Life Sciences 188 (2017) 10–16

Contents lists available at ScienceDirect

Life Sciences

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

Specific immune responses in mice following subchronic exposure to acetamiprid

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ARTICLE INFO

Keywords:
Acetamiprid
Curcumin
Subchronic exposure
Immunotoxicity
Specific immune response

ABSTRACT

Aims: Acetamiprid (ACE) is an insecticide of the neonicotinoid family, the most widely used in the world. Herein, we assessed the effect of ACE on either the humoral or cellular immune responses of rodents. We also evaluated the role of curcumin in the restoration of altered immune responses after ACE treatment.

Methods: Five groups of five Swiss Albino mice were immunized intraperitoneally with the recombinant form of CFP32, a virulence factor of *Mycobacterium tuberculosis*. One group received ACE (5 mg/kg) during 61 days, a second one received ACE associated with curcumin (100 mg/kg). Three control groups were included; one untreated, the second received corn oil and the third received curcumin alone. The humoral immune response was assessed by ELISA testing the anti-rCFP32 antibody concentrations in the serum. The cellular immune response was assessed by analyzing the cellular proliferation of the splenocytes stimulated *in vitro* by a mitogen or rCFP32.

Results: The ACE-treated mice showed a significant immunosuppression of the specific humoral response with a restorative effect of curcumin when administered with ACE. Similarly, ACE significantly decreased the level of splenocyte proliferation after either a non specific or a specific activation. Curcumin partially restores the antigen specific cellular immune response. Moreover, when administered alone, curcumin significantly inhibits the proliferative responses to the mitogen confirming its anti-mitogenic effect. Histological analysis showed alteration of spleens of mice exposed to ACE.

Significance: Altogether, our data indicated that ACE could potentially be harmful to the immune system.

1. Introduction

The neonicotinoids (NCs) belongs to a new class of insecticides that are commonly used to protect crops (cereals, potatoes, vegetables, fruits) from pest insects [1]. The neonicotinoids have been reported to act as agonist of nicotinic acetylcholine receptors (nAChRs) and their high toxicities towards insects have been attributed to their selective binding affinity to insect nAChRs [1].

The ubiquitous use of NCs in modern agriculture and the consequent exposure of populations have become an increasing health concern. Indeed, NCs may enter a human's body through various exposure pathways, such as occupational exposure *via* inhalation and dermal absorption. In addition, consumption of contaminated aquatic and vegetable products can contribute to human exposure [2]. Also, NCs are classified as “slightly toxic” based on oral toxicity, yet, several studies have described the adverse effects on animals and humans [3,4]. NCs alter reproductive function [5,6], decrease hepatic enzymes activities in rats [7] and elicit potential adverse effects on the central nervous system [8]. In a recent epidemiological study, Lopez et al. indicated a possible correlation between occupational exposure to NCs and an increased incidence of various neoplastic diseases in Mediterranean region probably due to mutagenic properties of NCs [9]. The impairment

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http://dx.doi.org/10.1016/j.lfs.2017.08.022
Received 14 May 2017; Received in revised form 16 August 2017; Accepted 22 August 2017
Available online 24 August 2017

of the immune function after exposure to NCs has been proposed as another mechanism that possibly enhances the risk of infectious diseases or cancers [9].

Although, few reports have been interested on the study of neonicotinoid-induced toxicity in the immune system of vertebrates, and the majority of them were conducted principally with neonicotinoids, such as imidacloprid, thiamethoxam, and clothianidin [1,3]. Data studying the potential immunotoxicity of acetamiprid (ACE) are scarce. Devan et al. concluded that subchronic treatment of Wistar rats with acetamiprid significantly decreased the lymphocyte proliferation to B cell mitogen which might contribute to immunosuppression [10].

Because of the risk of adverse effects including immunotoxicity, there is growing interest in the development of antidotes for potential NCs toxicity. Old drugs like *N*-acetylcysteine [11] or natural products have been more and more examined for potential use in attenuation of toxicities induced by environmental toxicants such as curcumin. Curcumin has either *in vitro* or *in vivo* diverse biological activities such as anti-oxidant, anti-inflammatory, anti-carcinogenic and anti-angiogenic effects [12,13]. Moreover, immunomodulatory protective effects of curcumin have been reported in mice. Accordingly, curcumin restored the nicotine-mediated disruption of Th1/Th2 immune balance [14].

Herein, we aimed to investigate the effect of subchronic exposure to ACE on the specific mediated immune responses in mice and to evaluate the potential restoring effect of curcumin. For this purpose, ACE treated and untreated mice were specifically immunized with CFP32 (originally annotated as Rv0577), a virulence factor of *Mycobacterium tuberculosis* [18] which induces a durable Th1 cellular response and a strong humoral response in mice [19]. Either the humoral or the cellular immunity was evaluated.

2. Material and methods

2.1. Chemicals

Acetamiprid or ACE, (acetin 20 SL 200 g/l) (*E*)-N1-[(6-chloro-3-pyridyl) methyl]-N2-cyano-N1-methyl acetamidine, was procured from the Agricultural Struggle Center in Tunis (Tunisia). ACE, diluted in corn oil before use, was administered orally by gavage at 5 mg/kg body weight/day, a dose which does not induce any sign of toxicity [15]. Curcumin, 1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene- 3, 5-dione, present in the rhizome of the plant *Curcuma longa*, is a naturally occurring pigment and component of the spice. It was procured from Sigma–Aldrich Co. (Germany) and administered orally by gavage at 100 mg/kg body weight/day. This dose of curcumin was defined accordingly to previous toxicity studies on animals reporting that curcumin is safe even at high doses [16,17].

2.2. Animals

Male Swiss Albino mice (6–8 weeks old) were supplied by the animal unit of Pasteur Institute of Tunis after the approbation of institutional bio-medical ethics committee (n°2015/14/E/FST). The mice were housed during 10 days and then randomly allocated into five cages of five mice under a controlled temperature (22–25 °C) and relative humidity maintained at 40–70% with *ad libitum* access to purified water and standard pellet diet. Photoperiod of 12 h light and dark cycles was maintained. All the procedures were in accordance with Guidelines for ethical conduct in the care and use of animals.

2.3. Recombinant CFP32 expression and purification

The recombinant form of CFP32 was produced as a secreted recombinant protein in the yeast *Pichia (P). pastoris* as previously described [20]. Briefly, the recombinant clone *P. pastoris* strain, KM71H, expressing rCFP32 was used for extracellular expression. It was inoculated into 5 ml of YPD media with 5 μg of Zeocin and incubated at 30 °C in a shaker at 250 rpm over night. The culture was transferred into 250 ml of Buffered Glycerol Complex Media (BMGY: 1% yeast extract, 2% peptone, 100 mM potassium phosphate pH 6, 1.34% YNB, 4.10-5boitine, 1% glycerol) in a 2 l baffled flask and incubated at 30 °C in an incubator shaker at 250 rpm over night (OD_{600} = 6). Cells were harvested through centrifugation at 1200 g for 10 min then resuspended in a 25 ml of Buffered Methanol Complex Medium (BMMY: 1% yeast extract, 2% peptone, 100 mM potassium phosphate pH 6, 1.34% YNB, 4.10–5 boitine, 1% methanol) and incubated for another 72 h with the addition of 1% methanol after every 24 h to maintain induction conditions. Cultures were centrifuged at 1200 g for 10 min and the supernatant was collected. Histidine-tagged rCFP32 purification was made in one step using a Ni + -Sepharose (Sigma). The rCFP32 was then dialyzed against phosphate buffered saline (PBS).

Table 1
Mice groups and dose delivered everyday of ACE/Corn oil/curcumin per mouse during the 61 days of the experiment.

Groups	Type	Dose Levels	No. of Animal
Group 1: UNT	Untreated	_	5
Group 2: CO	Corn oil	0.5 ml	5
Group 3: ACE	Acetamiprid	5 mg/kg	5
Group 4: ACE-CUR	Acetamiprid + Curcumin	5 mg/kg + 100 mg/kg	5
Group 5: CUR	Curcumin	100 mg/kg	5

2.4. Treatment schedule

Animals were randomly allocated in five groups of five mice (Table 1): the first group (UNT) was untreated, the second group (CO) received orally 0.5 ml of Corn oil, the third group (ACE) received 5 mg/kg of body weight of Acetamiprid diluted in 0.5 ml of corn oil, the forth group (ACE-CUR) received the same dose of Acetamiprid supplemented with 100 mg/kg of body weight of Curcumin, the last group (CUR) received only Curcumin. Mice were treated *via* oral gavage once a day, for 61 consecutive days as described in Fig. 1. ACE or vehicle was administered in the morning between 09:00 and 10:00 am. Body weight were determined daily for each mouse.

2.5. Mice immunization

All the mice were immunized at day 15 intraperitoneally with 30 μg of rCFP32 in the presence of complete Freund's adjuvant (FCA) 1:1 (v/v) (Fig. 1). The choice of this antigen was based on a recent study which showed that CFP32-DNA based booster vaccine, induces a durable Th1 cellular response and a strong humoral response against CFP32 in mice [19]. On days 36 and 51 (Fig. 1), mice received an intraperitoneal injection of 50 μg of rCFP32 in the presence of incomplete Freund's adjuvant.

2.6. Blood sampling and spleen weight

Blood samples were taken from the retro-orbital plexus of each mouse using EDTA tubes at day 1, 36, 51 and 61 (Fig. 1). At day 61, all mice were euthanized 2 h after the last bleeding by cervical dislocation and spleens were collected aseptically and weighted.

2.7. Histological examination

The spleen tissues were placed in 10% buffered formalin. After washing and deshydratation in alcohol, paraffin-embedded sections were cut (5–6 μm thickness) and stained with hematoxylin (H) and eosin (E) for microscopic examination at 400 × magnification.