Journal of Hazardous Materials 163 (2009) 1052–1055

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Metabolic disorders of acute exposure to malathion in adult Wistar rats

Mohamed M. Lasram*, Alya Berrahal Annabi, Naziha El Elj, Slimen Selmi, Abdelaziz Kamoun, Saloua El-Fazaa, Najoua Gharbi

Laboratoire de physiologie des aggressions, Département de physiologie animale, Faculté des sciences de Tunis, Université el Manar I, Tunis 1060, Tunisia

ARTICLE INFO

Article history:
Received 10 March 2008
Received in revised form 11 July 2008
Accepted 15 July 2008
Available online 23 July 2008

Keywords:
Malathion
Hyperglycaemia
Triglyceride
Cholesterol
HDL
LDL

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

* Corresponding author at: Laboratoire de physiologie des agressions: étude endocrinienne et métabolique, Département de physiologie, Faculté des sciences de Tunis, Université el Manar II, Tunis, Tunisia. Tel.: +216 98 25 61 46.
E-mail addresses: lasram_montassar@yahoo.fr (M.M. Lasram), najoua.gharbi@planet.tn (N. Gharbi).

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doi:10.1016/j.jhazmat.2008.07.059

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 $p < 0.01$.