Chemosphere 183 (2017) 18–26

Contents lists available at ScienceDirect

Chemosphere

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

Microbial detoxification of carvedilol, a β-adrenergic antagonist, by the filamentous fungus *Cunninghamella echinulata*

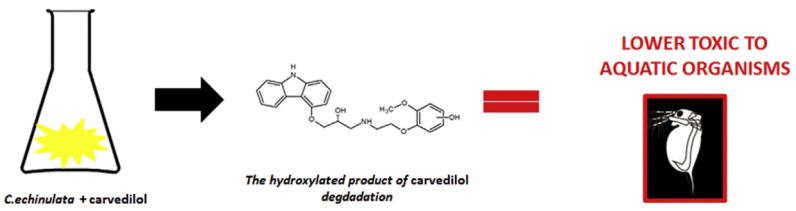
Katarzyna Zawadzka, Przemysław Bernat, Aleksandra Felczak, Katarzyna Lisowska*

Department of Industrial Microbiology and Biotechnology, Faculty of Biology and Environmental Protection, University of Lodz, 12/16 Banacha Street, 90-237, Lodz, Poland

HIGHLIGHTS

- The ability of *C. echinulata* to eliminate carvedilol was noted.
- Three carvedilol derivatives were detected in *C. echinulata* cultures.
- Detoxification of carvedilol by *C. echinulata* was showed.
- The changes in the fungal phospholipids profile in response to carvedilol were observed.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 20 March 2017
Received in revised form
8 May 2017
Accepted 13 May 2017
Available online 16 May 2017

Handling Editor: Klaus Kümmerer

Keywords:
Carvedilol
β-Blocker
Microbial degradation
Filamentous fungi
Detoxification
Phospholipids

ABSTRACT

Beta adrenergic antagonists like carvedilol are typical environmental pollutants detected in wastewater and surface water. Human metabolism of carvedilol is well investigated, while its environmental fates are still unknown. In recent years, there have been appearing reports on high toxicity of β-blockers toward aquatic organisms. In this paper the ability of the filamentous fungus *C. echinulata* to eliminate the β-blocker has been described for the first time. An 83% loss of carvedilol was observed after 120 h incubation of the tested fungus with the compound, where hydroxylated carvedilol metabolites were identified as the major biotransformation products. Carvedilol degradation by *C. echinulata* was proceeded by hydroxylation and conjugation reactions similar to its mammalian metabolism. Glucose conjugate was found in the fungi cultures, whereas glucuronide conjugates were detected in mammals. The impact of carvedilol on the functionality of fungal cells was also evaluated. A 2-fold decrease in the PC/PE ratio was noticed in the *C. echinulata* cell membrane after the exposition to carvedilol compared to control mycelium incubated without the β-blocker. The change can denote perturbation of fungal cell membrane integration by carvedilol. Moreover, 2.8-fold lower toxicity of postcultures supernatants toward *D. magna* were shown in contrast to abiotic control.

1. Introduction

Various groups of pharmaceuticals, such as antibiotics, beta adrenergic antagonists (β-blockers), steroids, hormones and products of their metabolism are commonly qualified as emerging pollutants. Although these compounds are detected at low levels in surface water, they may have an impact on diverse organisms including human because of their constant release to the environment (Farré et al., 2008; Geissen et al., 2015). Wastewater treatment plants are often recognized as the main emission source of pharmaceuticals into the environment as they are not able to completely eliminate drugs and their metabolites (Farré et al., 2008; Rodríguez-Navas et al., 2013). Therefore, it is important to develop new methods of removing the xenobiotic from the environment. In recent years, many promising results in the field of

* Corresponding author.
E-mail address: katarzyna.lisowska@biol.uni.lodz.pl (K. Lisowska).

http://dx.doi.org/10.1016/j.chemosphere.2017.05.088
0045-6535/

biotechnological and nanotechnological methods of eliminating pollution have been obtained (Chen et al., 2016; Cheng et al., 2016).

A few authors indicate that the presence of pharmaceutical residues in ecosystems poses new challenges in their monitoring and elimination from environment (Rodríguez-Navas et al., 2013; Gurke et al., 2015; Gavrilescu et al., 2015). β-blockers are one of the most important classes of medicines detected in wastewater, surface water and hospital sewage (Ramil et al., 2010; Gurke et al., 2015; Gavrilescu et al., 2015). Gurke et al. (2015) demonstrated occurrence of metoprolol in an urban sewage treatment plant in Germany, where its concentrations in the influent and the effluent reached 4.1 ± 1.0 and 4.4 ± 0.9 μg L^{-1}, respectively (Gurke et al., 2015). During seasonal monitoring of cardiovascular drugs in stream waters in Hungary the concentrations of carvedilol reached the values of 12–58 ng L^{-1} (Varga et al., 2013). Gavrilescu et al. (2015) summarized literature data concerning emerging pollutants and emphasized the frequent detection of β-blockers such as celiprolol, metoprolol, propranolol and satolol both in the wastewater influent and the effluent. Moreover, the authors paid attention to relatively low removal rates (from 36.4 to 55.8%) of these compounds from wastewater treatment plants (Gavrilescu et al., 2015). Atenolol and salbutamol were detected in the wastewater-treatment plant effluents and the landfill leachate in the range of 0.025–2.269 ng L^{-1} (Rodríguez-Navas et al., 2013).

In recent years, there have been many reports on aquatic ecotoxicity of β-blockers (Cleuvers, 2003; Huggett et al., 2002). Propanolol demonstrated high toxicity toward *Ceriodaphnia dubia* and *Daphnia magna*. The LC_{50} values for this compound were 0.8 ± 0.02 and 1.6 ± 0.3 mg L^{-1}, respectively. In the case of metoprolol, the LC_{50} values for *C. dubia* and *D. magna* were 8.8 ± 1.9 and 63.9 ± 6.2 mg L^{-1}, respectively (Huggett et al., 2002).

Carvedilol [(±)-1-[carbazolyl-4-oxy]-3-[(2-methoxy phenoxyethyl) amino]-2-propanol] is a nonselective beta-adrenergic antagonist used in the treatment of cardiovascular diseases including hypertension, angina and congestive heart failure. Moreover, this compound exhibits antioxidant activity (Chander et al., 2013). Products of carvedilol metabolism in dogs, rats and mice were described by Schaefer et al. (1998). Hydroxylated intermediates and glucuronide conjugates of carvedilol are main derivatives of its biotransformation in mammals (Lim et al., 2007).

Physicochemical degradation of carvedilol has been documented. Five degradation products of carvedilol were formed under varied stress conditions. The 1-(9*H*-carbazol-4-yloxy)-3-aminopropan-2-ol and 2-(2-(2-metoxyphenoxy) ethyloamino) ethanol were identified as main products of acidic, basic and photolytic degradation (Chander et al., 2013). However, there is no information about the behavior of carvedilol in the environment or its degradation by microorganism.

Filamentous fungi of the *Cunninghamella* species are commonly isolated from soil, seeds, nuts and plant-based materials (Asha and Vidyavathi, 2009). Furthermore, they have also been detected in the samples of surface water (Oliveira et al., 2013). *Cunninghamella* fungi are known for their ability to degrade different xenobiotics such as phenanthrene, quinoline, carbazole or tributyltin (Lisowska and Długoński, 2003; Bernat et al., 2014; Zawadzka et al., 2015; Felczak et al., 2016). On the other hand, fungi of *Cunninghamella* species have been described as a microbial model of drugs metabolism because of their enzymatic system analogous to mammals cytochrome P450 involved in pharmaceutical transformation (Asha and Vidyavathi, 2009; Murphy, 2015). The ability of *C. echinulata* to degrade naproxen, retinol, protryptilline, lapachol or diclofenac was shown. However, despite studies on *C. echinulata* potential to drugs elimination, this fungus is not known as extensively as *C. elegans* strain (Asha and Vidyavathi, 2009).

In this work for the first time we investigated the ability of *Cunninghamella echinulata* to remove carvedilol from its growth medium and identified the products of its fungal metabolism. Furthermore, we analyzed the changes in the phospholipid profile of *C. echinulata* in response to carvedilol. Detoxification of the tested compound by *C. echinulata* was also studied.

2. Materials and methods

2.1. Chemicals

Carvedilol was purchased from Sigma-Aldrich (USA). The other reagents with the analytical purity grade were obtained from Sigma-Aldrich (USA), POCH (Poland) and JT Baker (USA). Stocks solution of carvedilol was prepared in DMSO (20 mg mL^{-1}).

2.2. Microorganism, media and cultures preparation

Cunninghamella echinulata (no IM 2611) came from the collection of the Department of Industrial Microbiology and Biotechnology, University of Lodz (Poland). Spores of *C. echinulata* cultured for 10 days on ZT slants according to Długoński et al. (1984) were used to prepare a preculture in Sabouraud dextrose broth liquid medium (Difco, USA) supplemented with 2% glucose. The ZT slants consisted of 4 g L^{-1} yeast extract powder (Difco, USA); 4 g L^{-1} glucose (POCH, Poland); 25 g L^{-1} agar (BTL, Poland); 500 mL L^{-1} 12 °Blg malt extract (100 mL 1 °Blg malt extract contained 1 g soluble substances extracted from the grain. The spores were washed Sabouraud medium with 2% glucose to achieve 8–10 × 10^7 spores mL^{-1} and incubated at 28 °C for 24 h on a rotary shaker (160 rpm) in 100 mL Erlenmeyer flasks. The precultures were resuspended in 40 mL fresh Sabouraud medium with 2% glucose and cultivated for subsequent 24 h.

Carvedilol elimination, fungal dry weight and changes of the lipid profile were examined in modified Czapek-Dox liquid medium, composed of 3 g L^{-1} $NaNO_3$; 1 g L^{-1} KH_2PO_4; 0.5 g L^{-1} KCl; 0.5 g L^{-1} $MgSO_4 \times 7H_2O$; 0.01 g L^{-1} $FeSO_4 \times 7H_2O$; 40 g L^{-1} glucose. The Erlenmeyer flasks (100 mL) containing 18 mL Czapek-Dox medium were supplemented with carvedilol (2, 20, 50, 100 and 200 mg L^{-1}) and 2 mL of previously prepared fungal biomass. Biotic controls were prepared without the addition of the β-blocker and abiotic controls were prepared without mycelium. The cultures were cultivated on a rotary shaker at 28 °C for 48 h to achieve the stationary phase of fungal growth and used to evaluate the tolerance of the microorganism toward various concentrations of the β-blocker.

In the next step, the cultures of *C. echinulata* with carvedilol (20 mg L^{-1}) and corresponding biotic and abiotic controls were cultivated on a rotary shaker at 28 °C for 5 days to estimate *C. echinulata* growth and biotransformation of the β-blocker.

The fungal cultures and adequate biotic samples used in the studies of phospholipid change were supplemented with carvedilol (20 mg L^{-1}) and cultivated on a rotary shaker at 28 °C for 48 h to achieve the stationary phase of *C. echinulata* growth.

The mycelium was separated by filtration, washed twice with distilled water and dried at 100 °C to reach a constant weight for dry biomass determination. All samples were prepared in three independent repetitions.

2.3. Carvedilol extraction and LC-MS/MS-analysis

A Mixer Mill MM400 (Retsch, Germany) was used for disintegration of the filamentous fungi cultures after incubation. Portions of the homogenated suspensions of volume 10 mL were shaken for 2 min on a vortex mixer with 10 mL of ACN (acetonitrile) according to the modified QuEChERS protocol. In the next step, 2 g of