Chemosphere 93 (2013) 1029–1034

Contents lists available at SciVerse ScienceDirect

Chemosphere

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

Early life permethrin treatment leads to long-term cardiotoxicity

M.S. Dhivya Vadhana [a], S. Siva Arumugam [b], Manuel Carloni [c], Cinzia Nasuti [c], Rosita Gabbianelli [c,*]

[a] School of Advanced Studies, University of Camerino, Via Lili, 62032 Camerino, MC, Italy
[b] School of Environmental and Natural Sciences, University of Camerino, Via Gentile III da Varano, 62032 Camerino, MC, Italy
[c] School of Pharmacy, University of Camerino, Via Gentile III da Varano, 62032 Camerino, MC, Italy

HIGHLIGHTS

- Early life pesticide exposure has long-term consequences on heart.
- A significant decrease in heart surface area was observed in treated rats.
- Calcium and Nrf2 gene expression levels were increased in old age.

ARTICLE INFO

Article history:
Received 20 December 2012
Received in revised form 23 May 2013
Accepted 25 May 2013
Available online 24 June 2013

Keywords:
Early-life
Permethrin
Cardiac hypotrophy
Old age
Nrf2
Calcium

ABSTRACT

Environmental, nutritional or hormonal influences in early life may have long-term effects changing homeostatic processes and physiological parameters in adulthood. NF-kB and Nrf2, two of the main transcription factors regulating genes involved in pro-inflammatory and antioxidant responses respectively, can be modified by various stimuli. NF-kB controls immediate early genes and is required for cardiomyocyte hypertrophic growth, while Nrf2 protects the heart from oxidative stress-induced cardiovascular complications.

The aim of this study was to investigate the impact of early life permethrin treatment (1/50 of LD_{50}, from 6th to 21st day of life) on the development of cardiotoxicity in 500-day-old rats. Nrf2 and NF-kB gene expression, calcium level and heart surface area were chosen as biomarkers of toxicity.

Six candidate reference genes were first examined and GAPDH resulted the most stable one for RT-qPCR. The comparative expression analysis of the target genes showed 1.62-fold increase in Nrf2 mRNA level, while the NF-kB mRNA in treated rats was not significantly changed compared to control ones. A significant decrease in heart surface area was observed in treated rats (296.59 ± 8.09, mm^2) with respect to the control group (320.86 ± 4.93, mm^2). Finally, the intracellular calcium influx in heart of early life treated rats increased 4.33-fold compared to the control one.

In conclusion, early life pesticide exposure to low doses of permethrin insecticide, has long-term consequences leading to cardiac hypotrophy, increased calcium and Nrf2 gene expression levels in old age.

1. Introduction

There is increasing evidence that the cardiovascular system is susceptible to external influences throughout gestation and after birth. Fetal, early childhood and adolescent environmental exposures can impair cardiovascular health and function. In addition, biological and lifestyle factors can strongly affect cardiovascular health, sometimes by interacting with the effects of environmental exposures (Mone et al., 2004). Cardiomyocytes are highly differentiated cells that rarely replicate after birth; thus, any agent that harms them during the fetal period can cause lasting damage (Mone et al., 2004). Epidemiological studies have shown a strong correlation between stressful events (nutritional, hormonal or environmental) in early life and development of adult diseases such as obesity, diabetes and cardiovascular failure (Trevenzoli et al., 2007). Environmental, nutritional or hormonal influences in early life (during gestation and lactation) may change some physiological parameters in adulthood; this phenomenon is known as programming (Barker, 1995; Waterland and Garza, 1999). Several animal models of programming have been studied to explain how imprinting factors in early life may modulate physiological parameters and metabolism in a later permanent manner (Vickers et al., 2005; Zambrano et al., 2006). There are multiple reasons for the delayed appreciation of cardiovascular toxicity as a significant outcome of environmental pollutants (Bhatnagar, 2004).

Permethrin, a synthetic pyrethroid with a broad-spectrum insecticidal activity, is used for outdoor/indoor pest control and

* Corresponding author. Address: School of Pharmacy, Via Gentile III da Varano, UNICAM, 62032 Camerino, MC, Italy. Tel.: +39 0737 403208; fax: +39 0737 403290.
E-mail address: rosita.gabbianelli@unicam.it (R. Gabbianelli).

0045-6535/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.chemosphere.2013.05.073

as anti-wood worm agent (Bradberry et al., 2005). Permethrin is characterized by pronounced lipophilicity, which makes it able to easily cross membranes leading to human contamination, as demonstrated by the presence of permethrin metabolites in urine (Bradberry et al., 2005). In medicine, it has been shown to be effective and safe for treatment of body lice, head lice and scabies and children come in contact with this pesticide also following these uses (Taplin and Meinking, 1990).

A study by Blaylock et al. (1995) using oral exposure to permethrin in a mouse model, demonstrated immunotoxicity of permethrin in the form of inhibited T lymphocyte cytotoxic activity. Several reports from our laboratory show that chronic permethrin treatment brings to striatum oxidative stress and neurobehavioral disorders (Nasuti et al., 2007; Falcioni et al., 2010), immune system impartment (Gabbianelli et al., 2004, 2009; Vadhana et al., 2011; Fedeli et al., 2012) as well as heart cell damage (Vadhana et al., 2010). Moreover, previous findings demonstrate that early life permethrin treatment in rats induces biochemical changes correlated to heart disease and neurodegeneration in adulthood (Vadhana et al., 2011; Carloni et al., 2012).

Since environment-gene interactions can cause changes in genes involved in the redox system, here we analyzed the impact of early life permethrin exposure on gene expression profiles of the two main genes, Nrf2 and NF-kB, involved in the anti-inflammatory and pro-inflammatory responses. Nuclear factor E2-related factor 2 (Nrf2) is a transcription factor, ubiquitously expressed in the cardiovascular system, that controls the expression of a battery of antioxidant genes and other cytoprotective phase II detoxifying enzymes (Li et al., 2009a,b). In addition to the wealth of evidence showing Nrf2 to be a major regulator of cellular defenses against various pathological stresses in diverse organs, such as lung and kidney, it has been demonstrated that Nrf2 plays a critical role through the co-ordination of a group of its downstream antioxidant genes to suppress myocardial oxidative stress (Li et al., 2009a,b). Meanwhile, the transcription factor NF-kB regulates a wide variety of biological effects in diverse cell types and organs, particularly stress, immune and adaptive responses. In the heart, NF-kB has been found to be required for development of late preconditioning against myocardial infarction (Jones et al., 2005). NF-κB is important in regulating cellular responses because it belongs to the category of "rapid-acting" primary transcription factor that allows it to be a first responder to harmful cellular stimuli like reactive oxygen species (Chandel et al., 2000).

A better understanding of the heart transcriptional profiling following early life permethrin exposure, could be used to monitor the impact of pesticide exposure on the development of diseases associated with heart damage in adult age. For this reason, the present study aims at analyzing the expression of Nrf2 and NF-kB target genes in the heart tissue of 500-day-old rats treated with permethrin during early life (1/50 LD_{50}, from 6th to 21st day of life). Rats were sacrificed when they were 500-days-old because this age corresponds to 50-year-old humans (Andreollo et al., 2012) which represents the beginning of old age. Moreover, in order to evaluate permethrin cardiotoxicity, functional markers such as heart surface area and intracellular calcium level were measured.

2. Materials and methods

2.1. Materials

All reagents were of pure and analytical grade and were obtained from Sigma Chemical Co. (USA). Technical grade (75:25, trans:cis; 94% purity) 3-phenoxybenzyl-(1R,S)-cis,trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxyl-ate, (Permethrin) was generously donated by Dr. A. Stefanini of ACTIVA, Milan, Italy.

2.2. Animals

Male and female Wistar rats from Charles River (Calco, LC, Italy), weighing 250–270 g and about 90 d old were used. The animals were housed in plastic (Makrolon) cages (five rats/cage) in a temperature controlled room (21 ± 5 °C) and maintained on a laboratory diet with water ad libitum. The light/dark cycle was from 7 p.m. to 7 a.m. Animal use in this study complied with the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Rat pups born in our laboratory from primiparous dams were used in the study. The parturition day was set as Post Natal Day 0 (PND0). On PND1, all litters were examined externally for the presence of gross abnormalities, sexed, weighed and the female pups were discarded. Two male pups were assigned to each dam until weaning (PND21). No cross-fostering was employed. At 2 d of age, litters were randomly assigned to two experimental groups (n = 6 rats for each).

2.3. Treatment

Permerthin was dissolved in corn oil and administered orally by an intragastric tube (4 mL kg^{-1}) at a dose of 1/50 of LD_{50} corresponding to 34.05 mg kg^{-1} (Cantalamessa, 1993). The dosage was chosen based on the "no observed adverse effect level" (NOAEL) for permethrin of 25 mg kg^{-1}. The compounds were administered once a day in the morning from PND6 to PND21. Control rats were treated with vehicle (corn oil 4 mL kg^{-1}) on a similar schedule. The volume of the compound administered was adjusted daily based on body weight measured during the dosing period. On PND21, the offspring were weaned and the littermates were housed together. At old age (PND 500), six rats from each group (Permethrin treated and control groups) were sacrificed by exposure to CO_2, and their hearts were collected and pooled for analysis. For the experiments, the groups of animals were formed by drawing animals from different litters, so that no group contained siblings. All data were analyzed considering the litter as the smallest unit.

2.4. Total RNA isolation and quality assessment

Total RNA was isolated from a pool of tissue samples from control and treated rats using Trizol reagent (Gibco BRL), following the manufacturer's instructions, and resuspended in diethylpyrocarbonate (DEPC) treated water. RNA concentration was then quantified using the NanoDrop® ND-1000 UV–VIS spectrophotometer (Thermo Scientific Inc., USA). RNA quality was also assessed by running samples on a 1.7% agarose gel and staining with ethidium bromide to reveal two sharp bands in every sample.

2.5. cDNA synthesis and PCR amplification

Total RNA (2 μg) was reverse transcribed into single strand cDNAs using Maxima First Strand cDNA Synthesis Kit for RT-qPCR (Fermentas, Thermo Fisher Scientific Inc., USA) following the manufacturer's instructions. Serial dilutions of the cDNA in water were prepared (10^{-1}–10^{-4}) and were used to amplify 6 control genes used for normalization and 2 test genes (Table 1). The primers used for amplification (Table 1) were designed using the Primer Designer program, v3.0 (Scientific and Educational Software). The PCR was performed using Phire Hot Start II DNA Polymerase (Finnzymes Oy, Finland) in a total volume of 20 μl containing 100 ng of cDNA, 0.5 μM of sense and antisense gene-specific primers and 200 μM of dNTP Mix (Fermentas, Thermo Fisher Scientific Inc.,