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Antioxidant and anti-inflammatory effects of N-acetylcysteine against malathion-induced liver damages and immunotoxicity in rats

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ABSTRACT

Aims: Occupational exposure to organophosphate pesticides is becoming a common and increasingly alarming world-wide phenomenon. The present study is designed to investigate the preventive effect of N-acetylcysteine on malathion-induced hepatic injury and inflammation in rats.
Main methods: Adult male Wistar rats of body weight 200–230 g were used for the study. Malathion (200 mg/kg b.w./day) was administered to rats by oral intubation and N-acetylcysteine (2 g/l) in drinking water for 28 days. Rats were sacrificed on the 28th day, 2 h after the last administration. Markers of liver injury (aspartate transaminase, alanine transaminase, alkaline phosphatase and lactate desyhdogenase), inflammation (leukocyte counts, myeloperoxidase, immunophenotyping of $CD4^+$ and $CD8^+$, interleukin-1β, interleukin-6 and interferon-γ expression) and oxidative stress (lipid peroxidation, reduced glutathione and antioxidant status) were assessed.
Key findings: Malathion induced an increase in activities of hepatocellular enzymes in plasma, lipid peroxidation index, $CD3^+/CD4^+$ and $CD3^+/CD4^+$ percent and pro-inflammatory cytokines, when decreased antioxidant status in liver was noted. When malathion-treated rats were compared to NAC supplemented rats, leukocytosis, T cell count and IL-1β, IL-6, INF-γ expression were reduced. Furthermore, NAC restored liver enzyme activities and oxidative stress markers.
Significance: Malathion induces hepatotoxicity, oxidative stress and liver inflammation. N-acetylcysteine showed therapeutic effects against malathion toxicity.

Introduction

The policy of modern agriculture is based on the common use of pesticides as a defense against pests and weeds. The most commonly used class of pesticides currently in use is the organophosphorus (OP) insecticides because these are highly effective and exhibit relatively non-persistent characteristics (Galloway and Handy, 2003). Pesticides poisoning from occupational, accidental and intentional exposure is a major public health problem (Jeyaratnam, 1990). 2 million cases of human poisoning are reported each year with 200,000 deaths (Eddleston, 2000). Malathion is one of the largest selling and useful OPs 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., 2011), immunotoxicity (Nain et al., 2011), inflammation (Mostafalou et al., 2012) and hepatotoxicity (Kalender et al., 2010). Moreover, recent investigations have shown that malathion is a hepatotoxin (Moore et al., 2010; Josse et al., 2014). The pathological lesions caused by malathion affect the liver structure and function leading to hepatosteatosis. A growing body of evidence supports the role of hepatic inflammation in the pathogenesis of chronic liver disease (Hotamisligil, 2006). Indeed, liver inflammation leads to the secretion of pro-inflammatory cytokines which, in turn, contribute to a feed-forward amplification of inflammatory signaling and subsequent progression of hepatosteatosis (Li et al., 2012).

Otherwise, the generation of reactive oxygen species (ROS) and the instigation of oxidative stress are associated with liver damages (Moore et al., 2010). Malathion is known to induce extensive ROS in the liver when it is metabolized to malaoxon (Buratti et al., 2005). It was reported that malathion-induced oxidative stress can be detected after 2 h (Lasram et al., 2008) and this may contribute to toxicity in liver. A body of epidemiological, clinical and experimental evidences calls for hepatoprotective effects of antioxidants. Furthermore, some antioxidants

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showed an inhibition of inflammatory processes during hepatosteatosis (Demiroren et al., 2013).

N-acetylcysteine (NAC), a thiol containing amino acid, has been in clinical use for over 30 years, as an antidote for acetaminophen overdose (Baumgardner et al., 2008). NAC is a precursor for glutathione (GSH) synthesis and its hepatoprotective ability has been linked to prevention of liver diseases characterized by decreased GSH and/or increased oxidative stress such as alcoholic liver disease (Thong-Ngam et al., 2007). NAC acts as a scavenger of free radicals due to its direct interaction with ROS (Dodd et al., 2008). In addition, NAC is a potent anti-inflammatory and immune-modulatory compound. Thus, NAC enhanced T-cell function, and delayed the reduction in $CD4^+$ levels in HIV patients (Breitkreutz et al., 2000). Administration of NAC to postmenopausal women improved immune functions as exhibited by enhanced phagocytic capacity, leukocyte chemotaxis and decreased TNF-α and interleukin-8 (IL-8) levels (Arranz et al., 2008).

Based on these findings, we designed this study to evaluate the protective effects and possible immune-modulatory mechanisms of N-acetylcysteine in malathion-induced hepatic injury and immunotoxicity. To the best of our knowledge, this is the first study reporting the possible interactions, at the molecular level, between malathion and NAC in vivo.

Methods

Chemicals

Malathion (fyfanon 50 EC, 500 g/l) was obtained from the Agricultural Struggle Center, Tunis, Tunisia. N-acetyl-L-cysteine (C_5H_9–NO_3S, 99% purity) and all others chemicals were purchased from Sigma–Aldrich Co. (Germany).

Animals

Adult male Wistar rats weighting 200 ± 30 g (ten weeks old) were procured from Tunisian Society of Pharmaceutical Industries and housed two per clean plastic cage and allowed to acclimatize in the laboratory environment. Animals were maintained in a mass air displacement room with a 12-h light: 12-h dark cycle at 24 ± 2 °C with a relative humidity of 50 ± 10%. Balanced food and drinking water were given to the animals ad libitum. Animal experiments were carried out under strict compliance with the Guidelines for Ethical Control and Supervision in the Care and Use of Animals.

Determination of optimum dose

The choice of malathion dose was based on previous works of our group (Lasram et al., 2008, 2009) and corresponds to an acceptable dose that did not cause any sign of toxicity until the end of the experiment period. The used dose of malathion is calculated directly from commercial grade and corresponds to 1/10 DL50 (since LD50 = 2000 mg/kg of body weight for rat (IPCS, 1996)).

The dose of NAC was selected on the basis of previously published reports suggesting that NAC was not toxic to humans or animals at this dose (Ortolani et al., 2000). Furthermore, NAC does not show any signs of toxicity at doses even higher than the one administered in the present study (El Midaoui et al., 2008).

Experimental design

A total of 48 animals were randomized into four groups of 12 rats each and were treated as below for 28 consecutive days. Malathion or vehicle (corn oil) was administered in the morning (between 09:00 and 10:00 h) to non-fasted rats. To investigate the protective effect of NAC, a dose of 2 g/l was administered to rats in distilled drinking water since the first day of treatment and maintained during the experimental period. Control group (CTR) received 1 ml of corn oil. NAC-treated group (CTN) was administered NAC in distilled drinking water. The malathion-treated group (MAL) received by daily intragastric intubation 200 mg/kg of malathion dissolved in a total volume of 1 ml of corn oil. Finally, malathion plus NAC -treated group (NAC) received both malathion once a day and continuous treatment of N-acetylcysteine during 28 day.

Fasted animals were decapitated without preliminary anesthesia on the 28th day, 2 h after the last administration of malathion. Arteriovenous blood was quickly collected in EDTA tubes for peripheral leukocyte count. Plasma was separated by centrifuging at 3000 ×*g* for 15 min and used for marker enzymes of liver injury. At the same time, liver and spleen were removed, cleared of blood and immediately transferred into ice-cold saline. Liver tissue was weighed and homogenized in appropriate buffers and used for various estimations. Spleen tissue was used for cytometric analysis. Further, 8 rats per group were randomly selected for molecular and cellular studies.

Biochemical markers of liver injury

Supernatants of liver homogenates homogenized in phosphate buffer (0,1 M ; pH = 7,4) were taken for the analysis of malondialdehyde and the oxidative related parameters, catalase, superoxide dismutase, glutathione S-transferase and glutathione peroxidase. These parameters were measured by the methods as the following. All results were corrected using the level of protein contents in the samples.

Marker enzymes

To assess the liver injury, activities of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) in plasma were assayed using kits obtained from Randox Laboratories Diagnostics, Ltd. (UK).

Lipid peroxidation

Lipid peroxidation was evidenced by measuring the formation of thiobarbituric acid reactive substances hydroperoxides using the method of Ohkawa et al. (1979). Briefly, 0,1 ml of liver supernatant and 1,9 ml of 0,1 M sodium phosphate buffer (pH 7,4) was incubated at 37 °C for 1 h. After the incubation, the mixture was precipitated with 10% TCA (trichloroacetic acid) and centrifuged (2300 ×*g* for 15 min at room temperature) to collect supernatant. Then 1 ml of 1% TBA was added to the supernatant and placed in the boiling water for 15 min. After cooling to room temperature absorbance of the mixture was taken at 532 nm and was converted to MDA and expressed in nmoles per mg protein using molar extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$.

Reduced glutathione

Reduced glutathione (GSH) level was determined in hepatic liver tissue (homogenized directly in 10 volumes of 5% sulfosalicylic acid) by the methods of Griffith (1980) based on an enzyme recycling assay using glutathione reductase, 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB) and NADPH. Absorbance change at 412 nm was followed in a spectrophotometer for 5 min. The rate of yellow color accumulation is the result of 2-nitro-5-thiobenzoate (TNB) formed from DTNB. GSH levels are expressed as μmol per mg protein.

Superoxide dismutase

Superoxide dismutase activity (SOD) was assayed according to Misra and Fridovich (1972). The assay procedure involves the inhibition of epinephrine auto-oxidation in an alkaline medium (pH 10,2) to adrenochrome, which is markedly inhibited by the presence of SOD. Epinephrine was added to the assay mixture, containing tissue supernatant and the change in extinction coefficient was followed at 480 nm in a spectrophotometer. The unit of enzyme activity is defined as the enzyme required for 50% inhibition of auto-oxidation of epinephrine and expressed as unit per mg of protein.