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Effects of non-steroidal anti-inflammatory drugs on cyanobacteria and algae in laboratory strains and in natural algal assemblages☆

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ABSTRACT

In recent years measurable concentrations of non-steroidal anti-inflammatory drugs (NSAIDs) have been shown in the aquatic environment as a result of increasing human consumption. Effects of five frequently used non-steroidal anti-inflammatory drugs (diclofenac, diflunisal, ibuprofen, mefenamic acid and piroxicam in 0.1 mg ml^{-1} concentration) in batch cultures of cyanobacteria (*Synechococcus elongatus*, *Microcystis aeruginosa*, *Cylindrospermopsis raciborskii*), and eukaryotic algae (*Desmodesmus communis*, *Haematococcus pluvialis*, *Cryptomonas ovata*) were studied. Furthermore, the effects of the same concentrations of NSAIDs were investigated in natural algal assemblages in microcosms. According to the changes of chlorophyll-a content, unicellular cyanobacteria seemed to be more tolerant to NSAIDs than eukaryotic algae in laboratory experiments. Growth of eukaryotic algae was reduced by all drugs, the cryptomonad *C. ovata* was the most sensitive to NSAIDs, while the flagellated green alga *H. pluvialis* was more sensitive than the non-motile green alga *D. communis*. NSAID treatments had weaker impact in the natural assemblages dominated by cyanobacteria than in the ones dominated by eukaryotic algae, confirming the results of laboratory experiments. Diversity and number of functional groups did not change notably in cyanobacteria dominated assemblages, while they decreased significantly in eukaryotic algae dominated ones compared to controls. The results highlight that cyanobacteria (especially unicellular ones) are less sensitive to the studied, mostly hardly degradable NSAIDs, which suggest that their accumulation in water bodies may contribute to the expansion of cyanobacterial mass productions in appropriate environmental circumstances by pushing back eukaryotic algae. Thus, these contaminants require special attention during wastewater treatment and monitoring of surface waters.

1. Introduction

Nowadays drugs are widely used in human and veterinary medicine, causing increasing concern in the environment. The drugs present at low concentrations (from ng l^{-1} to μg l^{-1}) can also have a significant impact on the aquatic and terrestrial systems (Daughton and Ternes, 1999). Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most frequently used drugs (painkillers, antipyretics, treatment of inflammations and prevention of myocardial infarction). Primary effect of NSAIDs is the inhibition of cyclooxygenase (COX) enzymes, thus the conversion of arachidonic acid or eicosapentaenoic acid to prostaglandins. Specific NSAID toxicity is related to the non-selective inhibition of COX enzymes in organisms having these proteins (Dorne et al., 2007).

The yearly consumption of NSAID differs significantly in different countries. Certain drugs can be purchased only with prescription, while others are freely available. Therefore, the actual NSAID consumption is hardly definable. The most popular NSAIDs are ibuprofen, diclofenac, acetyl-salicylic acid, piroxicam, ketoprofen, or naproxen, although it showed variability by countries (Hudec et al., 2008, 2012; Mijatović et al., 2010). The consumption of ibuprofen and other well tolerable NSAIDs as paracetamol increased in many countries in the past few years (Hudec et al., 2012).

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Drugs can get to the environment from numerous sources. In most cases, the majority of the used drugs are not fully metabolized in the human body, so these drugs and their metabolites are excreted with urine or feces (Jjemba, 2006). Most of NSAIDs contain functional groups (e.g. aromatic groups, –F, –Cl, $-CF_3$, etc.), which are highly resistant to metabolism. This increases the half-life not only in the human body, but also prolongs the degradation time in the environment: e.g. diclofenac inhibits the normal development of bacterial flora in wastewater treatment plants (Ziylan and Ince, 2011). The excreted, mostly biologically active components get into the environment from wastewater and sewage sludge, due to insufficiently effective treatment technologies (Santos et al., 2010). Veterinary medicines can get into the environment with animal feces, the accumulated manure can leach into surface and ground waters from landfills (Koutsouba et al., 2003). However, by-products and discarded (and therefore not metabolized) drug molecules also can be significant sources of pollution (Ziylan and Ince, 2011). Thus, these compounds can come in contact with the community of different life forms (Jones et al., 2005).

The removal efficiency of NSAIDs vary within a wide range and depends on the chemical structure of the given drug molecule. Ibuprofen can be removed with 90–100% efficiency during wastewater treatment (Stumpf et al., 1999), Feng et al. (2013) reported an average 74% removal rate in their review. Diclofenac can be characterised by the lowest removal efficiency among NSAIDs (17–23% according to Heberer, 2002; Quintana et al., 2005; an average 35% removal rate is reported by Feng et al., 2013). These compounds can be present in wastewater treatment effluents from 0.04 (diclofenac) up to 95 μg l^{-1} (ibuprofen) concentration (Feng et al., 2013). Data for Hungarian surface waters are known from Danube: 1.47–3.28 μg l^{-1} diclofenac and 1.53–2.87 μg l^{-1} ibuprofen was detectable (Helenkár et al., 2010). These values meet or even exceed the data given in international literature for treated wastewater. Conventional wastewater treatment consists of a combination of physical, chemical, and biological processes. The removal rate varies from “very poor” to “complete” in different treatment plants and seasons, depending strongly on the applied process, the character of drugs or external influences (Feng et al., 2013).

The acute and chronic effects of NSAID to invertebrates and vertebrates are more or less well known (Santos et al., 2010) and can be attributed to the non-specific inhibition of COX enzymes (see above). Much less information is available about the impacts of drugs and pharmaceutical residues on microorganisms and producer organisms (which – at least to our knowledge – do not contain COX-like enzymes). Farré et al. (2001) studied the acute toxicity of NSAIDs on luminescent bacteria (*Vibrio fischeri*). A 50% decrease of luminescence was observed in the presence of 13.5 mg l^{-1} diclofenac, 19.2 mg l^{-1} ibuprofen or 35 mg l^{-1} ketoprofen (30 min EC_{50}). Among NSAIDs, diclofenac showed the strongest acute toxicity to aquatic species, toxic effect usually appeared under 100 mg l^{-1} concentration (Fent et al., 2006). Diclofenac caused acute toxicity to the waterweed *Lemna minor* (7 days EC_{50} was 7.5 mg l^{-1}; Cleuvers, 2003).

The key role of phytoplankton in aquatic habitats and even in the whole biosphere is not questionable. The primer production of phytoplankton provides the basis of the aquatic food chain and generates nearly 70% of the atmospheric oxygen supply (Reynolds, 1984). On the other hand, increasing excessive algal production (especially in standing freshwaters: lakes and reservoirs) generate economical problems in water use of human society (Reynolds, 1984). Recently the invasion and proliferation of cyanobacteria, especially the toxic ones is a well-known worldwide phenomenon in many types of aquatic habitats. The main problem with the expansion of cyanobacterial blooms is that they have the potential to alter significantly the structure of native algal and eventually the whole aquatic community, and consequently modify the functioning of the ecosystem (Sukenik et al., 2015). The expansion of non-toxic and toxic cyanobacteria to a wide geographic range may have an impact on the ecosystems, trophic cascades and geochemical cycles (Sukenik et al., 2015). Therefore, to study the possibility that organic contaminants may contribute to cyanobacterial dominance or not, is exceptionally important.

Despite the fact, that NSAIDs frequently occur in aquatic environments, there are very limited number of studies dealing with their effects on isolated strains of cyanobacteria and eukaryotic algae or on natural phytoplankton assemblages. In this study, the effects of five frequently used NSAIDs on the growth of common cyanobacteria and eukaryotic algae were tested in laboratory experiments. Furthermore, the effects of NSAIDs on the composition of algal assemblages of two shallow ponds with different algal dominancy also were studied in microcosm experiments. The changes of NSAIDs concentrations to the end of the treatments also were evaluated. On the basis of the few available information in literature, our hypotheses were the following:

- We assumed that NSAID has notable effects on the growth of isolated cyanobacterial and eukaryotic algal strains, and presumably cyanobacteria may be more tolerant then eukaryotic algae (on the basis of Pomati et al., 2004).
- We assumed that quantitative and qualitative changes occur in natural assemblages due to the presence of NSAIDs, but with a weaker influence on cyanobacteria (on the basis of Proia et al., 2013 and Corcoll et al., 2014).
- Decrease of initial NSAID concentration is presumable at least in the case of potentially mixotrophic isolates and natural assemblages (on the basis of Groning et al., 2007; Kunkel and Radke, 2008; Murdoch and Hay, 2013).

2. Materials and Methods

2.1. Strains, culturing conditions and laboratory experimental setup

Axenic cultures of *Synechococcus elongatus* (BG-101, a derivative of PCC 6301), *Microcystis aeruginosa* (BG-243) and *Cylindrospermopsis raciborskii* (BG-266) cyanobacteria are maintained in BG (*C. raciborskii*) and Allen (*S. elongatus* and *M. aeruginosa*) media at 28 °C in the algal culture collection of the Department of Botany. Axenic *Desmodesmus communis* (ACCDH-UD1004), monoalgal *Haematococcus pluvialis* (ACCDH-UD1205) and axenic *Cryptomonas ovata* (ACCDH-UD1003, a derivative of CCAP 979/61) cultures are maintained in Jaworski's medium (CCAP media recipes; *D. communis* and *C. ovata*) and in Optimized Haematococcus Medium (OHM, Fábregas et al., 2000; *H. pluvialis*), at 24 °C in the algal culture collection of the Department of Hydrobiology.

The experiments were carried out in triplicates, in shaken cultures (110 rpm) in 100 ml Erlenmeyer flasks with a final volume of 30 ml, under continuous irradiation (fluorescent light), at 28 °C for cyanobacteria and at 24 °C for eukaryotic algae. During the experiments, the preferences of the used cyanobacterial and eukaryotic algal strains were taken into consideration. Different culturing media and temperatures were used because of the different optima of cyanobacteria and eukaryotic algae (especially *H. pluvialis* and *C. ovata*). Diclofenac, diflunisal, ibuprofen, mefenamic acid and piroxicam NSAIDs were applied in 0.1 mg ml^{-1} concentration during the treatments. The applied concentration was chosen on the basis of available data about NSAID concentrations effective on algal growth (Cleuvers, 2003, 2004; Lin et al., 2009). The drugs were diluted in 10 g l^{-1} sodium-carbonate buffer. Cultures without the addition of the buffer and NSAIDs served as controls. To check