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Cellular effect evaluation of micropollutants using transporter functions of renal proximal tubule cells

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ABSTRACT

Issues pertaining to the effects of micropollutants in reclaimed water are arising in terms of their effect on human health. However, current cellular methodologies face some difficulties to detect subtle effects of waterborne micropollutants at environmental concentrations (ng L^{-1}–μg L^{-1}) on human and animal cells. In this study, an appropriate cellular model capable of detecting the subtle effects of aquatic micropollutants at environmental concentrations using the functions of primary cultured rabbit renal proximal tubule cells (PTCs) is proposed. Tris-(2-chloroethyl)-phosphate (TCEP) was chosen as the representative micropollutant from eight typical micropollutants via lactate dehydrogenase assay. TCEP significantly decreased not only ion (sodium, calcium, and phosphate) uptake from 10^{-2} mg L^{-1} (64.8–82.5%, 60.4–68.8%, and 91.9–93.8% of the control, respectively), but also the expression of ion transporters (NHE-3 and L-type Ca channel) from 10^{-2} mg L^{-1} (53.9–87.4% and 38.6–63.6% of the control, respectively). Moreover, TCEP significantly decreased both the non-ion (glucose, fructose, and L-arginine) uptake and the expression of non-ion transporters (SGLT 1, GLUT 5, and rBAT) from 10^{-2} mg L^{-1}. Therefore, the results demonstrated that the function of PTCs as a cellular model can be used to determine subtle effects of environmental micropollutants at low concentrations.

1. Introduction

Wastewater reclamation and reuse have been used as alternative methods of overcoming water scarcity throughout the world (Asano et al., 2007). Reclaimed water can be used as river recharge water, agriculture water, middle level water, industrial water, and drinking water through various treatment systems (Andresen and Bester, 2006; Loraine and Pettigrove, 2006; Ternes et al., 2007). However, reclaimed water still contains low concentration of pharmaceuticals, personal care products and miscellaneous chemicals in the ng L^{-1}–μg L^{-1} range (usually called micropollutants) (Ternes et al., 2003; Vieno et al., 2006; Kim et al., 2007). The concerns regarding waterborne micropollutants, either single or in complex mixtures in reclaimed water have been steadily increasing due to their potential effects on ecosystems and human health (Daughton and Ternes, 1999; Schwab et al., 2005; Muñoz et al., 2009).

The majority cellular studies of aquatic micropollutants have been undertaken to investigate the toxic effects on fish primary cells and cell lines in terms of their ecotoxicological effects, with major studies focused on cytotoxicity (Henschel et al., 1997; Caminada et al., 2006; Fent et al., 2006) and minor studies on molecular toxicology (Kilemade et al., 2002; Dorval et al., 2003; Caminada et al., 2008). Only a few studies pertaining to either the single or mixed conditions of micropollutants at environmental concentrations were conducted focusing their potential effects on human health, by explaining cytotoxic and molecular toxic effects on human and animal cell lines (Föllmann and Wober, 2006; Pomati et al., 2006). However, one possible reason for this minimal number of studies is that researchers have found difficulties to investigate the subtle effects of environmental concentrations on cytotoxicity and molecular toxicity in vitro, other than a study by Pomati et al. (2006) who found only cytotoxic effects at environmental concentrations.

Due to the low level concentrations of micropollutants in environmental water systems, investigating toxicological effects is difficult due to the fact that existing methodologies are not sensitive enough to detect the subtle effects of micropollutants at environmental concentrations even using different animal cell lines (Föllmann and Wober, 2006; Pomati et al., 2006). Moreover, the transporter or enzyme activities of cell lines may have changed or decreased compare to primary cultured cells (Lee et al., 2007). Thus, finding appropriate methods for investigating subtle changes of micropollutants at environmental concentrations in vitro is one of the focuses of present subjects.

To develop a new model system to fit the study of the subtle effects of micropollutants at environmental levels in vitro, we previously reported one cellular model consisted of cell cyclic regulatory protein expression and primary cultured rabbit proximal

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tubule cells (PTCs) (Ren et al., 2008). In this study, we also chose well-characterized primary cultured rabbit renal proximal tubule cells as the target cells, which can overcome limitations of sensitivity and accuracy in cell lines (Lee et al., 2007), but investigated ion and non-ion transportation as the target functions in the proximal tubule cells because one of main function of kidney PTCs was to reabsorb most of the fluid and solutes filtered from the glomerulus (Boron and Boulpaep, 2003; Han et al., 2005). For application of this new model system, we selected eight micropollutants generally found in reclaimed water, and then investigated the basic toxic effects of their micropollutants before choosing one of them as the target micropollutant for a more detailed test of molecular functional effects. Thus, the purpose of this study is to develop a novel model system for investigating the possible application of this model to the study of subtle effects of micropollutants at environmental concentrations via the functional study of a representative micropollutant on primary cultured rabbit renal proximal tubule cells.

2. Materials and methods

2.1. Micropollutant ingredients

Eight chemicals were selected as typical aquatic environmental micropollutants in reclaimed water based on a paper review and the actual environmental concentration of each chemical is listed in Table 1.

2.2. Cell proliferation and culture condition

The primary rabbit renal PTC cultures were prepared using the method reported by Chung et al. (1982). In brief, the cells were grown in a D-MEM/F-12 medium (Gibco-BRL, Gaithersburg, MD) with 15 mM HEPES and 20 mM sodium bicarbonate (pH 7.4) with three growth supplements (5 μg mL^{-1} insulin, 5 μg mL^{-1} transferrin, and 5×10^{-8} M hydrocortisone) being incorporated into the medium immediately before use. Rabbit kidneys were perfused through the renal artery, first with Phosphate Buffered Saline (PBS), and then with medium containing iron oxide (0.5%). Renal cortical slices were prepared and homogenized. The homogenate was first poured through a 253 μm mesh filter and then through an 83 μm mesh filter. Tubules and glomeruli were recovered from the top of the 83 μm filter and transferred into a sterile medium, and the glomeruli-containing portion (containing iron oxide) was removed using a magnetic stir bar. The remaining proximal tubules were briefly incubated in a medium containing collagenase (0.125 mg mL^{-1}) and a 0.025% soybean trypsin inhibitor. The tubules were then washed by centrifugation, resuspended in a medium containing the three supplements, and transferred into tissue culture dishes, in which the medium was changed one day after plating and every two days thereafter. Finally, the primary cultured rabbit kidney PTCs were maintained at 37 °C in a 5% CO_2 humidified environment in a serum-free basal medium supplemented with the three growth supplements that were mentioned earlier.

2.3. Cell count, lactate dehydrogenase (LDH) and sulforhodamine B (SRB) assay

The total number of cells was counted using the following methodology. The cells were washed twice with PBS and trypsinized from the culture dishes. This cell suspension was then mixed with a 0.4% (wt./vol.) trypan blue solution and the number of live cells was determined using a hemocytometer. Cell injury was assessed by LDH activity. The level of LDH activity in the medium was measured using a LDH assay kit. For measurement of LDH activity, the cells were treated with different concentrations of chemical for 24 h, and LDH activity was expressed as the fold-increase of the control. Cell viability was examined using SRB assay, where BE(2)-M17 cells with 180 μL of MEM culture media were cultivated to each well in 96 well plate and maintained in a CO_2 incubator for 12 h. These cells were then treated by chemical for 24 h. Fifty microlitre of 50% cold TCA was then slowly added to each well, which was placed in a refrigerator for 1 h at 4 °C. After 1 h, the plate was washed five times with tap water, and then left to dry for 1 h at room temperature. After drying, 100 μL of 0.4% SRB was added to each well and the plate was left for dry more than 30 min. Again, the plate was washed five times using 1% acetic acid, and then dried for 2 h at room temperature. Finally, 150 μL of 10 mM unbuffered Tris was added to each well, which was detected using a microplate reader at 550 nm.

2.4. [^{3}H] Thymidine incorporation

The medium was changed for the last time when the cells reached 70–80% confluence. The thymidine incorporation experiments were conducted according to the method described by Gabelman and Emerman (1992). The cells were incubated in the medium in the presence or absence of TCEP for 24 h and pulsed with 1 μCi of [methyl-^{3}H] thymidine for one hour at 37 °C. The cells were then washed twice with PBS, fixed in 10% trichloroacetic acid (TCA) at room temperature for 15 min, and then washed twice in 5% TCA. The acid-insoluble material was dissolved in 2 N NaOH at room temperature, and the level of radioactivity was determined using a liquid scintillation counter (model LS 6500, Beckman Instruments, Fullerton, CA). All experiments were performed in triplicate, and values were converted from absolute counts to percentage of control to allow for comparisons between the experiments. The number of viable cells was determined using a hemocytometer using a 0.4% (wt./vol.) trypan blue solution.

2.5. Ion and non-ion uptake assay

After chemical exposure for 24 h, the medium was discharged from the culture plate and the plate with monolayer of PTCs cells

Table 1
Characteristics of the eight selected waterborne micropollutants found in reclaimed water.

Micropollutants	CAS number	Category	Concentrations in reclaimed water	Reference
DEET	134-62-3	Insecticide	ND[b] – 0.0037[a]	Loraine and Pettigrove (2006), Lee and Rasmussen (2006)
Atenolol	29122-68-7	Pharmaceutical	4.0(E-5)[a] – 4.4(E-4)[a]	Ternes et al. (2003), Vieno et al. (2006)
Caffeine	58-08-2	Pharmaceutical	2.0(E-6)[a] – 0.022[a]	Spongberg and Witter (2008), Gagné et al. (2006)
Diclofenac	15307-79-6	Pharmaceutical	<1.0(E-6)[a] – 0.0055[a]	Kim et al. (2007), Bendz et al. (2005)
Ibuprofen	15687-27-1	Pharmaceutical	<1.0(E-6)[a] – 0.0014[a]	Loraine and Pettigrove (2006), Kim et al. (2007)
Sodium diatrizoate	737-31-5	Pharmaceutical	0.0033[a] – 0.0057[a]	Ternes et al. (2003), Ternes et al. (2007)
Sulfamethoxazole	723-46-6	Pharmaceutical	ND[b] –4.07(E-4)[a]	Spongberg and Witter (2008), Kim et al. (2007)
TCEP	115-96-8	Flame retardant	6.0(E-7)[a] – 0.0026[a]	Kim et al. (2007), Andresen and Bester (2006)

[a] mg L^{-1}.
[b] Not detected.