

Pharmacology, toxicology / Pharmacologie, toxicologie

Effect of subchronic exposure to malathion on metabolic parameters in the rat

Raja Rezg^{*}, Bessem Mornagui, Abdelaziz Kamoun, Saloua El-Fazaa, Najoua Gharbi

*Laboratoire de physiologie des agressions, département de biologie, faculté des sciences de Tunis, campus universitaire,
2092 Manar II, Tunis, Tunisie*

Received 30 October 2006; accepted after revision 14 November 2006

Available online 8 December 2006

Presented by Jean Rosa

Abstract

This study investigates the effects of subchronic exposure to organophosphate insecticide Malathion (Fyfanon 50 EC 500 g/l) of commercial grade. It was administered intragastrically by stomach tube in the amount of 1 ml of corn oil containing 100 mg/kg body weight (BW) daily for 32 days. At the end of the experiment, acetylcholinesterase activity (AChE), haematocrit value, haemoglobin content, and blood glucose concentration were estimated. The liver and the skeletal muscle were removed to determine hepatic and muscular glycogen, hepatic proteins and lipids contents. No sign of toxicity was observed until the end of experiment. No significant change in the haematocrit value was observed, in spite of the significant increase in haemoglobin content, which can be considered as an adaptive situation in order to guarantee a good oxygenation in response to pulmonary damage induced following subchronic exposure to organophosphorus compound. Malathion intoxication decreased significantly hepatic proteins and lipid contents that could be associated to liver gluconeogenesis. This result was coupled with a significant decrease in muscular glycogen rate, which indicates a stimulated glycogenolysis in favour of glucose release into the blood until reaching hyperglycaemia. Several studies indicate that hyperglycaemia is temporary, which is probably due to a stimulated glycogenesis that increases hepatic glycogen deposition and return of glucose to control levels, as demonstrated in our study. One possible explanation for these results could be the turnover of glucose by a succession between its release via glycogenolysis and gluconeogenesis, which involves abnormal hyperglycaemia, and its storage via glycogenesis in subchronic exposure to malathion. **To cite this article:** R. Rezg *et al.*, *C. R. Biologies 330 (2007)*.

© 2006 Académie des sciences. Published by Elsevier Masson SAS. All rights reserved.

Résumé

Incidence métabolique du malathion après une exposition subchronique chez le rat. Cette étude s'intéresse aux effets hématologiques et métaboliques d'un insecticide organophosphoré, le malathion (Fyfanon 50 EC 500 g/l), lors d'une exposition chronique chez le rat. Le malathion, solubilisé dans 1 ml d'huile de maïs à raison de 100 mg/kg de poids corporel par jour, est administré par gavage durant 32 jours. À la fin de l'expérience, nous avons déterminé l'activité de l'acétylcholinestérase (AChE), le taux d'hématocrite, la concentration en hémoglobine et la glycémie. Le foie et le muscle ont été isolés pour la détermination du glycogène hépatique et musculaire, ainsi que les taux des lipides et des protéines hépatiques. Aucun signe de toxicité n'est enregistré jusqu'à la fin de l'expérience. Nous n'avons pas enregistré de différence significative des taux d'hématocrite, malgré l'élévation significative de la concentration en hémoglobine, qui pourrait être une situation adaptative en réponse aux dommages pulmonaires

^{*} Corresponding author.

E-mail address: raja.rezg@laposte.net (R. Rezg).

induits par l'administration chronique du malathion afin d'assurer une bonne oxygénation. L'intoxication par le malathion entraîne une diminution significative des taux des protéines et des lipides hépatiques qui pourrait être due à une néoglucogenèse active au niveau du foie. Par ailleurs, ces résultats sont associés à une diminution significative du taux du glycogène musculaire, indiquant une glycogénolyse active en faveur de la libération du glucose dans le sang jusqu'à atteindre une hyperglycémie. Plusieurs études ont montré que l'hyperglycémie enregistrée est transitoire, ce qui provoquerait ultérieurement une activation de la glycogénèse, qui entraînerait l'élévation du taux de glycogène hépatique et le retour de la glycémie à son niveau normal. Nous proposons, dans cette étude, une explication possible pour ces résultats, qui pourrait être l'installation du mécanisme de *turnover* du glucose durant l'exposition chronique au malathion. En effet, il semble que l'administration du malathion entraîne quotidiennement la succession de la libération du glucose dans le sang, jusqu'à atteindre une hyperglycémie, et de son stockage, jusqu'au retour de la glycémie à son niveau normal. L'hyperglycémie est probablement due à l'activation de la glycogénolyse dans plusieurs organes et la néoglucogenèse dans le foie, qui aura pour conséquence une hyperglycémie anormale, qui sera affrontée par l'activation de la glycogénogenèse. **Pour citer cet article :** R. Rezg et al., C. R. Biologies 330 (2007).

© 2006 Académie des sciences. Published by Elsevier Masson SAS. All rights reserved.

Keywords: Haemoglobin; Malathion; Glycogenolysis; Gluconeogenesis; Glycogenesis; Rat

Mots-clés : Hémoglobine ; Malathion ; Glycogénolyse ; Néoglucogenèse ; Glycogénèse ; Rat

1. Introduction

Pesticides are occasionally used indiscriminately in large amounts, causing environmental pollution; they are therefore a cause of concern. The use of organophosphorus pesticides (OP) has increased considerably due to their low potency and durability as compared to organochlorine pesticides [1]. Residual amounts of (OP) pesticides have been detected in the soil, water bodies, vegetables, grains, and other foods products [2,3]. The mechanism of the toxicity of (OP) compounds consists mainly in blocking acetylcholinesterase, an enzyme that decomposes acetylcholine [4,5]. Immobilisation of this enzyme results in an accumulation of excessive amounts of acetylcholine in nervous tissue and muscular motor plates, as well as in symptoms of endogenic poisoning by this neurohormone. The OP compound malathion [S-1,2(bis ethoxycarbonyl) ethyl O, O dimethyl phosphorodithioate] is used extensively throughout the world to control major arthropods in public health programs, animal ectoparasites, human head and body lice, household insects and to protect grain in storage [6]. Among the targets for malathion toxicity is the liver, which is the most important organ in glucose homeostasis and production of related enzymes [7].

It has been reported in several studies that hyperglycaemia is one of the side effects in poisoning by OP in acute treatment and in subchronic exposure via glycogenolysis and gluconeogenesis [1,8–11]. Nevertheless, Rezg et al. have noted for the first time an activation of glycogen storage and stimulation of enzymes of glycogenesis, accompanied with normoglycaemia in subchronic exposure to malation [12].

The main question of the present study was: what will happen to metabolic parameters in subchronic exposure to malathion to establish these antagonist reactions, glycogenesis and glycogenolysis associated with gluconeogenesis? In addition, the present investigation examines malation effects on some haematological parameters.

2. Materials and methods

2.1. Chemicals

Malathion (fyfanon 50 EC 500 g/l) of commercial grade was used in this study.

Acetylthiocholine iodide, 5,5-dithio bis(2-nitrobenzoic acid) (DTNB), trichloroacetic acid (TCA), KOH, ethanol, ether, Coomassie G250, bovine serum albumin, orthophosphoric acid 85%, chloroform, methanol, and NaCl were obtained from Sigma-Aldrich Co. (Germany).

2.2. Animals and treatments

Adult male Wistar rats (170–180 g), procured from the Tunisian Society of Pharmaceutical Industries, were kept in clean plastic cages and allowed to acclimatize in the laboratory environment for 7 days. Balanced food and drinking water were given to the animals ad libitum.

Animals were randomly divided into two groups of 12 animals and treated for 32 days. The first group received orally 1 ml of corn oil by force-feeding. The second one received 1 ml of corn oil containing 100 mg Mal/kg body weight per day. The experiment was performed in ethical conditions. At the end of the treatment, 24 h after the last dose of malathion, animals

were killed by decapitation, without preliminary anaesthesia, and arteriovenous blood was taken quickly. The liver and the skeletal muscle were removed by transverse abdominal incision and kept frozen at -80°C .

2.3. Acetylcholinesterase (AChE) activity assay

Acetylcholinesterase activity of plasma was estimated by the method of Ellman [13], using acetylthiocholine iodide as a substrate. The rate of hydrolysis of acetylthiocholine iodide is measured at 412 nm through the release of a thiol compound, which, when reacted with DTNB, produces the colour-forming compound TNB.

2.4. Haematological parameters

2.4.1. Haemoglobin concentration

Haemoglobin was measured through its transformation to cyanmethemoglobin, which is measured spectrophotometrically at 540 nm under the action of potassium ferricyanide and potassium cyanide [14].

2.4.2. Haematocrit content

This assay was done directly using a centrifugal machine to haematocrit.

2.5. Metabolic parameters

2.5.1. Plasma glucose assay

Glucose was measured by the glucose oxidase and peroxidase using quinoneimine as a chromogen. The amount of plasma glucose is related to the amount of quinoneimine, which is measured spectrophotometrically at 505 nm [15].

2.5.2. Hepatic and muscular glycogen assay

For the determination of glycogen, we followed the technique of Good [16]: 0.5 g of liver or muscle was extracted with 3 ml of 30% KOH, incubated for 30 min at 100°C , cooled and 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 are expressed in g of glycogen per 100 g of liver.

2.5.3. Hepatic protein assay

The protein content in liver was quantified by the method of Bradford [17], using bovine serum albumin as a standard.

2.5.4. Hepatic lipid assay

The lipid content in liver was quantified by the method of Folch [18], using a mixture of chloroform and methanol for the extraction. Lipid was precipitated by ethanol and weighed. The results were expressed in g of glycogen per 100 g of liver.

2.6. Statistical analysis

All data are expressed as mean \pm SEM by factorial ANOVA, followed by Student test. Differences between groups were considered significant when $P < 0.05$.

3. Results

3.1. Physical observation

No signs of toxicity were observed in malathion-treated animals until the end of experiment.

3.2. Acetylcholinesterase (AChE) activity and haematological parameters

Malathion reduced significantly AChE activity. No significant change was observed in haematocrit values, in spite of the significant increase in haemoglobin concentration (Table 1).

3.3. Effect of malathion on metabolic parameters

Malathion at dose of 100 mg/kg/day decreased significantly hepatic proteins and lipids contents and muscular glycogen rate, in spite of the significant increase in hepatic glycogen rate. Blood glucose concentration was not significantly different between groups (Table 2).

Table 1
Effect of subchronic administration of malathion on AChE activity, haematocrit value and haemoglobin concentration

Treatment	AChE (nmol/min/mg protein)	Haematocrit value (%)	Haemoglobin concentration (mmol/l)
Control ($n = 12$)	12.20 ± 0.79	42.28 ± 0.76	1.64 ± 0.13
Malathion ($n = 12$)	$6.62 \pm 0.07^{***}$	42.03 ± 0.63	$2.00 \pm 0.09^*$

Values are presented as the mean \pm SEM, n : number of rats used.

* $p < 0.05$ as compared with control.

*** $p < 0.001$ as compared with control.

Table 2

Effect of subchronic administration of malathion on plasma glucose concentration, hepatic and muscular glycogen rates and hepatic proteins and lipids' contents

Treatment	Blood glucose concentration (g/l)	Muscular glycogen rate (g/100 g of liver)	Hepatic glycogen rate (g/100 g of liver)	Hepatic proteins rate (g/100 g of liver)	Hepatic lipids rate (g/100 g of liver)
Control ($n = 12$)	1.16 ± 0.24	2.7 ± 0.10	3.91 ± 0.34	$4.20 \pm 0.21^*$	5.92 ± 0.16
Malathion ($n = 12$)	1.20 ± 0.15	$1.88 \pm 0.06^{***}$	$5.88 \pm 0.43^{**}$	3.60 ± 0.09	$4.65 \pm 0.18^{***}$

Values are presented as the mean \pm SEM; n : number of rats used.

* $p < 0.05$ as compared with control.

** $p < 0.01$ as compared with control.

*** $p < 0.001$ as compared with control.

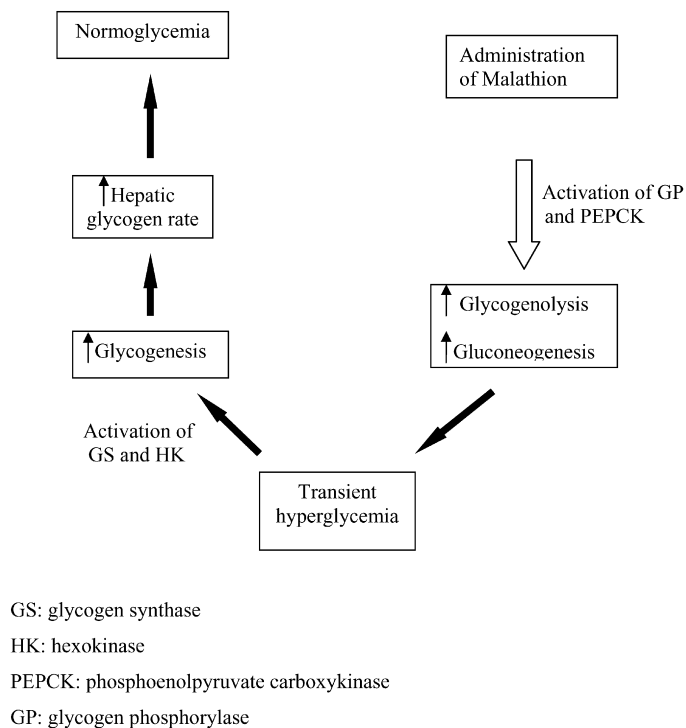


Fig. 1. The mechanism of turnover of glucose in subchronic exposure to malathion: A time-dependent variation of different metabolic parameters.

4. Discussion

4.1. Effect of malathion on haematological parameters

The results of this study indicate that malathion, in subchronic exposure at a dose of 100 mg/kg per day, decreases significantly acetylcholinesterase (AChE) activity on plasma, which is often taken as an indicator of (OPs) pesticide intoxication. In addition, our results showed a marked increase in haemoglobin concentration, which can be considered as an adaptive situation in order to guarantee a good oxygenation in response to pulmonary damage induced by subchronic exposure to malathion. Indeed, a rise in the haemoglobin content is generally among the signs of the pulmonary fibrosis [19,20], which several studies suggested to be among

the injurious consequences of (OP) compound administration [21–23]. This explanatory assumption needs to be examined in more detail with appropriate biochemical and hormonal determinations. On the other hand, the haematocrits' count has not been changed significantly.

4.2. Effect of malathion on metabolic parameters

The results of this study indicate that malathion in subchronic exposure decreased significantly hepatic proteins and lipid contents. This reduction could be associated to liver gluconeogenesis. Indeed, Abdollahi et al. [11] have showed an increase in the hepatic gluconeogenic enzyme activity, phosphoenolpyruvate carboxykinase (PEPCK), in response to malathion exposure, which indicates a stimulated gluconeogenesis.

In addition, several studies indicate that malathion exposure causes hyperglycaemia, as an immediate consequence of malathion administration [8–10], which was explained by stimulation of glycogenolysis in several organs [1,10,11] and of gluconeogenesis in liver [11], provoking glucose release into the blood. This explained the significant decrease in hepatic proteins and lipid contents and the observed muscular glycogen rate.

However, it is interesting to note that hyperglycaemia is temporary [8,9]. Indeed, a time-dependent variation in blood glucose concentration was probably repeated daily, independently of the duration of the treatment (acute or chronic). Thus, malathion induced a gradual increase followed by a decrease in blood glucose, which can even reach hypoglycaemia [12]. On the other hand, it has been shown that acute exposure to malathion leads to the increase of glycogen deposition in liver during 6 to 24 h after malathion treatment [8]. It is well established that the increase in hepatic glycogen rate is due to the stimulation of key enzyme of glycogenesis (glycogen synthetase) [12].

One possible explanation for these results could be the turnover of glucose by its release through a succession of glycogenolysis and gluconeogenesis [1,11], which involves abnormal hyperglycaemia and its storage via glycogenesis [12] in subchronic exposure to malathion (Fig. 1).

Consequently, the divergence observed between studies may be due to the difference between the experimental protocols, especially the duration separating sampling and last administration of malathion. However, finding out the exact mechanism of glucose turnover in subchronic exposure needs appropriate sampling and molecular analysis. This explanatory assumption is currently under study by our team.

References

- [1] S. Pournourmohammadi, B. Farzami, S.N. Ostad, E. Azizi, M. Abdollahi, Effects of malathion subchronic exposure on rat skeletal muscle glucose metabolism, *Environ. Toxicol. Pharmacol.* 19 (1) (2004) 169–191.
- [2] IARC, Miscellaneous Pesticides, Monograph on the Evaluation of Carcinogenic Risk of Chemicals to Man, vol. 30, International Agency for Research on Cancer, Lyons, France, 1983.
- [3] S. John, M. Kale, N. Rathore, D. Bhatnagar, Protective effect of vitamin E in dimethonate and malathion induced oxidative stress in rat erythrocytes, *J. Nutr. Biochem.* 12 (2001) 500–504.
- [4] K.S. Harlin, J.A. Dellinger, Retina, brain and blood cholinesterase levels in cats treated with oral dichlorvos, *Vet. Hum. Toxicol.* 35 (1993) 201–203.
- [5] J. Lewalter, U. Korallus, Erythrocyte protein conjugates as a principle of biological monitoring for pesticides, *Toxicol. Lett.* 33 (1986) 153.
- [6] M. Maroni, C. Colosio, A. Ferioli, A. Fait, Biological monitoring of pesticide exposure: a review, *Introduction, Toxicology* 7 (2000) 1–118.
- [7] M.C. Yang, A.J. McLean, L.P. Rivory, D.G. Le Couteur, Hepatic disposition of neurotoxins and pesticides, *Pharmacol. Toxicol.* 87 (2000) 286–291.
- [8] P.K. Gupta, Malathion induced biochemical changes in rats, *Acta Pharmacol. Toxicol.* 35 (3) (1974) 191–194.
- [9] M.R. Rodrigues, F.R. Puga, E. Chenker, M.T. Mazanti, Short-term effect of malathion on rats' blood glucose and on glucose utilization by mammalian cells in vitro, *Ectotoxicol. Environ. Saf.* 12 (2) (1986) 110–113.
- [10] M.A. Matin, K. Husain, Cerebral glycogenolysis and glycolysis in malathion-treated hyperglycaemic animals, *Biochem. Pharmacol.* 36 (11) (1987) 1815–1817.
- [11] M. Abdollahi, M. Donyavi, S. Pournourmohammadi, M. Saadat, Hyperglycemia associated with increased hepatic glycogen phosphorylase and phosphoenolpyruvate carboxykinase in rats following subchronic exposure to malathion, *Comp. Biochem. Physiol. C* 137 (4) (2004) 343–347.
- [12] R. Rezg, B. Mornagui, M. El-Arbi, A. Kamoun, S. El-Fazaa, N. Gharbi, Effect of subchronic exposure to malathion on glycogen phosphorylase and hexokinase activities in rat liver using native PAGE, *Toxicology* 223 (2006) 9–14.
- [13] G.L. Ellman, K.D. Courtney, V. Andres Jr., R.M. Featherstone, A new and rapid colorimetric determination of acetylcholinesterase activity, *Biochem. Pharmacol.* 7 (1961) 88–95.
- [14] W.G. Zijlstra, V.E. Kampen, Standardization of hemoglobinometry. I. The extinction coefficient of hemiglobincyanide, *Clin. Chim. Acta* 5 (1960) 9–26.
- [15] J.A. Lott, K. Turner, Evaluation of Trinder's glucose oxidase method for measuring glucose in serum and urine, *Clin. Chem.* 21 (12) (1975) 1754–1760.
- [16] C.A. Good, H. Krames, M. Somogyi, Chemical procedures for analysis of polysaccharides, *Methods Enzymol.* VII (1933) 34.
- [17] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, *Anal. Biochem.* 72 (1976) 248–254.
- [18] J. Folch, M. Lees, G.H. Sloane Stanley, A simple method for the isolation and purification of total lipides from animal tissues, *J. Biol. Chem.* 226 (1) (1957) 497–509.
- [19] J.P. Kehrer, Y.C. Lee, Pulmonary hydroxyproline content and production following treatment of mice with *O,S,S*-trimethyl phosphorodithioate, *Toxicol. Lett.* 38 (3) (1987) 321–327.
- [20] A. Tsantes, S. Bonovas, S. Tassiopoulos, K. Filioussi, A. Vlachou, J. Meletis, S. Papadimitriou, G. Vaiopoulos, A comparative study of the role of erythropoietin in the pathogenesis of deficient erythropoiesis in idiopathic pulmonary fibrosis as opposed to chronic obstructive pulmonary disease, *Med. Sci. Monit.* 11 (4) (2005) 177–181.
- [21] A. Samimi, J.A. Last, Inhibition of lysyl hydroxylase by malathion and malaxon, *Toxicol. Appl. Pharmacol.* 172 (2001) 203–209.
- [22] N. Konno, T.R. Fukuto, T. Imamura, Lung injury and delayed toxicity produced by *O,S,S*-trimethyl phosphorodithioate, an impurity of malathion, *Toxicol. Appl. Pharmacol.* 15 75 (2) (1984) 219–228.
- [23] T. Imamura, N.L. Schiller, T.R. Fukuto, Malathion and phenthoate carboxylesterase activities in pulmonary alveolar macrophages as indicators of lung injury, *Toxicol. Appl. Pharmacol.* 70 (1) (1983) 140–147.