Food and Chemical Toxicology 109 (2017) 60–67

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

Microcystin-LR exposure induces oxidative damage in *Caenorhabditis elegans*: Protective effect of lutein extracted from marigold flowers

Paula Rossini Augusti [a, *], Allana Von Sulzback Brasil [a], Caroline Souto [b], Gabriela Göethel [b], Alessandro de Oliveira Rios [a], Tatiana Emanuelli [c], Marilise Escobar Bürger [d], Solange Cristina Garcia [b]

[a] Instituto de Ciência e Tecnologia de Alimentos, Universidade Federal do Rio Grande do Sul (UFRGS), Av. Bento Gonçalves, 9500, Prédio 43.212, Campus do Vale, Porto Alegre, CEP 91501-970, RS, Brazil
[b] Laboratório de Toxicologia (LATOX), Departamento de Análises, Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul (UFRGS), Av. Ipiranga, 2752, Porto Alegre, CEP 90610-000, RS, Brazil
[c] Núcleo Integrado de Desenvolvimento em Análises Laboratoriais (NIDAL), Departamento de Tecnologia e Ciência dos Alimentos, Centro de Ciências Rurais, Universidade Federal de Santa Maria (UFSM), Av. Roraima, 1000, Santa Maria, CEP 97105-900, RS, Brazil
[d] Laboratório de Farmacologia e Toxicologia (FARMATOX), Departamento de Fisiologia, Centro de Ciências da Saúde, Universidade Federal de Santa Maria (UFSM), Av. Roraima, 1000, Santa Maria, CEP 97105-900, RS, Brazil

ARTICLE INFO

Article history:
Received 15 May 2017
Received in revised form
28 August 2017
Accepted 29 August 2017
Available online 1 September 2017

Keywords:
Survival rate
Dichlorofluorescein diacetate assay
Reactive oxygen species
Cyanotoxins

ABSTRACT

Microcystin-LR (MIC-LR) is a hepatotoxin, with toxicity mechanisms linked to oxidative stress. Besides, neurotoxic effects of MIC-LR have recently been described. Herein, we evaluated the effects of environmentally important concentrations of MIC-LR (1, 10, 100, 250, and 500 μg/L) on oxidative stress markers and the survival rate of the nematode *Caenorhabditis elegans (C. elegans)*. In addition, a possible protective effect of the carotenoid lutein (LUT) extracted from marigold flowers against MIC-LR toxicity was investigated. Higher concentrations (250 and 500 μg/L) of MIC-LR induced the generation of reactive oxygen species (ROS) and resulted in a survival loss in *C elegans*. Meanwhile, all MIC-LR concentrations caused an increase in the superoxide dismutase (SOD) expression, while catalase (CAT) expression was only affected at 500 μg/L. The carotenoid LUT prevented the ROS generation, impairment in the CAT expression, and the survival loss induced by MIC-LR in *C. elegans*. Our results confirm the toxicity of MIC-LR even in a liver-lacking invertebrate and the involvement of oxidative events in this response. Additionally, LUT appears to be able to mitigate the MIC-LR toxic effects.

1. Introduction

Safe water is important for public health and a better management of water resources can boost the economy and greatly contribute to poverty reduction (WHO, 2016). When exposed to high temperatures and intense sunlight, polluted water offers optimal conditions for the growth of cyanobacteria (Pitois et al., 2000). Besides affecting the water odor and taste, as well as leading to the loss of water supply and its recreational and fishing value, the presence of cyanobacteria is of a special importance because of the ability of these organisms to produce toxins (Tyagi et al., 1999). In fact, cyanotoxins have been responsible for almost all known cases of fresh and salt water intoxication caused by algae-produced toxins, including the death of more than 50 patients attributed to the contaminated dialysis water in Caruaru, Brazil (Pouria et al., 1998).

Microcystins (MICs) are cyanotoxins with hepatotoxic and genotoxic properties, produced by freshwater cyanobacteria such as *Microcystis aeruginosa* (Dawson, 1998). Among liver injuries, MICs are involved in liver cancer and hepatitis. Since oral intake seems to be the main route of exposure to MICs by humans, chronic exposure to low doses in drinking water has been associated with

Abbreviations: ANOVA, analysis of variance; CAT, catalase; C. *elegans*, *Caenorhabditis elegans*; DCFH-DA, 2′-7′-dichlorofluorescin diacetate; DMSO, dimethyl sulphoxide; Nrf2, E2-related factor 2; HPLC, high performance liquid chromatography; LD_{10}, lethal dose to 10%; LD_{50}, lethal dose to 50%; LUT, lutein; MIC-LR, microcystin-LR; MICs, microcystins; NF-κB, nuclear factor kappa-B; NGM, nematode growth media; ROS, reactive oxygen species; SOD, superoxide dismutase; SEM, standard error of the mean; TBARS, thiobarbituric acid reactive substances; WHO, World Health Organization.

* Corresponding author.
E-mail address: paularaugusti@gmail.com (P.R. Augusti).

http://dx.doi.org/10.1016/j.fct.2017.08.045
0278-6915/

the ability of these toxins to promote tumours (Valério et al., 2016). Although several MICs have been identified, microcystin-LR (MIC-LR) is the most ubiquitous one (Ding and Ong, 2003), and its provisional guideline value in drinking water was established by the World Health Organization as 1 μg/L (Chorus and Bartram, 1999).

The inhibition of the phosphatases PP1 and PP2A is the most likely mechanism of action of these toxins, which initiate a cascade of events leading to the necrosis and apoptosis of animal cells (Campos and Vasconcelos, 2010). These events include DNA damage, cytoskeleton disruption, mitochondria dysfunction, endoplasmic reticulum disturbance and cell cycle deregulation (Chen and Xie, 2016). Besides all these mechanisms of toxicity, MIC-LR can also lead to oxidative stress through an increase in the generation of reactive oxygen species (ROS) and/or antioxidant depletion (Kondo et al., 1992). Moreover, in rats treated intraperitoneally with MIC-LR, hepatic glutathione peroxidase, glutathione reductase, superoxide dismutase (SOD), and catalase (CAT) decreased, along with the increased levels of lipid peroxidation (Moreno et al., 2005).

Although the liver is the primary target organ of MIC-LR, the toxin can also affect some other organs, such as the brain in zebrafish (*Danio rerio*) (Kist et al., 2012). Li et al. (2014) reported MIC-LR-induced pathological damage in the hippocampus, as well as the learning and memory loss in rats. MIC-LR and other environmental toxins are able to cause physiological changes similar to those observed during neurodegenerative processes in glial cells via oxidative stress and excitotoxicity (D'Mello et al., 2017). Moreover, Meng et al. (2013) reported that ROS generated by MIC-LR in neuroendocrine cells were involved in the Tau protein phosphorylation, a key event in neurodegenerative processes.

Besides classical models (cells and animals), the nematode *Caenorhabditis elegans (C. elegans)* has emerged as an important model in neurobiology, developmental biology, and genetics since this nematode has a centralized nervous system and shares many similarities with vertebrates in terms of neural physiology (Cole et al., 2004). This nematode has also been used to study apoptotic events induced by MIC-LR (Wang et al., 2012) and neurological modulation by environmentally relevant concentrations of MIC-LR (1–100 μg/L) (Saul et al., 2014). Despite the similarities between *C. elegans* and mammals regarding the neurobiology and stress responses (Kaletta and Hengartner, 2006), the effects of environmentally relevant concentrations of MIC-LR on oxidative stress events in *C. elegans* remain to be elucidated.

Since the injury caused by MICs is severe, fast, and non-reversible, therapeutic approaches have little or no effect. Thus, effective prevention appears to be of great importance (Dawson, 1998). Accordingly, agents such as rifampin and cyclosporin A showed good results in the prophylaxis of MIC-LR intoxication in mice (Hermansky et al., 1990; Wannemacher et al., 1990). Since ROS generation and/or antioxidant depletion may be potential mechanisms of MIC-LR toxicity, diet supplementation with antioxidants could be helpful. In fact, vitamin E prevented the death and reversed the increase in the liver weight in mice exposed to a MIC-LR extract [at 70% of the lethal dose, 50% (LD_{50})] (Gehringer et al., 2003). Additionally, quercetin was also able to prevent the MIC-LR-induced immunotoxicity by eliminating the oxidative stress in fish lymphocytes (Zhang et al., 2014). Thus, a search for new compounds would potentially provide additional options to manage MIC-LR intoxication.

Lutein (LUT) is a carotenoid present in high amounts in dark leaves of plants such as spinach, broccoli, and kale, as well as in the egg yolk (Santocono et al., 2006). This xanthophyll is more polar than known carotenoids found in the serum, such as lycopene and β-carotene. This hydrophilic property allows LUT to more efficiently remove ROS from an aqueous phase than nonpolar carotenoids (Ojima et al., 1993). LUT has well-known effects against age-related macular degeneration and cataract, for which oxidative stress is known to be an important risk factor. Besides the strong ROS-scavenging capacity, LUT also prevents lipid peroxidation (Gammone et al., 2015). All these properties were demonstrated to be involved in LUT protection against cardiac and renal toxicity caused by the chemotherapy drug doxorubicin (Sindhu et al., 2016), β-amyloid peptide toxicity in cerebrovascular endothelial cells (Liu et al., 2017), and hepatic toxicity induced by arsenic in mice (Niu et al., 2015). However, no studies have been carried out on a protective effect of LUT against MIC-LR toxicity. In addition, no data have been available on the effect of LUT itself on the ROS generation and survival of *C. elegans*.

To address the involvement of oxidative stress in MIC-LR toxicity in liver-lacking animals and to find possible protective agents, this study aimed to evaluate the effect of MIC-LR on oxidative damage markers in *C. elegans* and the possible protective effect of the carotenoid LUT against MIC-LR toxicity.

2. Materials and methods

2.1. Materials

French marigold flowers were purchased from a local market in Porto Alegre (Brazil) to prepare a LUT extract. MIC-LR was obtained from Enzo Life Sciences, Inc. (Farmingdale, NY, USA). All solvents used were of analytical grade, and dimethyl sulfoxide (DMSO) was used to dilute MIC-LR and LUT.

2.2. Extraction of LUT from marigold petals

LUT was prepared from marigold petals by the extraction with tetrahydrofuran and saponification with 10% KOH (w/v), as described by Nachtigall (2007). Then, LUT was crystallized, and the crystals formed were dried under reduced pressure (temperature <30 °C) (Vechpanich and Shotipruk, 2011). The purity of the LUT extract was evaluated by high-performance liquid chromatography (HPLC).

2.2.1. Chromatographic conditions

To evaluate the purity of the LUT extract, a chromatograph (Series 1100; Agilent, Santa Clara, CA, USA) equipped with an online degasser, quaternary pump, solvent system, UV–visible detector, and automatic injector, coupled with a C30 polymeric column YCM (250 × 4.6 mm, 3 μm), was used at 33 °C. The gradient elution was performed at a flow rate of 1 mL/min, and the eluent consisted of water/methanol/methyl *tert*-butyl ether (v/v/v), starting at a ratio of 5/90/5 and reaching the ratios of 0/95/5 at 12 min, 0/89/11 at 25 min, 0/75/25 at 40 min, and 0/50/50 at 60 min. The LUT purity was verified at a wavelength of 445 nm.

2.3. C. elegans strains, culture, and synchronization

The *C. elegans* N2 (wild type), CF1553 (muls84), and GA800 (wuls154) strains, provided by the Caenorhabditis Genetics Center (Twin Cities, MN, USA), were maintained at 20 °C on *Escherichia coli (E. coli)* OP50/NGM (nematode growth medium) plates (60 × 10 mm), as previously described (Brenner, 1974). For the synchronization process, an L1 population was obtained by isolating embryos from gravid hermaphrodites using a bleaching solution (1% NaOCl and 0.25 M NaOH) and a 30% sucrose solution to separate eggs by flotation. The eggs were washed with M9 buffer (0.02 M KH_2PO_4, 0.04 M Na_2HPO_4, 0.08 M NaCl, and 0.001 M $MgSO_4$) and incubated overnight on NGM agar plates without bacteria, as previously described (Ávila et al., 2012).