Food and Chemical Toxicology 88 (2016) 105–111

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

Curcumin attenuates Mancozeb-induced toxicity in rat thymocytes through mitochondrial survival pathway

Voja Pavlovic [a, *], Snezana Cekic [a], Milan Ciric [a], Dane Krtinic [b], Jelena Jovanovic [c]

[a] Institute of Physiology, Medical Faculty University of Nis, Bulevar Dr. Zorana Djindjica 81, Nis, 18000, Serbia
[b] Medical Faculty University of Nis, Bulevar Dr. Zorana Djindjica 81, Nis, Serbia
[c] Institute for Treatment and Rehabilitation Niska Banja, Srpskih Junaka 2, 18205, Niska Banja, Serbia

ARTICLE INFO

Article history:
Received 14 November 2015
Received in revised form
20 December 2015
Accepted 31 December 2015
Available online 5 January 2016

Keywords:
Mancozeb
Curcumin
Thymocytes
Toxicity
Mitochondrial survival pathway

ABSTRACT

The widely used fungicide Mancozeb (Man) has been shown to cause genotoxic effects in rodents and toxicological manifestations in different cells, mainly by altering the antioxidant defense in cells. On the other hand, curcumin (Cur), a natural phenolic compound, is thought to possess anti-inflammatory and antioxidant properties. Here, we investigated the possible protective role of Cur on Man-induced toxicity in rat thymocytes and potential mechanism involved. Rat thymocytes were treated with Man(0.01 μg/ml) and/or increasing Cur(0.3, 1, 3 μM) concentrations and levels of cell viability, apoptosis, mitochondrial membrane potential (MMP),Bcl-2, Bax protein expression, caspase-3 and -9 activity and p38 MAPK signaling involvement were examined. Cells treated with Man displayed increased cell toxicity, hypodiploid cells, caspase-3 and -9 activity, Bax protein expression, followed with decreased MMP and Bcl-2 protein expression. Inhibition of p38 MAPK signaling pathway markedly reduced apoptosis rate and caspase-3 activity in thymocytes exposed to Man. Application of increasing Cur (1, 3 μM) concentrations resulted with significantly reduced cytotoxicity, apoptosis, caspase-3, -9 activity, Bax protein expression, together with increased MMP and Bcl-2 protein expression in rat thymocytes. These result suggest that certain Cur concentrations may mediate Man-induced rat thymocytes toxicity through mitochondrial survival pathway, which may be useful in preventing possible secondary immunological consequences induced by Man.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Over past decades, pesticides are widely used through the world for global food production. Widespread usage of pesticides results with continuous exposure of humans to these substances and their residues. Due to persistent accumulative pesticides residues in environment, food intake represents key source for contamination of general population (Paro et al., 2012). Mancozeb is a metal-containing fungicide of ethylene-bis-dithiocarbamate (EBDC) family. These fungicides have been though to show low acute toxicity to humans and thus are used globally (Corsini et al., 2005). However, Mancozeb has been reported to induce toxic effects in cells of the immune system (Calviello et al., 2006; Srivastava et al., 2012) and in other non-immune cells (Domico et al., 2006; Tsang and Trombetta, 2007). Ethylene thiourea (ETU), a major metabolite of EBDCs, is a key factor in Mancozeb-induced toxicity, since it has been documented its carcinogenic and teratogenic effect in rodents (Srivastava et al., 2012). Also, Mancozeb exposure can induce genotoxicity and apoptosis through formation of reactive oxygen species (ROS), since this fungicide mainly targets mitochondrial enzymes (Domico et al., 2006; Leiphon and Picklo, 2007). Curcumin is a naturally occurring yellow pigment isolated from ground rhizomes of the plant *Curcuma longa* and has been known since ancient times to possess therapeutic effects (Borra et al., 2014). Several studies have reported that curcumin induced apoptosis and inhibited proliferation of different cancer cells under *in vitro* condition (Liao et al., 2008; Mackenzie et al., 2008; Bill et al., 2009). Also, it has been reported to scavenge oxygen free radicals and protect the cellular macromolecules from oxidative damage (Yucel et al., 2011) as well as to block drug-induced apoptotic changes *in vitro*, including ROS accumulation and loss of mitochondrial membrane (MMP) potential (Jaruga et al., 1998). On the other hand, some studies suggest that curcumin show pro-apoptotic effect by inducing the DNA damage in cells (Banerjee et al., 2008), indicating that molecular mechanisms and action of this compound still remains elusive. Therefore, in the current study, we tested the

* Corresponding author.
E-mail address: vojapav@yahoo.com (V. Pavlovic).

http://dx.doi.org/10.1016/j.fct.2015.12.029
0278-6915/© 2016 Elsevier Ltd. All rights reserved.

hypothesis that curcumin could protect rat thymocytes against Mancozeb-induced toxicity along with the potential mechanisms involved.

2. Material and methods

2.1. Animals

Experiments were performed on adult male Wistar rats (180–200 g), 9–11 weeks old, bread at the Vivarium of the Institute of Biomedical Research, Medical Faculty, Nis, under conventional laboratory conditions and in accordance with national animal protection guidelines.

2.2. Preparation of thymocytes

Rat thymocytes were isolated as described previously (Pavlovic et al., 2007). Briefly, thymus was extirpated using sterile technique and placed in culture medium (CM) with 10% FCS. Thymocytes were released by teasing the thymus through a steel mesh. Cell suspensions were filtered through sterile nylon filter to remove the stroma and then the cells were washed twice with CM/10% FCS. The viability of isolated cells, as determined by trypan blue dye exclusion test, was always over 95%. Isolated thymocytes were counted and adjusted to a density of 5×10^6 cells/mL of CM. CM was prepared using RPMI 1640 (Sigma–Aldrich, St. Louis, Mo., USA), according to the manufacturer's instructions. CM contained 25 mM HEPES, 2 mM glutamine, penicillin (100 U/mL), streptomycin (100 μg/mL) and 10% fetal calf serum (FCS).

2.3. Cell culture and treatments

Isolated rat thymocytes were cultivated in 96-well round-bottom plates (NUNC, Aarhus, Denmark), containing a 100 μL of cell suspension (5×10^5 cells) in each well (Pavlovic et al., 2015). After treatment with Mancozeb (0.01 μg/mL), the cells were co-incubated with increasing concentrations of curcumin (0.3, 1, 3 μM). Control cells were cultivated with appropriate amounts of vehicle alone, diluted in CM. All cell cultures are done in triplicates and cultured for 24 h in an incubator (Galaxy, Wolf Laboratories, USA) with 5% CO_2 at 37 °C.

To test the role of protein kinase p38 MAPK in Mancozeb-induced cytotoxicity, rat thymocytes were cultured with Mancozeb in the presence or absence of SB203580 (Sigma–Aldrich, St. Louis, Mo., USA), a p38 MAPK inhibitor, at final concentration of 1 μM/L (Du et al., 2012). Apoptosis and caspase-3 activity in rat thymocytes were evaluated after 24 h of incubation.

Mancozeb was purchased from Galenika-Fitofarmacija a.d., Belgrade, Serbia. Pesticide solutions were prepared immediately before use in dimethyl sulfoxide (DMSO) and diluted in CM. Control cells were treated with the same amount of vehicle alone. The final DMSO concentration never exceeded 0.5% (v/v).

Based on the results in our previous study (Pavlovic et al., 2015), regarding the dose dependent toxicity in rat thymocytes induced by Mancozeb, as well as on proposed acceptable daily intake (0.05 mg/kg body weight) of Mancozeb in humans (Rohm, 1995), in our experiment we used 0.01 μg/mL of Mancozeb which corresponds to an *in vivo* exposure 0.1 mg/kg body weight (Calviello et al., 2006; Vaccari et al., 1999). Curcumin was obtained from Sigma–Aldrich (St. Louis, Mo., USA) and dissolved in DMSO as a stock solution. The stock solution was stored at -20 °C and diluted in CM before use. The final concentration of DMSO, applied to the cells, was less than 0.5%. Incubation of increasing concentrations of curcumin (0.3, 1, 3 μM) was chosen due to our preliminary experiments and previous study results in rat thymocytes (Koizumi et al., 2011), which showed that 3 μM was the lowest concentration which was not able to induce any cytotoxic actions in rat thymocytes.

2.4. Analysis of cell viability

Cell viability of rat thymocytes, after cultivation period, was evaluated by CCK-8 assay (Sigma–Aldrich, St. Louis, Mo., USA) as was previously described (Hori et al., 2002). Ten microliter of reaction mixture was added in each well. After 2 h of incubation, the solubilized formazan product was quantified spectrophotometrically. Absorbance was measured at 450 nm. For each sample, basal intensity values were subtracted from those obtained after different treatments. Absorbances were presented as a ratio of control for further comparison (Pavlovic et al., 2013).

2.5. Apoptotic DNA analysis

Thymocytes undergoing apoptosis were identified by their reduced relative nuclear DNA content, as previously described (Nicoletti et al., 1991). Single apoptotic cells were detected using an Epics XL flow cytometer (Coulter, Krefeld Germany) as a reduction in fluorescence of the DNA-binding dye Propidium Iodide (PI-Santa Cruz, Biotechnology, Santa Cruz, CA, USA) in apoptotic nuclei. The percentage of apoptotic cells (subdiploid DNA) was determined and presented as a ratio of control for further comparison.

2.6. Determination of mitochondrial membrane potential

Changes of mitochondrial membrane potential (MMP) of rat thymocytes were evaluated by uptake of lipophilic cation Rhodamine 123 (Sigma–Aldrich, St. Louis, Mo., USA) into mitochondria, as previously described (Wang et al., 2007). The fluorescence of intracellular Rhodamine 123 was determined by flow cytometry, as published earlier (Singh et al., 2013). For each sample, basal intensity values were subtracted from those obtained after different treatments and results were presented as ratio of mean fluorescence intensity for further comparison.

2.7. Caspase-3 and caspase-9 activity assay

The enzymatic activity of the caspases were determined by a colorimetric assay (by using the chromogenic substrate DEVD-pNA and LEHD-pNA) from R&D Systems (Minneapolis, USA), according to the manufacturer's protocol. The reaction was measured by determining the change in absorbance at 405 nm. The activity was expressed as fold change of treated cell over the non-treated cells. The background values were subtracted from the experimental results before calculation the fold induction.

2.8. Flow cytometric evaluation of Bcl-2 and Bax expression

The expression was evaluated as we described previously (Pavlovic et al., 2007), by using anti-Bcl-2FITC (Santa Cruz, Biotechnology, Santa Cruz, CA, USA) and anti-BaxFITC (Santa Cruz, Biotechnology, Santa Cruz, CA, USA) mouse monoclonal antibodies. Labeled cells were fixed in 4% formalin and analyzed (5000 analyzed cells/per sample) on Epics XL flow cytometer (Coulter, Krefeld Germany). Non-specific binding was detected by the control cells, which were incubated with the irrelevant antibody.

2.9. Statistical analysis

Results are presented as mean ± SD. The comparisons among groups were carried out using the analysis of variance (ANOVA)

Download English Version:

https://daneshyari.com/en/article/5849442

Download Persian Version:

https://daneshyari.com/article/5849442

Daneshyari.com