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Association of inflammatory response and oxidative injury in the pathogenesis of liver steatosis and insulin resistance following subchronic exposure to malathion in rats

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ABSTRACT

Insulin resistance and risk of type 2 diabetes are the most important complications following exposure to organophosphorous (OPs) pesticides. Regarding the importance of liver on metabolic pathways regulation, in particular blood glucose homeostasis, we focused on liver inflammation and oxidative damages in a subchronic model of toxicity by malathion. Adult male Wistar rats of body weight 200–250 g were used for the study. Malathion (200 mg/kg b.w./day) was administered to rats by oral intubation for 28 days. Glycemic and insulin resistance indices, markers of liver injury, markers of inflammation and oxidative stress were assessed. Malathion-treated rats showed increased glycemia, insulinemia and glycated hemoglobin level, HOMA-IR and HOMA-β indices, plasma activities of hepatocellular enzymes, lipid peroxidation index, $CD3^+/CD4^+$ and $CD3^+/CD4^+$ and pro-inflammatory cytokines when decreased antioxidant status in liver was noted. Most of our study indicates that malathion promotes insulin resistance, inflammation and Hepatosteatosis in subchronic model of exposure. On the basis of biochemical and molecular findings, it is

Abbreviations: ALP, Alkaline phosphatase; ALT, Alanine transaminase; AP1, Activator Protein 1; AST, Aspartate transaminase; BChE, Butyrylcholinesterase; BLR, Bilirubin; CAT, Catalase; $CD3^+$, Cluster of differentiation 3 (T-cell coreceptor); $CD4^+$, Cluster of differentiation 4 (cell-surface glycoprotein); $CD8^+$, Cluster of differentiation 8 (transmembrane glycoprotein); CTR, control rats; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GPx, Glutathione peroxidase; GSH, Reduced glutathione; GST, Glutathione S-transferase; HOMA-IR, Homeostasis model assessment for insulin resistance; HOMA-β, Pancreatic β-cell function; IKK, IκB kinase; IL-1β, Interleukin-1 beta; IL-6, Interleukin-6; INF-γ, Interferon gamma; IRS, Insulin receptor substrate; JAK, C-Jun N-terminal kinases; KO, Knock-out; LDH, Lactate deshydrogenase; MAL, malathion-treated rats; MDA, Malondialdehyde; MPO, Myeloperoxidase; NF-κB, Nuclear Factor-kappa B; OPs, Organophosphorus; ROS, Reactive Oxygen Species; SOCS-3, Suppressor cytokine signaling 3; SOD, Superoxide dismutase; SP-1, Specific protein 1; STATs, Signal Transducers and Activators of Transcription; TBARS, thiobarbituric acid reactive substances; TNF-α, Tumor necrosis factor α.

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concluded that insulin resistance induced by malathion occurs through oxidative stress and related pro-inflammatory markers in a way to result in a reduced function of insulin in liver cells.

1. Introduction

In recent years, organophosphate pesticides (OPs) use is increasing exponentially in order to protect crops from pests and weeds. Recent investigations focused on the effects of these pesticides on metabolism since OPs are associated with elevated risk of developing diabetes (Lassiter et al., 2008). In a case study research reported during 5 years (1999–2003) including 33,457 applicators, Montgomery et al. (2008) have suggested that long-term exposure to handling OPs is particularly diabetogenic. Actually, there are an increasing number of animal and human studies that show an association between pesticide exposure and diabetes. This is concerning, as pesticide use is not only well entrenched but has dramatically increased.

The OP compound malathion [S-1,2(bis ethoxycarbonyl) ethyl O,O-dimethyl phosphorodithioate] is extensively used as insecticide and acaricide in agricultural, veterinary, medical and public health practices (Hazarika et al., 2003). Malathion exposure has been associated with metabolic disorders (Lasram et al., 2009), oxidative stress (Alp et al., 2012), immunotoxicity (Nain et al., 2011), inflammation (Mostafalou et al., 2012) and hepatotoxicity (Kalender et al., 2010; Moore et al., 2010). Recently, Raafat et al. (2012) have investigated the effect of malathion in Egyptian farmers and demonstrated a strong correlation between insulin resistance and malathion blood concentration. In previous works, we have demonstrated a time dependent variation in blood glucose concentration in acute malathion intoxication. In addition, we have suggested a turn over mechanism of glucose following malathion administration, explained by a succession between its release via glycogenolysis and gluconeogenesis (Lasram et al., 2008, 2009). More other studies reported glucose metabolism disturbance and insulin resistance in experimental animals exposed to acute or chronic malathion dosage (Pournourmohammadi et al., 2007; Vosough-Ghanbari et al., 2007). The mechanisms involved in the pathogenesis of insulin resistance following malathion exposure are, still under investigation.

Remarkable advances in our understanding of mechanisms responsible for malathion-induced insulin resistance have arisen from molecular, cellular and functional studies in animals. These studies have established that malathion primarily affects the liver of treated animals (Aboul-Soud et al., 2011; Al-Othman et al., 2012). The pathological lesions caused by malathion lead to hepatotoxic injury. As proposed by Kalender et al. (2010), malathion initially damages the liver which is involved on its conversion to toxic intermediates, followed by Reactive Oxygen Species (ROS) production, lipid peroxidation and release of pro-inflammatory cytokines. The intense expression of several pro-inflammatory cytokines has been implicated in inflammation, fibrogenesis and hepatosteatosis (Fujii and Kawada, 2012).

In fact, increasing evidence suggests chronic inflammation as an important pathogenetic factor in the development of insulin resistance and type 2 diabetes (Donath and Shoelson, 2011). It was further demonstrated that elevated hepatic IKK (IκB kinase) and NF-κB (Nuclear Factor-kappa B) activation is associated with insulin resistance and systemic inflammation (Cai et al., 2005). Similarly, over-expression of hepatic IKK and constitutive activation of NF-κB increase pro-inflammatory cytokine expression, including IL-1β (Interleukin-1 beta), IL-6 (Interleukin-6), and INF-γ (Interferon gamma) (Cai et al., 2005). By contrast, NF-κB inhibition by abrogation of liver IKK activity led to decreased pro-inflammatory cytokine levels (Arkan et al., 2005). Together, these studies suggest that an increased active hepatic IKK/NF-κB pathway can drive the onset of insulin resistance. Thus, the current study is designed to investigate the diabetogenic, oxidative and inflammatory effects of malathion in rats. Furthermore, we aimed to determine the role of malathion in hepatotxicity and its relationship with insulin resistance.

2. Methods

2.1. Chemicals

Malathion was obtained from the Agricultural Struggle Center (Tunis, Tunisia), molecular formula: $C_{10}H_{19}O_6PS_2$, molecule weight: 330.4 g/mol. Its purity is 98.9% by HPLC analysis. 1-Chloro-2,4-dinitrobenzene (CDNB); 2,6,-di-tert-butyl-4-methylphenol (BHT); 2-thiobarbituric acid (TBA); 5,5-dithio bis(2-nitrobenzoic acid)(DTNB); Ammonium chloride (NH_4Cl); Bovine serum albumin; Epinephrine; Glutathione reductase; Hydrogen peroxide (H_2O_2); Potassium hydroxide (KOH); Reduced glutathione (GSH); S-butyrylthiocholine iodide; Sodium azide; Sulfosalicylic acid; Trichloroacetic acid (TCA) and β-Nicotinamide adenine dinucleotide phosphate (NADPH) were obtained from sigma-Aldrich Co (Germany). Deoxyribonucleotide (dNTP) mix; DNA ladder; DNase; Free RNase; Oligo-dT; $MgCl_2$; Random hexamer; RNAse inhibitor; RPMI-1640; Taq DNA polymerase; TRIzol reagent and Primers for IL-1β, IL-6 and INF-γ were obtained from Invitrogen (St Thomas Aubin, France). Alanine transaminase (ALT); Alkaline phosphatase (ALP); Aspartate transaminase (AST); Bilirubin (BLR); Glucose oxidase and Lactate deshydrogenase (LDH) kits were provided from Randox laboratories diagnostics, Ltd. (UK). Rat insulin ELISA kit was purchased from (Biosource, Belgium). The complementary DNA (cDNA) synthesis kit was provided from Invitrogen (St Thomas Aubin, France). Myeloperoxidase (MPO) kit was obtained CytoStore (Calgary, Canada). Monoclonal anti-rat antibodies used for flow cytometry analysis: