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Metabolic disorders of acute exposure to malathion in adult Wistar rats

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ABSTRACT

Malathion is a widely organphosphorus insecticide used in agriculture, which shows strong insecticidal effects. However, the use of this insecticide leads to disruption in metabolic pathways. The aim of this study is to evaluate the acute effects of malathion on metabolic parameters in Wistar rats. Malathion was administered orally to rats at a dose of 400 mg/kg body weight dissolved in corn oil. Glucidic and lipidic status were analyzed in plasma, cholinesterase activities were also determined. Malathion induces a transitory hyperglycaemia which correlated with depletion on glycogen content. Plasma triglycerides and LDL level increased significantly in malathion treated-rats. HDL rate was unchanged and cholesterol plasma content decrease transitory but rapidly reached a normal level. Results of this study indicate, clearly, that malathion in acute exposure leads to a disruption of lipid metabolism with an enhancement in LDL and triglyceride contents and may play an important role in the development of atherosclerosis and cardiovascular disease. Disruption in plasma lipid profile may leads to a kind of insulin resistance which results in hyperglycaemia.

1. Introduction

Organophosphorus (OP) insecticides are among the most commonly used compounds to control pests and unwanted insects. They have largely replaced organochlorine insecticides, since they have the advantage of being more readily biodegradable and less persistence in different environmental compartments [1]. However, they are currently responsible for more poisonings than any other single class of pesticides [2]. OPs act by binding to a specific serine residue at the active site of certain esterase enzymes, including acetylcholinesterase (AChE) [3]. Numerous complications were reported in many cases of intoxications by this family of pesticides in both human and animal [4]. Apart inhibition of AChE and cholinergic effects, hyperglycemia has been reported as one of the adverse effects in poisoning by OPs in both humans and animals [5–7].

Malathion [*S*-1,2(bis-ethoxycarbonyl) etyl *O*,*O*-dimetyl phosphorodithioate] is one of the most widely used organophosphate pesticides for agriculture and public health programs [8]. It is known to induce excitotoxicity through its bioactivated analog, malaoxon [9]. Toxicity of malathion affects many systems, particularly the nervous system [10]. Others organs that could be affected by OPs intoxication include liver, pancreas and kidney [11,12]. As studied in hen, mouse, rat, cow and men, malathion is highly lipid soluble and it stored in liver and other lipophilic tissues [13]. Additionally, malathion was found to have a rapid but asymmetrical transmembrane uptake by the liver. Therefore, the liver which is the most important organ in glucose and lipid homeostasis and production of related enzymes can be a target for malathion toxicity [14].

The liver is known to be the intermediary metabolism site of lipids and energy and hence, regulation of hepatic gene expression may play a central role in the adaptive response to altered digestion by changing the capacity of enzymes in relevant metabolic pathways [15]. Lipogenesis takes place primarily in the liver and the liver account for 95% of the *de novo* fatty acid synthesis and there is apparently a general assumption that almost all the fat that accumulates in broiler adipose tissue is synthesized in the liver or is derived from the diet [16]. Hyperlipidemia or high levels of serum triglycerides (TG) and cholesterol are a risk factor for premature atherosclerosis [17].

However, the information available on the effects of pesticides, at biochemical level, particularly on lipid metabolism is scanty. The present investigation is taken up to analyze the effect of acute

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Table 1
Effects of acute administration of malathion on rat erythrocyte acetylcholinesterase, plasma butyrylcholinesterase activities and plasma protein concentrations

Parameters	CTR	MAL			
		2 h	6 h	12 h	24 h
AChE (nmol/(min mg) of haemoglobin)	12.20 ± 0.79	4.50 ± 0.38**	5.22 ± 0.37**	6.16 ± 0.39**	6.62 ± 0.76**
BChE (nmol/(min mg protein))	2.34 ± 0.23	0.86 ± 013**	1.20 ± 0.12**	1.27 ± 0.04**	1.28 ± 0.16**
Proteins (mg/dl)	5.52 ± 0.35	7.46 ± 0.55**	6.45 ± 0.44	6.61 ± 0.12*	6.56 ± 0.02

AChE, acetylcholinesterase; BChE, butyrylcholinesterase; CRT, control; MAL, malathion treated rats. Values are mean ± S.E.M. (n = 12). *Significantly different from control at $p < 0.05$. **Significantly different from control at $p < 0.01$.

administration of malathion on metabolic parameters, particularly, on plasma lipid profile and glucose homeostasis in Wistar rats and to evaluate what relationship is established between hyperglycaemia and alteration of lipid metabolism.

2. Materials and methods

2.1. Chemical

Malathion (fyfanon 50 EC 500 g/l) of commercial grade was used in this study, Acetylthiocholine iodide (ATCh), *S*-butyrylthiocholine iodide (BTCh), 5,5′-dithiobis-2-nitrobenzoic acid (DTNB), trichloroacetic acid (TCA), KOH, ethanol, ether, Coomassie G250, bovine serum albumin, orthophosphoric acid 85%, and NaCl were obtained from Sigma–Aldrich Co. (Germany).

2.2. Animals and treatments

Adult male Wistar rats (150–170 g) were procured from the Tunisian Society of Pharmaceutical Industries. The animals were housed in polypropylene cages, fed a standard laboratory diet and water *ad libitum*. Rats were exposed to a 12 light/dark cycle, at a room temperature of 18–22 °C. Animals were quarantined for 10 days before beginning of the experiments.

Rats were divided into two groups, control (n = 12) and experiment groups (n = 48). Rats in experiment groups were divided into four groups (12 rats per group). Malathion was administered orally to fasted rats at a dose of 400 mg/kg of body weight (corresponding to 1/5 of LD_{50} value: 2000 mg/kg b.w. determined in a preliminary study) dissolved in corn oil and animals were killed at 2, 6, 12 and 24 h after dosing. Control group received equal amount of corn oil. The experiment was performed in ethical conditions.

At the end of treatment, animals were decapitated without preliminary anesthesia, and arteriovenous blood was taken quickly. Plasma and erythrocytes were separated by centrifuging at 2000 rpm for 15 min. Plasma was stored at −20 °C for biochemical analyzes. Liver was removed for the determination of hepatic glycogen rate. Normal sterile saline (NSS) was used for diluting plasma. Erythrocyte pellets were washed twice with physiological saline and Aliquots were kept at −20 °C.

2.3. Biochemical determinations

Plasma glucose assay was measured by the glucose oxidase and peroxidase using quinoneimine as a chromogen. The amount of plasma glucose is related to amount of quinoneimine which measured spectrophotometrically at 505 nm [18].

For determination of glycogen, 0.5 g of liver was extracted with 3 ml of 30% KOH, incubated for 30 min at 100 °C, then brought to acid pH by addition of 20% trichloroacetic acid. Precipitated protein was removed by centrifugation for 10 min at 3000 × *g*. glycogen was precipitated by ethanol and weighed. The results were expressed in g of glycogen/100 g of liver [19].

For determination of serum total cholesterol (TC), low-density lipoprotein-cholesterol (LDL), high-density lipoprotein-cholesterol (HDL) and TG concentrations, the corresponding diagnostic kits, set by Randox Laboratories Ltd. (UK) were used according to the instructions of the manufacturer. The lipoproteins LDL and HDL were fractionated by a dual precipitation technique [20]. After fractional precipitation, lipoprotein cholesterol was estimated. In exploitation of lipid metabolism, we evaluated the cardiovascular risk factors TC/HDL ratio, TG/HDL [21] and the atherogenic index (AI) was calculated as (TC–HDL)/HDL.

Acetylcholinesterase (E.C.3.1.1.7) and butyrylcholinesterase (E.C.3.1.1.8) activities were determined at 25 °C in 0.1 M phosphate buffer (pH 7.4) with 0.3 mM DTNB and 1.0 mM ATCh or BTCh using the Ellman spectrophotometric method [22].

Protein concentrations in plasma were determined by the Coomassie reagent using serum bovine albumin as a standard [23].

2.4. Statistical analysis

Mean and standard error values were determined for all the parameters and the results were expressed as mean ± S.E. All data were analyzed employing analysis of variance ANOVA followed by Student's test. Differences between groups were considered significant when $p < 0.05$.

3. Results

No signs of toxicity were observed in malathion-treated rats until end of experiment. Our results showed that malathion administration caused a significant decrease in both acetylcholinesterase and butyrylcholinesterase activities in spite of significant increase in plasma protein content (Table 1).

Malathion at dose of 400 mg/kg of PC induced a significant increase in blood glucose, with a fold peak 2 h after administration of OP. The hepatic glycogen level was considerably increased 6–24 h after malathion administration, reaching a maximum increase at about 12 h (Table 2).

Table 2
Effects of acute administration of malathion on blood glucose level and hepatic glycogen rate

Parameters	CTR	MAL			
		2 h	6 h	12 h	24 h
Glycaemia (mg/ml)	0.98 ± 0.04	2.27 ± 0.05**	1.09 ± 0.10	1.07 ± 0.03	1.10 ± 0.05
Liver glycogen rate (g/100 g of liver)	3.17 ± 0.87	1.46 ± 0.99**	4.02 ± 0.34*	5.94 ± 0.18**	6.12 ± 0.17**

CRT, control; MAL, malathion treated rats. Values are mean ± S.E.M. (n = 12). *Significantly different from control at $p < 0.05$. **Significantly different from control at $p < 0.01$.

Table 3
Effects of acute administration of malathion on lipid status and cardiovascular index

Parameters	CTR	MAL			
		2 h	6 h	12 h	24 h
TG (mg/ml)	0.72 ± 0.03	1.02 ± 0.15*	0.84 ± 0.10*	1.27 ± 0.17**	0.95 ± 0.10*
TC (mg/ml)	0.63 ± 0.04	0.50 ± 0.03*	0.75 ± 0.02	0.70 ± 0.05	0.62 ± 0.03
HDL (mg/ml)	0.19 ± 0.01	0.21 ± 0.01	0.34 ± 0.02	0.14 ± 0.01	0.20 ± 0.03
LDL (mg/ml)	0.15 ± 0.01	0.15 ± 0.03	0.32 ± 0.04**	0.30 ± 0.05*	0.23 ± 0.03*
TG/HDL	2.37 ± 0.15	5.93 ± 1.19*	4.72 ± 0.93*	5.94 ± 0.97**	6.19 ± 1.64**
TC/HDL	2.35 ± 0.10	3.19 ± 0.69	2.72 ± 0.42	4.16 ± 0.38**	4.03 ± 0.51**

CRT, control; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MAL, malathion treated rats; TC, total cholesterol; TG, triglyceride; TC/C-HDL, total cholesterol (CT)/cholesterol of high-density lipoprotein (HDL) ratio; TG/HDL, triglycerides/ hight density lipoprotein ratio. Values are mean ± S.E.M. (n = 12). *Significantly different from control at $p < 0.05$. **Significantly different from control at $p < 0.01$.

In addition, malathion increased significantly plasma triglycerides content. There was no change in total cholesterol (TC) in malathion-treated rats except at 2 h, where the rate of TC decreased significantly. In malathion-treated rats, there was no change in rate of HDL, while, LDL level presents a significant increase when compared to control rats. Also, our results showed an increase in cardiovascular factors TG/HDL and TC/HDL (Table 3).

Table 4 presents the effect of acute administration of malathion on the AI of control and treated rats. There was a significant ($p < 0.05$) increase in the AI of treated rats compared with control rats indicating an enhancement of atherosclerosis risk.

4. Discussion

Organophosphorus pesticides show neurotoxic effects directly associated with cholinesterase inactivation [3]. Epidemiologic research for acute and chronic toxicity of malathion indicate that is highly toxic to mammals [4]. In the present study, acute exposure to high dose of malathion resulted in a significant increase in blood glucose and a depletion of hepatic glycogen. The values remained appreciably high up to 6 h, and then began decreasing until reaching control levels. It is supported by many previous works a pronounced increase in blood glucose level which was going parallel to the inhibition of the ChE and the manifestation of cholinergic stimulation as a result of OPs intoxication [6,7,12,24–26]. The mechanisms involved in the blood glucose alterations following OPs exposure are under investigation in the recent years. In explanation of the malathion-induced hyperglycemia, there is evidence that OPs influence metabolic pathways in brain, skeletal, muscles and liver in favor of increased glucose production [24]. Recently, it was reported that malathion-induced hyperglycemia by activation of hepatic cells glycogenolysis and gluconeogenesis [27]. Interestingly, stimulated glycogen metabolism and augmented activities of Phosphoenolpyruvate carboxykinase (PEPCK) are seen in most diabetic cases and could be a primary or acquired defect in the pathogenesis of diabetes [28]. Furthermore, enhanced expression of the hepatic PEPCK gene is seen in most models of diabetes [29]. Also, disturbed glycogen metabolism and glucose transport has been suggested as a cause of insulin resistance in patients with diabetes which could be a primary or acquired defect in the pathogenesis of diabetes [30]. Additionally, malathion administration showed a significant enhancement in the plasma protein content which may be explained by the increase in liver production of defence enzymatic system against toxin.

Table 4
Effects of acute administration of malathion on atherogenic index

Parameters	AI
CTR	1.35 ± 0.10
MAL	
2 h	2.19 ± 0.69*
6 h	1.52 ± 0.34
12 h	5.15 ± 1.05**
24 h	2.72 ± 0.54*

AI, atherogenic index; CRT, control; MAL, malathion treated rats. Values are mean ± S.E.M. (n = 12). *Significantly different from control at $p < 0.05$. **Significantly different from control at $p < 0.01$.

In the other hand, results from the serum lipid status of rats treated with malathion showed increased concentrations of serum triglycerides (TG), and LDL, whereas HDL was unchanged.

Lipid abnormalities play an important role in the development of atherosclerosis in type 2 diabetic patients [31]. The dyslipidemia observed in type 2 diabetes includes both quantitative and qualitative lipid abnormalities [32–34]. Quantitative lipid abnormalities are represented by hypertriglyceridemia and decreased plasma HDL levels [33]. Qualitative abnormalities include the presence of small dense LDL particles [35,36], increased triglycerides content of LDL and HDL [33], glycation of apolipoproteins [37], and increased susceptibility of LDL to oxidation [38,39]. These lipid qualitative abnormalities are likely to promote atherosclerosis. Among these, the oxidative process, particularly the oxidative conversion of native LDL to oxidized LDL, is now considered to be an essential step in the atherogenic process [40].

The AI, defined as the ratio of TC–HDL and HDL, is believed to be an important risk factor of atherosclerosis. Our data clearly demonstrate that malathion significantly increase this ratio. Malathion increased, also, a ratio TG/HDL which is a pertinent index of incidence of cardiovascular risk [21].

All these findings support the idea that OPs induce insulin resistance. As a matter of fact, hyperglycaemia, disorder of glycogen uptake, dysregulation of lipid and fatty acid metabolism, as well as the increase of stress signalling might contribute to the development of insulin resistance [41]. Recently, combination of in vivo and in vitro findings have proved that malathion induce a kind of insulin resistance [12].

The insulin produced by the pancreas is metabolized mainly by the liver. Decreased hepatic insulin metabolism alone or in association with peripheral insulin resistance is seen in individuals with cirrhosis who are also hyperinsulinemic [42]. On the other hand, the abnormality in the transport of lipoprotein diminishes the catabolism of the very low-density lipoprotein (VLDL) and increases the catabolism of the high-density lipoprotein (HDL), which creates insulin resistance [43,44]. Liver resistance to insulin might leads to an increase in hepatic glucose production, which is correlated with fasting hyperglycaemia in diabetic cases [45].

The liver has been shown to play a central role in the maintenance of glucose homeostasis. It is the primary site of endogenous glucose output which produces glucose either *de novo* from 3-carbon precursors such as glycerol, lactate and alanine (gluconeogenesis) or via the breakdown of glycogen stores (glycogenolysis). At the cellular level, regulation of these pathways is mainly mediated by hormonal and nutritional signals. Several key enzymes

integrate these signals to manipulate glucose production in the liver [46]. Liver is also involved in lipoprotein synthesis and lipid homeostasis by stimulating the breakdown of excess cholesterol [47]. The liver which is the most important organ in glucose lipid homeostasis can be a target for malathion toxicity [14].

In conclusion, malathion in acute model intoxication showed a kind of insulin resistance which may result in hyperglycaemia and lipotoxicity. Underlying mechanisms of malathion-induced hyperglycaemia are yet to be elucidated. It is not surprising if to conclude that disruption in lipid metabolism and induction of insulin resistance may lead to the development of hyperglycaemia. At the moment, it is difficult to establish how the observed changes are controlled by physiological mechanism. However, more detailed functional data will be needed to further elucidate the mechanisms by which malathion interact with lipid and glucose pathways.

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