ELSEVIER

Toxicon 50 (2007) 1105–1115

TOXICON

www.elsevier.com/locate/toxicon

No induction of structural chromosomal aberrations in cylindrospermopsin-treated CHO-K1 cells without and with metabolic activation ☆

A. Lankoff[a,*], A. Wojcik[a,b], H. Lisowska[a], J. Bialczyk[c], D. Dziga[c], W.W. Carmichael[d]

[a]*Department of Radiobiology and Immunology, Institute of Biology, Swietokrzyska Academy, ul. Swietokrzyska 15, 25-406 Kielce, Poland*
[b]*Department of Radiobiology and Health Protection, Institute of Nuclear Chemistry and Technology, Warsaw, Poland*
[c]*Department of Plant Physiology and Development, Faculty of Biotechnology, Jagiellonian University, Kraków, Poland*
[d]*Department of Biological Sciences, Wright State University, Dayton, OH, USA*

Received 29 May 2007; received in revised form 25 July 2007; accepted 26 July 2007
Available online 8 August 2007

Abstract

Cylindrospermopsin (CYN) is a cyanobacterial alkaloid that has been implicated in outbreaks of human morbidity and animal mortality. The principal mode of action for CYN is inhibition of protein and glutathione synthesis, and its toxicity seems to be mediated by cytochrome P-450-generated metabolites. It was also shown that CYN might be responsible for tumor initiation in animals; nevertheless, mechanisms leading to CYN-induced carcinogenesis are scarce and equivocal. The aim of the present study was to investigate the impact of metabolic activation on CYN-induced DNA damage. The effect of different doses of CYN (0.05–2 μg/ml) on DNA damage was determined in CHO-K1 cells after 3, 16 and 21 h of the treatment. The chromosome aberration assay with and without metabolic activation was applied to evaluate the clastogenic activity of CYN and its metabolite(s). In addition, the occurrence of apoptosis and necrosis was estimated by the annexin method using flow cytometry. The results revealed that CYN is not clastogenic in CHO-K1 cells irrespective of S9 fraction-induced metabolic activation. However, CYN significantly decreases the frequencies of mitotic indices and decreases proliferation irrespective of metabolic activation system. CYN increases the frequency of necrotic cells in a dose- and time-dependent manner, whereas it has a very slight impact on apoptosis. Moreover, the presence of metabolic activation influences a susceptibility to necrotic cell death but not an apoptotic one.

Keywords: Cylindrospermopsin; Metabolic activation; Chromosomal aberrations; Mitotic index; Apoptosis; Necrosis; CHO-K1 cells

1. Introduction

Cylindrospermopsin (CYN) is a hepatotoxin produced by the cyanobacterium *Cylindrospermopsis raciborskii* (*Woloszynska*) *Seenaya* and *Subba Raju*, *Umezakia natans*, *Aphanizomenon ovalisporum*; *Raphidiopsis curvata*, *Anabaena bergii*, *Aphani-*

☆The experiments were carried out in accordance with the current guidelines for in vitro investigation set by the Institute's ethical committee.

*Corresponding author. Tel.: +4841 349 62 98; fax: +4841 349 62 92.
E-mail address: alankoff@pu.kielce.pl (A. Lankoff).

0041-0101/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.toxicon.2007.07.021

zomenon flos-aquae and *Lyngbya wollei* (*Farlow ex Gomont*) *Speziale* and *Dyck* (Ohtani et al., 1992; Harada et al., 1994; Shaw et al., 1999; Li et al., 2001; Schembri et al., 2001; Preußel et al., 2006; Seifert et al., 2007). CYN has been implicated in outbreaks of human morbidity and animal mortality (Hawkins et al., 1985; Carmichael et al., 2001; Griffiths and Saker, 2003). The main target of CYN toxicity is the liver, but other organs such as the thymus, kidneys, adrenal glands, lungs, intestinal tract and heart may also be affected (Falconer et al., 1999; Shaw et al., 2000). CYN is an alkaloid consisting of a tricyclic guanidine moiety combined with hydroxymethyluracil (Ohtani et al., 1992), which possesses an ability to accumulate in a tissue (Saker and Eaglesham, 1999; White et al., 2007). The principal mode of action for CYN is inhibition of protein synthesis (Terao et al., 1994) and glutathione synthesis (Runnegar et al., 1995). Its toxicity seems to be mediated by cytochrome P-450-generated metabolites (Runnegar et al., 1995; Norris et al., 2002; Froscio et al., 2003; Humpage et al., 2005). There is also preliminary evidence that CYN may be responsible for tumor initiation in mice (Falconer and Humpage, 2001); nevertheless, mechanisms leading to CYN-induced carcinogenesis are not well understood. The induction of DNA adducts in hepatocytes of animals treated with the extract of CYN *in vivo* was reported by Shaw et al. (2000). DNA fragmentation was observed in the extracts of mouse livers exposed to CYN *in vivo* (Shen et al., 2002). Humpage et al. (2000) showed that CYN increased the incidence of centromere-positive micronuclei in WIL-NS cells, indicating the aneugenic activity of CYN, whereas the results dealing with the frequency of centromere-negative micronuclei, indicating a possible clastogenic activity of CYN, were equivocal. It was also shown that CYN produced significant DNA breakage in primary mouse hepatocytes *in vitro*, which was inhibited by the cytochrome P-450 inhibitors (Humpage et al., 2005). In contrast, no DNA damage was found in CHO-K1 cells following exposure to CYN (Fessard and Bernard, 2003).

Considering the scarce and unclear information regarding the genotoxic activity of CYN and suggestions that its metabolism may be a prerequisite for genotoxicity, we decided to evaluate the impact of the toxin on DNA damage in CHO-K1 cells. We performed the chromosome aberration assay with and without metabolic activation to evaluate the clastogenic activity of CYN and its metabolite(s). To examine whether CYN or CYN-derived metabolite(s) are involved in cell cycle progression, we determined the frequency of mitotic cells. In addition, we assessed the occurrence of apoptosis and necrosis in CHO-K1 cells following CYN exposure with and without metabolic activation using the annexin method and flow cytometry.

2. Materials and methods

2.1. Cylindrospermopsin extraction and purification

CYN was isolated from two cultures of *C. raciborskii*. The first, AWT 205, was isolated by Dr. Peter Hawkins of the Australian Water Technology center at EnSight (Falconer et al., 1999). The second was a strain (CYN-Thai) isolated from a fish pond in Thailand (Li et al., 2001). Cells were removed from the medium by filtration through a Pellicon (Millipore, Milford, MA) filter. The filtered medium was passed through a column of YMC-ODS-A. The filtrate was collected and successively passed through a 10×250 mm HPLC preparative column using a preparative HPLC system (Waters, Milford, MA). A solution of 5% formic acid in methanol was used to elute the A_{262} absorbing material from the Carbograph column. This fraction was dried and it was later subjected to preparative HPLC on a 10×250 mm column of YMC-ODS-A. The mobile phase was 3% methanol in water and the flow rate was 2 mL/min. The A_{262} peak having the same retention time as a CYN standard (cylindrospermopsin "R" conformation, CAS NO. 10143545-90-8, HPLC/NMR, as certified by Dr. Greg Boyer, 2004) was collected and dried. The pure CYN was redissolved in water and the concentration of CYN was determined using LC/MS (Finnigan Thermo-Quest Micromass LCQduo benchtop LC/MS with electrospray ionization (ESI) (4.0 kV spray voltage) probe with Detector-UV6000 PDA, col. YMC-ODS) according to our previous results (Friday et al., 2002). Select ion mode (SIM) was done on each sample to confirm CYN and the total ion concentration (TIC) determined for the SIMs of 415.5–416.5 (Eaglesham et al., 1999). Signal strengths of 10^5 or higher was considered significant. The TIC peak area was used to calculate the amount of CYN present as compared with standard CYN (a gift of Dr. Glen Shaw-NRCET, Brisbane, Australia). The level of detection on column is about 200 pg.