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Physiopathology of multiple organ dysfunctions in severely monocrotophos-poisoned rabbits

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ABSTRACT

Objective: Acute organophosphorus pesticides poisoning has a serious threat on people's health. This study aimed to investigate the pathogenesis and molecular mechanism of multiple organ dysfunction syndrome (MODS) in severely monocrotophos-poisoned rabbits.

Methods: Chinchilla rabbits were used to build the monocrotophos-poisoned animal model via subcutaneous abdominal injection. Acetylcholinesterase activity was determined using the dithiobisnitrobenzoic acid enzyme kinetics method, and the free organophosphorus (FOP) toxic substances content was analyzed using the enzyme inhibition method. The contents of tumor necrosis factor (TNF-α), interleukin 1-β (IL-β) and thromboxane B_2 (TXB_2) in the plasma and tissue homogenates were determined via radioimmunoassay.

Results: Twenty-four hours after exposure, in comparison to the plasma, blood cells and homogenates of various tissues, the bile had a significantly different FOP content ($P < 0.05$). In different phases, HE staining results confirmed that several degrees of pathological lesions (such as hemorrhage, edema, degeneration and necrosis) were detected in FOP poisoned rabbits. The TXB_2 and TNF contents in plasma were significantly higher than those of the control ($P < 0.05$). Except for the intercostal muscle, all of the tissues had significantly higher TXB_2 contents than the control. The TNF contents of the liver and lung and the IL-1β contents of the liver and kidney were significantly higher than those of the control ($P < 0.05$).

Conclusion: FOP stored in the gallbladder may play important role in enterohepatic circulation. In MODS rabbits, caused by OP poisoning, the TXB_2 and TNF-α may play important role in inflammatory response and complement and coagulation systems respectively.

1. Introduction

Organophosphorus pesticides (OPs) are currently the most commonly produced and used pesticides [1]. OPs require a small application amount and have high toxicity and a broad spectrum of activity. While these pesticides are widely applied in agriculture and forestry, they also cause poisoning in approximately 300,000 people worldwide each year due to inadequate protection or suicide [2]. Despite the existence of specific antidotes, mortality has remained as high as 40% [3], with even higher mortality for severely poisoned victims. For a long time, the mechanisms that underlie the central and peripheral respiratory failure and organ dysfunction that are caused by acute OP pesticide poisoning were explained by the poisoning enzyme theory [4,5], and only a few studies have investigated the functional organ damage caused by OP poisoning from the perspective of histopathology [6–9]. However, in these studies, the dose of the poisoning was rather low and thus did not reflect the characteristics of severe OP poisoning. Clinical practice has found that severe OP poisoning is usually caused by oral ingestion to commit suicide or inadvertent ingestion [10], in which a large dose of toxic substances is consumed, leading to severe poisoning and many patients dying of multiple organ dysfunction syndrome (MODS) or multiple organ failure (MOF) despite active rescue. The role and status of the distribution and redistribution of the free agent in the bodies of animals with severe OP poisoning and the pathological damage and molecular damage

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that are induced by this type of poisoning in MODS have been reported infrequently [11]. In this study, a Chinchilla rabbit poisoning model with 10 × LD_{50} of monocrotophos was established to investigate the status and role of the repository of free toxic substances after severe monocrotophos poisoning, as well as the pathological injuries and molecular damage (thromboxane B_2, TXB_2, tumor necrosis factor-α, TNF-α and interleukin-1β, IL-1β) that occur in multiple organs in MODS.

2. Materials and methods

We aimed to investigate the pathogenesis and molecular mechanism of MODS in severely monocrotophos-poisoned rabbits, a Chinchilla rabbit poisoning model was performed according to Fig. 1. The details were stated as following.

2.1. Materials

Radioimmunoassay kits for IL-1β, TXB_2 and TNF-α were provided by the Beijing North Biotechnology Institute in China. The automatic-γ RIA counter (model XH-6010) was obtained from Factory 262 of Xi'an, China. The low-temperature centrifuge (LDR4-8·4C) was a product of Beijing Medical Centrifuge Factory, Beijing, China. The monocrotophos standard (99.1%) was purchased from the state-owned Qingdao Pesticide Plant. Quinoline hydrochloride ethyl amyl ether (Changxiao Tuoning) was provided by the Institute of Pharmacology and Toxicology at the Military Medical Academy. Purified acetylcholinesterase (AChE) was provided by the Teaching and Research Section of Immunity at Binzhou Medical College. The experimental animals were Chinchilla rabbits that were 3.4 months of age and 2–2.5 kg in body weight. The rabbits were provided by the animal laboratory of Binzhou Medical College.

2.2. Methods

2.2.1. OP poisoning model and sample preparation

The Chinchilla rabbits were divided into four groups: three monocrotophos exposure (10 times of median lethal dose, 10 × LD_{50}, 22.24 mg/kg) groups, which were sampled 1, 6 and 24 h after exposure, and one control group. Each group included six animals. Monocrotophos was administered via subcutaneous abdominal injection at four locations. To prevent animal death, an intramuscular injection of Changxiao Tuoning was administered to the rabbits at a dose of 0.4 mg/kg of body weight 5 min before the OP application. Changxiao Tuoning is penequinine hydrochloride, mainly acting on M_1, M_2 and N_1 and N_2 receptors with strong central and peripheral anticholinergic effects. Then it was applied repeatedly every 10–12 h, with the doses adjusted according to the rabbits' condition.

2.2.2. Sampling method

The animals were sacrificed 1, 6 and 24 h after the animals were poisoned. Their blood samples (3 mL) was collected and treated with heparin. Plasma and blood cells were then separated and stored at −70 °C. The liver, kidney, brain stem, lung, intestine, heart and adipose tissues and the bile from the gallbladder were collected and immediately placed in a −70 °C freezer. One piece from each of the tissues and organs was sampled and fixed in 4% paraformaldehyde. To prepare homogenates of the tissues and the organs, the tissue and organ samples were retrieved from the −70 °C freezer. Water and blood on the surface of the samples were dried using filter paper. After the wet weights of the tissues and the organs were determined, the samples were cut into pieces and added to a glass homogenizer with 0.1 mol/L of PBS (1:9, W/V) to prepare the 1:10 tissue homogenate. The homogenate of adipose tissue was prepared using 98% ethanol.

2.2.3. AChE activity assay

Purified AChE activity was assayed using the dithiobisnitrobenzoic acid (DTNB) enzymatic kinetics method [4]. The assay results showed that after 8-fold dilution with 0.1 mol/L PBS, 98% ethanol had little inhibitory effect on AChE vitality; therefore, when using 98% ethanol to prepare homogenates from fat tissues for the AChE activity assay, the employed dilution was no less than 10-fold.

2.2.4. Dynamic FOP measurements of blood, bile and tissue homogenates

The FOP content of each specimen was measured using the enzyme inhibition method. In detail, the samples of blood, bile and tissue homogenates were diluted by 10 × 0.1 mol/L PBS, and each 0.15 mL of them was added with 0.15 mL AChE, incubated at 37 °C for 10 min. After attenuated the hemocytes in different sample by 1.9 mL 0.1 M PBS, 0.15 mL of them were treated with 0.15 mL AChE and incubated at 37 °C for 10 min. The standard sample was a mixture of 0.15 mL PBS and 0.15 mL AChE. Each sample was measured 2 times for average content of AChE. The FOP content was

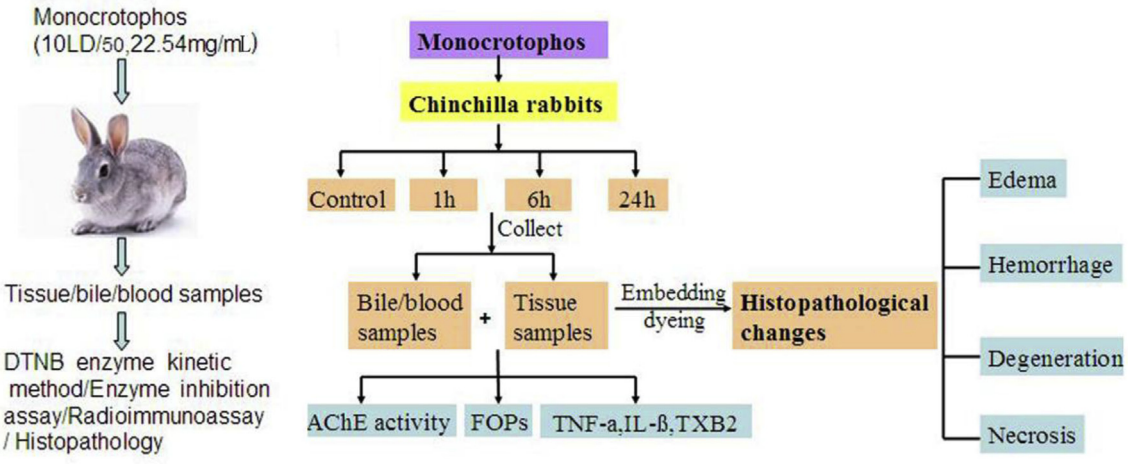

Fig. 1. Schematic description of that used in the research. A Chinchilla rabbit poisoning model is performed to investigate the pathogenesis and molecular mechanism of MODS in severely monocrotophos-poisoned rabbits.