Ecotoxicology and Environmental Safety 120 (2015) 279–285

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Environmental monitoring of urban streams using a primary fish gill cell culture system (FIGCS)

Sabine Schnell [a], Kafilat Bawa-Allah [b], Adebayo Otitoloju [b], Christer Hogstrand [a], Thomas H. Miller [c], Leon P. Barron [c], Nic R. Bury [a,*]

[a] Division of Diabetes and Nutritional Sciences, Faculty of Life Sciences and Medicine, King's College London, Franklin Wilkins Building, 150 Stamford Street, London SE1 9NH, United Kingdom
[b] Ecotoxicology Laboratory, Department of Zoology, Faculty of Science, University of Lagos, Akoka, 101017 Lagos, Nigeria
[c] Analytical and Environmental Sciences Division, Faculty of Life Sciences and Medicine, King's College London, Franklin Wilkins Building, 150 Stamford Street, London SE1 9NH, United Kingdom

ARTICLE INFO

Article history:
Received 30 April 2015
Received in revised form
6 June 2015
Accepted 8 June 2015
Available online 17 June 2015

Keywords:
cyp1a1
cyp3
Metallothionein
Fish
Environmental monitoring
Cell culture
3Rs

ABSTRACT

The primary fish gill cell culture system (FIGCS) is an *in vitro* technique which has the potential to replace animals in whole effluent toxicity tests. In the current study FIGCS were transported into the field and exposed to filtered (0.2 μm) river water for 24 h from 4 sites, on 2 different sampling dates. Sites 1 and 2 are situated in an urban catchment (River Wandle, London, UK) with site 1 downstream of a sewage treatment work; site 3 is located in a suburban park (River Cray, Kent, UK), and site 4 is more rural (River Darent, Kent, UK). The change in transepithelial electrical resistance (TER), the expression of the metal responsive genes metallothionein A (*mta*) and B (*mtb*), cytochrome P450 1A1 (*cyp1a1*) and 3A27 (*cyp3a27*), involved in phase 1 metabolism, were assessed following exposure to sample water for 24 h. TER was comparable between FIGCS exposed to 0.2 μm filtered river water and those exposed to synthetic moderately soft water for 24 h. During the first sampling time, there was an increase in *mta*, *cyp1a1* and *cyp3a27* gene expression in epithelium exposed to water from sites 1 and 2, and during the second sampling period an increase in *cyp3a27* gene expression at sites 1 and 4. Urban river water is a complex mixture of contaminants (e.g., metals, pesticides, pharmaceuticals and polyaromatic hydrocarbons) and the increase in the expression of genes encoding *mta*, *cyp1a1* and *cyp3a27* in FIGCS is indicative of the presence of biologically active pollutants.

1. Introduction

A large number of fish are used each year for waste effluent toxicity testing, with an estimated 3 million being used in the US alone see Tanneberger et al. (2013). There is a desire worldwide to reduce the number of fish used in toxicity testing and thus reliable alternatives are being investigated. A number of studies have assessed fish cell lines as alternative methodologies with success (Davoren et al., 2005; Dayeh et al., 2009; Kinani et al., 2010; Schnell et al., 2013). However, a drawback to using cell lines for waterborne toxicity is that they are often unable to tolerate hypoosomotic water. To overcome this, water has to be modified by the addition of osmolytes to ensure osmotic tonicity between the external medium and the intracellular compartment. An alternative approach is the use of a primary FIsh Gill Cell culture System (FIGCS; Walker et al., 2008; Minghetti et al., 2014, Bury et al., 2014). This method uses a double seeding technique and ensures that the epithelium contains the different cell types characteristic of an intact gill (Fletcher et al., 2000; Walker et al., 2007). When grown on permeable supports, the membrane forms a polarised tight epithelium with transepithelial electrical resistance (TER) measurements exceeding 10 KΩ. At this stage the epithelium is able to tolerate the application of water on the apical surface for up to 48 h. The property of tolerating freshwater has led to the use of the system for physiological studies see Wood et al. (2002), the assessment of pharmaceutical uptake (Stott et al., 2015) and toxicity of pollutants within the aquatic environment (Sandbacka et al., 1999, Bury et al., 2014).

A recent study also explored the potential for FIGCS to be used for environmental monitoring of natural waters (Minghetti et al., 2014). In this study the cells were transported 1000 km in a temperature controlled container and were exposed in the field to metal-contaminated river water under non-sterile conditions. The

* Corresponding author.
E-mail address: nic.bury@kcl.ac.uk (N.R. Bury).

http://dx.doi.org/10.1016/j.ecoenv.2015.06.012
0147-6513/

membrane maintained integrity, showing comparable changes in TER after 24 h between those exposed to river water and those exposed to reconstituted sterile water. The cells also showed no signs of cell mortality, as measured by the Methylthiazol Tetrazolium (MTT) assay, but they did show an increase in expression of the genes encoding for the metal binding proteins metallothionein A and B (Minghetti et al., 2014), demonstrating the presence of bioreactive metals.

The previous study (Minghetti et al., 2014) specifically targeted rivers in Cornwall, South West England as they are known to have elevated metals with very little other pollutant load and in the laboratory the primary gill cells are known to respond to metals with increased expression of *mta* and *mtb* genes (Walker et al., 2007). The FIGCS output (gene expression) is an integrative response that takes into account the over lying water chemistry, which determines metal speciation, and the ability of the metal to enter the cell and bind to intracellular receptors in sufficient quantities to cause an effect. If this system is to be used more widely to detect the presence of compounds that may elicit a biological effect, it is necessary to evaluate the response of the cells to more complex aquatic matrices. Thus, the aims of the current study are to expose the primary gill cell culture to a further 3 sites on urban rivers in London, UK, and one site on a more rural river in Kent, UK, that potentially have a far complex mixture of pollutants than the metal contaminated rivers in Cornwall (Minghetti et al., 2014) and to measure the expression of genes encoding for *mta* and *mtb*, as well as *cyp1a1* and *cyp3a27*, enzymes which are involved in phase 1 organic compound metabolism (Uno et al., 2012), as well as TER following 24 h of exposure to the river water. An increased transcription of *mta* and *mtb* indicate transactivation through metal-responsive transcription factor-1 (Mtf1) (Olsson et al., 1995; Samson and Gedamu, 1995), whilst increased levels of mRNA for *cyp1a1* and *cyp3a27* are indicative of increased activity of the aryl hydrocarbon receptor (Ahr) and the pregnane-X-receptor /retinoic acid-X-receptor (Pxr/Rxr) heteroduplex, respectively (Uno et al., 2012).

2. Materials and methods

2.1. Study sites and water chemistry

The 4 study sites were on the River Wandle at Colliers Wood (site 1, latitude 51.420368; longitude −0.181487) and Beddington (site 2, 51.370284; −0.125072), the river Cray at Sidcup (site 3, 51.428425; 0.132730), all in South East London, and the River Darent at Lullingstone (site 4, 51.362372, 0.196315) in Kent, UK. Site 1 on the River Wandle is highly urbanised and is approximately 4.5 km downstream of Beddington Sewage Treatment Works (STW) which receives wastewater from approximately 360,000 people. Site 2 is above the input from Beddington STW, but is still within a heavily urbanised catchment, receiving drainage from the Borough of Croydon. Site 3 is within a suburban park, whilst Site 4 is within a rural setting; however the River Darent flows through suburban area of Sevenoaks, Kent. The first sampling date was 2.12.2013 and the second sampling on 11.12.2013.

Water pH, conductivity, temperature and suspended solids were measured using a Hanna Hi991300 probe. For chemical analysis water samples were collected in the field in low density polyethylene bottles and immediately frozen and stored at −20 °C on returning to the laboratory. Total and Mg hardness and alkalinity were measured colourimetrically, and for Cu and Zn analysis water samples were filtered (0.2 μm filters) and acidified prior to measurement via inductively coupled plasma mass spectrometer (Aglient 7700x ICP-MS). For analysis of pharmaceuticals, sample clean-up and pre-concentration was achieved by solid phase extraction (SPE) on Waters Oasis mixed-mode hydrophilic lipophilic balanced (HLB) cartridges, 6 cc, 200 mg sorbent (Waters Corporation, Milford, MA, USA), similarly to our previous works (Lacey et al., 2008; Barron, et al., 2008, 2009; Miller et al., 2015). Briefly, 100 mL aliquots of surface water samples were adjusted to pH 6.5 with ammonium acetate (1 mL of a 1 M solution). SPE cartridges were conditioned with 6 mL of MeOH and ultra-pure water followed by sample loading. Cartridges were then washed with 1 mL ultra-pure water and dried for ~30 min under a vacuum. Cartridges were eluted in 10 mL of 50:50 ethyl acetate: acetone and dried under N_2 and at 30 °C using a TurboVap (Biotage, Uppsala, Sweden). The dried extract residues were reconstituted in 0.5 mL of 90:10 (v/v) 10 mM ammonium acetate in water:acetonitrile and transferred to a septum capped vial. Analysis was performed on an Agilent 1100 high pressure liquid chromatography system interfaced to Waters Quattro triple quadrupole mass spectrometer according to the conditions listed in Miller et al. (2015). Separations were performed on a C_{18} reversed-phase column (Waters Sunfire $C_{18,}$ 2.1 × 150 mm, 2.5 μm). Multiple reaction monitoring was used to detect characteristic transitions of all targeted pharmaceutical compounds. Concentrations of all pharmaceuticals are expressed as single-shot quantitation measurements based on comparison to a single matrix matched calibrant at 200 ng L^{-1} spiking level (in triplicate). Therefore concentrations should be considered as semi-quantitiative. These were extracted alongside unspiked samples ($n=3$) for background correction purposes.

2.2. Cell culture and field exposures

Primary gill cell culture techniques and exposure methods followed the methods described in Minghetti et al. (2014). Rainbow trout (*Oncorhynchus mykiss*) were obtained from a local trout farm. Primary gill cell cultures were prepared from fish of 80–100 g. All fish were housed at King's College London where they were maintained in fibreglass tanks (1000 L) with flowing and aerated de-chlorinated City of London tap water ($[Na^+]$= 0.53 mM; $[Ca^{2+}]$=0.92 mM; $[Mg^{2+}]$=0.14 mM; $[K^+]$=0.066 mM; $[NH_4^+]$=0.027 mM), which was passed through activated carbon, mechanical and biological filters. Water temperature was maintained at 14 °C, while photoperiod was held constant (12 h light, 12 h dark). Fish were fed daily a one-percent (w/w) ration of trout pellets. The primary gill cells were isolated and cultured as described in Fletcher et al. (2000) and prepared using the double seeding technique as described in Kelly et al. (2000) and Walker et al. (2007). Sterile techniques were used throughout all cell culture procedures. Briefly, for each seeding, 2 fish were sacrificed (following local UK Home Office schedule 1), the gills were dissected out and the gill filaments were subject to cleaning and tryptic digestion (0.05% Trypsin-EDTA; Invitrogen). Isolated rainbow trout gill cells were seeded onto cyclopore polyethylene terephthalate membrane (cell, surface area 0.9 cm^2, pore size 0.4 μm, Falcon) at a cell density of 1.2×10^6 per insert, in Leibovitz (L-15) medium (Invitrogen) supplemented with antibiotics (5% foetal bovine serum (FBS); Sigma, 2% penicillin and streptomycin (PEST); Invitrogen and 2% gentamicin; GIBCO v/v). After 24 h incubation at 18 °C in an air atmosphere cool incubator (Sanyo Mir-253), the cells were washed twice in phosphate-buffered saline (PBS) to remove debris and another seeding of primary gill cells was added at the same density per insert, and cultured in supplemented L-15 medium. After a further 24 h incubation another PBS wash followed and supplemented L-15 was replaced at a volume of 1.5 mL in the apical chamber of the insert and 2.0 mL in the basolateral chamber. Cultures were grown at 18 °C. After 96 h the gill cell system was cultured using L-15 medium +5% FBS, but without antibiotics with complete medium changes every 48 h. The