toxic levels and its effects can be cumulative (Fegereisen, 1995; Hatjian et al., 2000). It is known that a longer duration and earlier initiation of malathion exposure results in more severe problems. However, to confirm the data, it would be useful to characterize further the metabolism of the insecticide and in vivo genotoxic effects in rodent species that have a metabolism close to that of humans. Although there are several reports on the mutagenicity of malathion exposure, very few concern the family-mediated effects of levels (especially GST). On the other hand, the induction of mammalian tissues by several pesticides or other toxic compounds can cause differentiation in the other enzyme levels that have effects on the protective and tolerant system such as glutathione-related enzymes and mixed function oxidases. The conjugation of GSH with 1-chloro-2, 4-dinitrobenzene is widely used for detecting GST activity, but it may not detect all GST isozymes. The forms of GST specifically involved in the mechanism of tolerance to each molecule cannot be identified by a CDNB reaction; thus it is possible that they differ or exhibit cross-reactivity with pesticide substrate. Apparently this differential induction might be due to the ability of differentxenobiotics to selectively activate transcription of GST genes coding per enzyme subunits that exhibit particular substrate specificities.

As an overview of the present results, the GST detoxification system may have the ability to manage malathion LD_{50} dose administration by the induction of the enzyme both in parent and neonatal rats. AChE inhibition increased significantly in the malathion-treated group. The hazardous effects of repeated exposure to this insecticide are thought to be so serious that differing doses have to be applied to determine the least hazardous effect for the insecticide metabolism.

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