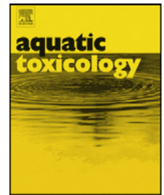
Aquatic Toxicology 98 (2010) 256–264

Contents lists available at ScienceDirect

Aquatic Toxicology

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



Endocrine disruption and consequences of chronic exposure to ibuprofen in Japanese medaka (*Oryzias latipes*) and freshwater cladocerans *Daphnia magna* and *Moina macrocopa*

Sunyoung Han[a], Kyungho Choi[a,*], Jungkon Kim[a], Kyunghee Ji[a], Sunmi Kim[a], Byeongwoo Ahn[b], Junheon Yun[c], Kyunghee Choi[c], Jong Seong Khim[d], Xiaowei Zhang[e,f], John P. Giesy[e,f,g,h,i]

[a] *School of Public Health, Seoul National University, 28 Yunkeon, Chongro, Seoul 110-799, Republic of Korea*
[b] *College of Veterinary Medicine, Chungbuk National University, Cheongju 361-763, Republic of Korea*
[c] *National Institute of Environmental Research, Incheon 404-708, Republic of Korea*
[d] *Division of Environmental Science and Ecological Engineering, Korea University, Seoul 136-713, Republic of Korea*
[e] *Toxicology Centre and Department of Veterinary Biomedical Sciences, University of Saskatchewan, Saskatoon, Saskatchewan S7J 5B3, Canada*
[f] *State Key Laboratory of Pollution Control and Resource Reuse & School of the Environment, Nanjing University, Nanjing 210093, China*
[g] *Zoology of Department, Center for Integrative Toxicology, Michigan State University, East Lansing, MI 48824, USA*
[h] *Department of Biology and Chemistry, City University of Hong Kong, Kowloon, Hong Kong SAR, China*
[i] *State Key Laboratory of Marine Environmental Science, College of Oceanography and Environmental Science, Xiamen University, Xiamen, China*

ARTICLE INFO

Article history:
Received 9 November 2009
Received in revised form 8 February 2010
Accepted 16 February 2010

Keywords:
Non-steroidal anti-inflammatory drug
Fish
Steroidogenesis
H295R cell
Aromatase
Hormones

ABSTRACT

Despite frequent detection of ibuprofen in aquatic environments, the hazards associated with long-term exposure to ibuprofen have seldom been investigated. Ibuprofen is suspected of influencing sex steroid hormones through steroidogenic pathways in both vertebrates and invertebrates. In this study, the effect of ibuprofen on sex hormone balance and the associated mechanisms was investigated *in vitro* by use of H295R cells. We also conducted chronic toxicity tests using freshwater fish, *Oryzias latipes*, and two freshwater cladocerans, *Daphnia magna* and *Moina macrocopa*, for up to 144 and 21 d of exposure, respectively. Ibuprofen exposure increased 17β-estradiol (E2) production and aromatase activity in H295R cells. Testosterone (T) production decreased in a dose-dependent manner. For *D. magna*, the 48 h immobilization EC50 was 51.4 mg/L and the 21 d reproduction NOEC was <1.23 mg/L; for *M. macrocopa*, the 48 h immobilization EC50 was 72.6 mg/L and the 7 d reproduction NOEC was 25 mg/L. For *O. latipes*, 120 d survival NOEC was 0.0001 mg/L. In addition, ibuprofen affected several endpoints related to reproduction of the fish, including induction of vitellogenin in male fish, fewer broods per pair, and more eggs per brood. Parental exposure to as low as 0.0001 mg/L ibuprofen delayed hatching of eggs even when they were transferred to and cultured in clean water. Delayed hatching is environmentally relevant because this may increase the risk of being predated. For *O. latipes*, the acute-to-chronic ratio of ibuprofen was estimated to be greater than 1000. Overall, relatively high acute-to-chronic ratio and observation of reproduction damage in medaka fish at environmentally relevant ranges of ibuprofen warrant the need for further studies to elucidate potential ecological consequences of ibuprofen contamination in the aquatic environment.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

Pharmaceuticals are developed and used for intended biological effects in human and veterinary medicine. The physiologically active nature of pharmaceuticals, however, raised concerns about their potential impacts to non-target species when they were inadvertently discharged into ecosystem (Ankley et al., 2007; Fent et al., 2006). Ibuprofen ((*RS*)-2-(4-isobutylphenyl)propanoic acid, CAS number 15687-27-1) is one of non-steroidal anti-inflammatory drugs (NSAIDs), and is widely used as analgesic, antipyretic and anti-inflammatory purposes to relieve symptoms of arthritis, rheumatic disorders and fever (Hayashi et al., 2008). It is one of the

Abbreviations: cAMP, cyclic adenosine monophosphate; CF, condition factor; COX, cyclooxygenase; CI, confidence interval; CYP, cytochrome P450; dph, day post-hatch; EC50, median effective concentration; ELISA, enzyme-linked immunosorbent assay; GSI, gonadosomatic index; HSI, hepatosomatic index; LOEC, lowest observed effect concentration; MCIG, minimum concentration to inhibit growth; NOEC, no observed effect concentration; NSAID, non-steroidal anti-inflammatory drug; PGE2, prostaglandin E2; PGR, population growth rate; STP, sewage treatment plant.

* Corresponding author.
E-mail address: kyungho@snu.ac.kr (K. Choi).

0166-445X/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquatox.2010.02.013

core medicines included in "Essential Drugs List" of World Health Organization, and therefore produced in large amounts worldwide (Heckmann et al., 2007).

Ibuprofen has frequently been detected in surface water, with as much as 0.1 μg/L detected in surface water of South Wales, UK (Kasprzyk-Hordern et al., 2008). The average concentration detected in major rivers of Korea was 0.03 μg/L (Kim et al., 2007). Relatively greater concentrations of ibuprofen have been reported for effluents (up to 22 μg/L) and influents (up to 84 μg/L) of sewage treatment plants (STPs) (Brun et al., 2006; Gömez et al., 2007). Hence municipal wastewater effluents are an important source of ibuprofen in aquatic environments, especially streams and rivers.

Due to the widespread occurrence of ibuprofen in aqueous environments, its potential for ecological impact has been of growing concern (Christensen et al., 2009). Ibuprofen is known to influence the cyclooxygenase (COX) pathway (Flippin et al., 2007; Heckmann et al., 2008). The COX enzyme could influence the synthesis of eicosanoids, which are important regulators of reproduction in both vertebrates and invertebrates (Hayashi et al., 2008). Therefore, ibuprofen contamination in water environment could affect reproduction of aquatic animals. However, most investigations have been limited to lethal effects during acute exposures. Studies involving long-term exposure have been limited to invertebrates such as *Hydra* (Quinn et al., 2008), mollusks (Pounds et al., 2008) and the freshwater cladoceran, *D. magna* (Halling-Sørensen et al., 1998; Han et al., 2006; Heckmann et al., 2007). In *D. magna*, reproduction and population growth were measured after 14 d exposure to environmentally realistic concentrations of ibuprofen (Heckmann et al., 2007). Only one study reported effects of longer term ibuprofen exposure in freshwater fish: In Japanese medaka (*Oryzias latipes*), reproduction-related endpoints such as blood size and number of broods were evaluated during a 6-week exposure (Flippin et al., 2007).

However, short-term chronic tests covering only partial life stages may not be sufficient for chemicals like ibuprofen that are constantly released from municipal STPs. A combination of a development test with a complementary fish reproduction test, for example, has been suggested in order to understand potential ecological risks of such contaminants, while full life cycle test could be used to refine such assessment (Hutchinson et al., 2000). Therefore, most assessments of the ecotoxicity of ibuprofen conducted to date were not sufficient to understand the potential for chronic effects of ibuprofen exposure on endpoints other than lethality. Significant knowledge gaps in the mechanism of toxicity also exist.

This study was conducted to identify the effects of ibuprofen on steroidogenesis. In addition, we evaluated the effects of chronic exposure of freshwater crustaceans and fish to environmentally relevant concentrations of ibuprofen. For this purpose, the H295R cells, human adrenocortical carcinoma cell line and three model freshwater species, including freshwater macroinvertebrates *D. magna* and *Moina macrocopa*, and fish *O. latipes*, were employed.

2. Material and methods

2.1. Chemicals

Test solutions were freshly prepared by diluting stock solutions with appropriate culture media, immediately before the test or before the renewal of test solutions. Ibuprofen (purity 98%) was purchased from Sigma–Aldrich (St. Louis, MO, USA). Solvent-free stock solution of ibuprofen was prepared by dissolving in MilliQ water (Millipore Asia, Yonezawa, Japan) with sonication. Measured concentrations of ibuprofen were 1.13 and 71.9 mg/L for the nominal concentrations of 1 and 100 mg/L, respectively. Concentrations of ibuprofen did not change more than 6% after the 48 h of exposure of *D. magna* or *O. latipes* (Supplement Table S1). Nominal concentrations are reported throughout the paper.

2.2. Cell culture and maintenance of test organisms

The H295R human adrenocortical carcinoma cell line was obtained from the American Type Culture Collection (ATCC # CRL-2128, ATCC, Manasas, VA, USA) and cultured at 37 °C in a 5% CO_2 atmosphere as previously described (Gracia et al., 2007). Briefly, the cells were grown in a 1:1 mixture of Dulbecco's Modified Eagle's Medium and Ham's F-12 Nutrient mixture (DMEM/F12) (Sigma D-2906; Sigma–Aldrich) supplemented with 1.2 g/L Na_2CO_3, 10 mL/L of ITS+ Premix (BD Bioscience; 354352), and 25 mL/L of Nu-Serum (BD Bioscience; 355100). Medium was changed every 4 d and cells were subcultured every week.

The two freshwater cladocerans (*D. magna* and *M. macrocopa)* and the fish (Japanese medaka fish; *O. latipes*) were cultured and maintained in the Environmental Toxicology Laboratory, Seoul National University (Seoul, Korea) following US Environmental Protection Agency (US EPA, 2002), Oh (2007), and Organization for Economic Cooperation and Development TG 203 (OECD, 1992a,b) protocols, respectively. Both crustaceans were fed daily with a 1:1:1 mixture of yeast (ACH Food Companies, Memphis, TN, USA), cerophyll (Nutraceutical Corporation, Park City, UT, USA), and Tetramin® (Tetra, Melle, Germany). In addition, algae (*Pseudokirchneriella subcapitata*) were also provided. Japanese medaka fish were cultured in filtered tap water after dechlorination by aeration for more than 24 h. Medaka were maintained at 25 ± 1 °C, under a 16:8-h light:dark photoperiod, and were fed twice a day with freshly hatched *Artemia nauplii* (Brine Shrimp Direct, Ogden, UT, USA).

Water quality parameters, including dissolved oxygen, pH, conductivity and temperature, were monitored and logged whenever new batches of media were prepared, following American Public Health Association, American Water Works Association, and Water Pollution Control Federation (1992) protocols. To confirm comparable sensitivity of the test organisms over time, acute lethality tests were conducted with the reference toxicant (zinc chloride) to determine the relative sensitivity of *D. magna*, *M. macrocopa*, and *O. latipes* on a monthly basis (data not shown).

2.3. Steroidogenesis assay using H295R cells

H295R cells were seeded into 24-well plates at a concentration of 3×10^5 cells/mL in 1 mL of medium per well. After 24 h, cells were exposed to ibuprofen dissolved in dimethyl sulfoxide (DMSO; Sigma–Aldrich, St. Louis, MO, USA). The final DMSO concentration in the exposure medium was less than 0.1% (v/v). H295R cells were exposed to various concentrations of ibuprofen for 48 h, and were inspected microscopically for viability. When the exposure resulted in cell viability less than 85% the data were not used to determine the effect on hormone production (Gracia et al., 2007). In addition, to identify the range of ibuprofen concentrations that are non-cytotoxic, a Live/Dead cell viability assay kit (Molecular Probes, Eugene, OR, USA) was used. The culture medium was collected after the exposure and stored at −80 °C for further measurement of hormones produced.

Hormone extraction and quantification were conducted as previously described (Hecker et al., 2006). Briefly, hormones were extracted from the culture medium twice with diethyl ether (5 mL) and the solvent was evaporated under a gentle stream of nitrogen. The residue was reconstituted in ELISA assay buffer and was measured by competitive ELISA following the manufacturer's recommendation (Cayman Chemical, Ann Arbor, MI, USA; Testosterone [T, Cat # 582701], 17β-Estradiol [E2, Cat # 582251]).

Activity of aromatase enzyme was measured using two different methods, direct or indirect assays as previously described

Download English Version:

https://daneshyari.com/en/article/4530193

Download Persian Version:

https://daneshyari.com/article/4530193

Daneshyari.com