A TELEOST IN VITRO REPORTER GENE ASSAY TO SCREEN FOR AGONISTS OF THE PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS

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Abstract—Several contaminants detected in aquatic ecosystems are agonists of peroxisome proliferator-activated receptors (PPARs). Peroxisome proliferator-activated receptors interact with the retinoid X receptor (RXR) to activate the transcription of genes that control a variety of physiological functions. We cloned and sequenced partial cDNA fragments of rainbow trout (Oncorhynchus mykiss) PPARα and PPARβ from rainbow trout (rt) gill1-W1 cells, a cell line derived from rainbow trout gills; predicted amino acid identities are 77% and 82% compared with their respective human homologs and 83 to 88% and 91 to 98% identical to fish homologs. A reporter gene assay was developed by transfecting rt-gill1-W1 cells with a reporter gene construct containing the peroxisome proliferator response element (PPRE) of the rat liver 3-ketoacyl-CoA thiolase B (TB) gene, which drives luciferase expression. Agonists of both PPARα (WY14,643 and gemfibrozil) and PPARβ (beza®brate) induced luciferase activity, while rosiglitazone, a PPARγ agonist, was not effective. The fibrate drug, beza®brate increased luciferase activity in a dose-dependent manner, but addition of 50 nM 9-cis-retinoic acid to the transfected rt-gill-W1 cell culture maximized the sensitivity of the assay so that beza®brate could be detected at concentrations as low as 6 nM. Extracts from treated domestic wastewater containing fibrate drugs induced luciferase activity in the transfected gill cells. This in vitro reporter gene assay shows promise as a rapid and sensitive technique for screening environmental samples for PPAR-active substances.

Keywords—Fibrate Peroxisome proliferator response element Retinoid X receptor

INTRODUCTION

A variety of environmental contaminants are agonists of the peroxisome proliferator activated receptor (PPAR). Peroxisome proliferator-activated receptors are transcription factors that control key cellular functions, including lipid metabolism, inflammation, and cell differentiation [1,2]. In sensitive rodent models, chronic exposure to PPAR-active substances causes cellular peroxisome proliferation, elevates enzymatic markers of oxidative stress, and is linked to the development of hepatic tumors [3,4]. Peroxisome proliferation is now recommended as a biomarker of exposure to PPAR-active compounds in environmental pollution assessment [5]. Many compounds that are known PPAR agonists are contaminants in the aquatic environment, including phthalate plasticizers [6] and fibrate drugs [7]. These compounds could affect the health of aquatic organisms, including fish, and a number of studies report that fish respond at the cellular level to PPAR agonists [8,9].

Detection of PPAR-active substances using analytical techniques (i.e., liquid chromatography-mass spectrometry) is expensive and time consuming. Cell-based reporter gene assays represent alternative methods to detect PPAR-active substances. Reporter gene assays with fish cells have been developed to determine the presence of estrogenic substances in the environment [10,11]. In this study, our goal was to develop a teleost reporter gene assay to determine the presence of PPAR-active compounds in environmental samples.

A variety of pharmaceuticals have been reported in aquatic ecosystems [12,13]. Members of the fibrate class of drugs, including clofibric acid, gemfibrozil, and bezafibrate, are present in treated domestic wastewater at concentrations ranging from high nanograms per liter to low micrograms per liter concentrations, and in surface waters at low nanogram per liter concentrations [7,14,15]. Fibrate drugs are clinically prescribed to reduce serum triglycerides and do so by acting as peroxisomal proliferators [3,4]. A teleost reporter gene assay would be especially useful for detecting the presence of fibrate drugs in samples of domestic wastewater or surface water. However, the assay would need to be sensitive enough to detect these compounds at nanogram per liter (i.e., nM or ppb) concentrations.

Peroxisome proliferator-activated receptors are members of the nuclear hormone receptor superfamily and include at least three isoforms identified in mammals as PPARα, PPARβ(δ), and PPARγ [16]. In mammals and fish, expression levels of PPARs differ between cell types [1,17,18]. Peroxisome proliferator-activated receptor α is expressed primarily in tissues with a high degree of fatty acid oxidation, including liver, heart, skeletal muscle, brain, and intestine, but slightly or not at all in other tissues. In contrast, PPARγ is largely expressed in adipose tissue, whereas PPARδ (also known as PPARδ) is ubiquitously distributed. The PPARs are ligand-regulated transcription factors that control gene expression by binding to specific peroxisome proliferator response elements (PPREs) within the promoter region of sensitive genes [16]. A second nuclear receptor, the retinoid X receptor (RXR), is also required to form a heterodimeric complex with PPAR to fully activate gene transcription [1,16,19]. A number of sequences of the three PPARs have been re-

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Temperature cycling proceeded as follows: 1 cycle at 94°C for 30 s and 35 cycles at 94°C for 25 s, 50°C for 30 s, and 68°C for 60 s, followed by 68°C for 120 s. The PCR products were then subjected to gel electrophoresis on a 1% agarose gel and stained with ethidium bromide to confirm the predicted sizes before extraction (Ultrafree-DA DNA extraction kit; Millipore, MA, USA) and insertion into the TOPO TA cloning vector. Positive colonies were selected following standard methods from the manufacturer. The DNA was isolated (Wizard plus SV miniprep; Promega) and analyzed using the LI-COR Automated DNA Sequencing System (Model4200) with simultaneous bidirectional sequencing by the Canadian Molecular Research Services (Ottawa, ON, Canada). At least three clones were sequenced to obtain the reported consensus partial sequences for trout PPARα and PPARβ.

Plasmid constructs

The PGLuc is a luciferase expression vector containing the β-globin promoter [23]. This served as a control in initial experiments. The PGLuc-TB was created by inserting synthetic double-strand oligonucleotide representing the putative PPRE of the rat 3-ketoacyl-CoA thiolase (TB) gene (top strand 5′AGCTCTCTAGAGACAGCTCTTTGAAAACCAGCTT3′) between the HindIII and BamHI sites of PGLuc [24]. This response element is referred to as PPRE1 in a later publication [25]. Both plasmid constructs were kindly provided by N. Latruffe and V. Nicolas-Francés, Université de Bourgogne, France. After culturing in Escherichia coli, plasmid DNA was isolated using the HiSpeed maxi kit (Qiagen; Mississauga, ON, Canada). The pSV-β-galactosidase control vector (Promega E1081) was cotransfected with PGLuc or PGLuc-TB and served as an internal positive control.

Cell culture, DNA transfection, and luciferase assay

The rt-gill-W1 cells were cultured in Leibovitz’s L-15 medium with 10% FBS at 20°C without CO₂ or antibiotics for 5 to 7 d in a 250-ml flask. Cells were seeded in 6-well plates at 1 × 10⁵ cells per well in L-15 medium and 1% FBS. Cells were allowed to attach overnight, and the medium changed the next morning. At 90 to 95% confluence (~4–5 d in culture), cells were transfected with the DNA-Lipofectamine complex using a 1:3, weight:volume ratio of DNA:Lipofectamine 2000. Five hundred microliters DNA-Lipofectamine complex was added to each well containing cells and 1 ml of fresh 1% FBS L-15 medium. The plate was mixed by gentle rocking. Transfection was allowed to proceed for 6 h at 20°C, the medium removed and 2 ml of fresh 1% FBS L-15 were added to each well and incubation continued overnight. On the next morning, drug solutions were freshly prepared and the cells were exposed to the drugs or dimethyl sulfoxide (DMSO) control vehicle (1 μl/ml medium) for an additional 72 h at 20°C. Thereafter, the cells were rinsed twice with cold phosphate-buffered saline, harvested into 200 μl reporter lysis buffer and centrifuged after two freeze-thaw cycles in liquid N₂. All exposures were performed in triplicate and in three independent transfection experiments. The samples were stored at ~80°C until assayed for transgene expression; luciferase and β-galactosidase activities were measured according to the manufacturer’s protocol. Samples (20 μl) were injected automatically into 100 μl luciferase assay reagent and light emission measured for 10 s using a Monolight 2010 Luminometer (Analytical Luminoscience Laboratory, San Diego, CA, USA). The β-galactosidase activities were determined by using O-nitrophenyl-β-
D-galactopyranoside as a substrate and product measured by spectrophotometry at a wavelength of 600 nm.

**Effects of 9-cis retinoic acid on bezafibrate-induced luciferase activity**

The rt-gill-W1 cells transfected with PGLuc-TB were exposed to 100 or 50 nM RA in DMSO (1 μL/mL) in combination with a range of concentrations of bezafibrate (0.006, 0.063, 0.625, 3.125, 6.25, 12.5, 25, 50, 100 μM). All other incubation conditions, cell harvesting, and luciferase determinations were as reported above.

**Expression of PPARα and PPARβ in rt-gill-W1 cells**

Cells were cultured and transfected under standard conditions as described above. Cells were seeded on 100-mm plates at 1 × 10⁷ cells per plate. Following an overnight incubation (20°C), the cells were exposed to bezafibrate or wastewater treatment plant (WWTP) effluent in the presence of 50 nM RA for an additional 72 h. The cells were washed twice with cold 1 × phosphate-buffered saline and harvested using Trizol reagent to extract total RNA. The quantification of gene expression was conducted using reverse transcriptase (RT)-PCR and PCR products were detected using the conditions noted above. Trout β-actin was amplified using the same PCR conditions as for the PPARs except the cycle number was 27 and the following primers were used: forward 5′-TTG GAT GGT GGG AAT GGG TCA-3′; reverse 5′-TTT GAT GTC ACG CAC GAT TTC C-3′.

**Response of rt-gill-W1 cells to extracts from effluents of wastewater treatment plants**

Samples of treated effluent were collected from WWTP in the Canadian cities of Peterborough, Ontario, and Calgary, Alberta, on April 24, 2002, and October 8, 2002, respectively. Acidic drugs, including fibrate compounds, were extracted from 250-mL aliquots of the samples by solid phase extraction and analyzed by liquid chromatography tandem mass spectrometry, as described by Miao et al. [26]. The concentrations of acidic drugs in the effluent sample from the Peterborough WWTP were previously reported in Metcalfe et al. [14], but the concentrations in the Calgary WWTP sample have not been previously reported. Levels of bezafibrate and gemfibrozil in WWTP effluents in Canada and Europe are typically in the nanomolar (i.e., ng/L or ppb) range. Concentrations of bezafibrate and gemfibrozil in the acidic extract prepared from the sample of Peterborough WWTP effluent were 6 nM and 5.9 nM, respectively [14]. In the acidic extract from the Calgary WWTP effluent, bezafibrate and gemfibrozil concentrations were 1.9 nM and 3.9 nM, respectively (C. Metcalfe, Trent University, Peterborough, ON, Canada, unpublished data). Neutral drugs were extracted from 250-mL aliquots of the samples using solid-phase extraction techniques and analyzed by liquid chromatography-mass spectrometry as described by Metcalfe et al. [14]. No fibrate drugs were detected in the neutral extracts. All extracts were concentrated to a volume of 0.25 mL in methanol (i.e., concentrated by 1000×) and were stored at 4°C until testing. At the time of the experiment, the methanol was evaporated to dryness under a stream of nitrogen at room temperature and the extract was redissolved in an equivalent volume of DMSO. Transfected cells were exposed to a 1-μL volume of extract per milliliter of L-15 medium (i.e., diluted by 1000×) or to 1 μL of DMSO (control) in the presence of 50 nM RA, as described above. As a positive control, cells were also exposed to a 6 nM concentration of bezafibrate. Exposure concentrations in vitro were equivalent to those in the original WWTP effluent samples.

**Statistical analysis**

All data are expressed as relative luciferase activity (fold increase above basal control values). Levels of β-galactosidase were used to correct luciferase activity for between-experiment variations in transfection efficiency. Data were analyzed by the analysis of variance procedure as described in Statistical Analysis Systems followed by Tukey’s student range test (SAS 9.1; Cary, NC, USA) for each set of experiments. Differences at p < 0.05 were considered statistically significant.

**RESULTS**

**Cloning of PPAR subtypes from rt-gill-W1 cells**

Partial cDNAs for PPARα (342 base pairs; GenBank Accession number AY494835) and PPARβ (573 base pairs; Accession number AY356399) were cloned and sequenced from rt-gill11-W1 cells using RT-PCR. Comparisons to other known PPAR sequences in GenBank using BLASTX (www.ncbi.nlm.nih.gov/blast) confirmed their identity as trout PPARα and PPARβ. The trout sequences shared 77 and 82% amino acid identity to the human and approximately 85 and 92% with other fish homologs of PPARα and PPARβ, respectively. Using the cloning strategy noted in the Materials and Methods section, we were unable to clone a PPARγ from the rt-gill-W1 cells.

**Response of promoter activity to specific PPAR agonists**

A construct containing the rat liver TB-PPRE or the original PGLuc construct without the PPRE (as control) linked to a luciferase reporter gene was transfected into cultures of the rt-gill-W1 cell line. The transfected cell line was exposed to various PPAR agonists. When compared with the respective controls, promoter activities increased up to eightfold in response to the PPARβ agonist, bezafibrate (Fig. 1A and B), while the PPARα agonists, WY14,643, and gemfibrozil only increased activities twofold (Fig. 1A). The PPARγ agonist, rosiglitazone at 1 μM, did not increase luciferase activity relative to the response in the DMSO control (Fig. 1B). Higher rosiglitazone concentrations significantly impaired cell viability and were not used.

**Dose-dependent response of promoter activity to bezafibrate**

In further tests with bezafibrate, luciferase activity increased in a dose-dependent manner. Cells were treated with 3.125, 6.25, 12.5, 25, 50, and 100 μM bezafibrate for 72 h, a time period selected to give maximum activation. Concentrations less than 12.5 μM induced no response, whereas concentrations of 12.5 μM or higher increased activity by 3- to 25-fold relative to the DMSO control (Fig. 2). These results show that the assay was only moderately sensitive to bezafibrate. As PPARs must heterodimerize with RXR for full activation of transcription [1,16,27], we reasoned that an optimum response by the rt-gill-W1 cells may require the addition of RA.

With the addition of 100 nM RA to the medium, 25 μM bezafibrate increased luciferase activity by approximately twofold compared with the same concentration of bezafibrate alone and shifted the concentration-response curve to the left (Fig. 2).
Agonist-induced promoter activities of transfected rainbow trout (rt)-gill-W1 cell cultures. Two plasmid constructs, PGluc (control) and PGluc-TB, containing the peroxisome proliferator response element of the rat liver 3-ketoacyl-CoA thiolase B and the luciferase reporter gene, were transfected into cultures of rt-gill-W1 cell line as described in the Materials and Methods section. (A) The transfected cell lines were exposed for 72 h to various peroxisome proliferator-activated receptor (PPAR) agonists: WY14,643 (WY) and gemfibrozil (GEM) for PPARα and bezafibrate (BZ) for PPARβ. (B) In a second experiment, the transfected cell lines were exposed for 72 h to BZ and the PPARγ agonist rosiglitazone (RO). Dimethyl sulfoxide (DM) served as the control treatment. Each agonist was applied at 25 μM, except RO, which was applied at 1 μM. Luciferase activities were measured using the luciferase assay system (Promega, Madison, WI, USA). Data are expressed as the mean ± standard error of three independent experiments. The * indicates significant differences (p < 0.05) compared with DM control group.

In the presence of 100 nM RA, all bezafibrate concentrations except 0.063 μM significantly elevated luciferase activities above controls (p < 0.05). This RA-mediated increase in sensitivity to bezafibrate permitted detection of concentrations of bezafibrate in the 0.063 to 0.625 μM range. However, these concentrations of bezafibrate are approximately 10 to 100 times higher than the concentrations reported in WWTP effluents [7,14,15]. In addition, the presence of 100 nM RA alone in the test medium induced a small but significant increase in basal luciferase activity (Fig. 3B) that could potentially mask the response to low concentrations of bezafibrate. Therefore, we reasoned that lower RA concentrations may enhance sensitivity without affecting the basal response. Indeed, 50 nM RA alone did not affect basal luciferase activity (Fig. 3B). By reducing the concentration of RA to 50 nM, we observed that bezafibrate concentrations as low as 0.006 μM (6 nM) significantly increased luciferase activity above controls (Fig. 3B).

Response of promoter activity to WWTP effluent samples

Acid and neutral extracts prepared from treated WWTP effluents from the Canadian cities of Peterborough and Calgary were tested for their effects on TB promoter activity. In this experiment, exposure to 50 nM RA alone or the positive control (6 nM bezafibrate) alone did not elevate promoter activity relative to the DMSO control (Fig. 4). However, exposure to the combined treatment of 50 nM RA and the positive control induced promoter activity greater than fourfold (Fig. 4), confirming previous results (Fig. 3). The acid extract (1 μl/ml) alone from either the Peterborough or the Calgary WWTP did not increase luciferase activity. However, in the presence of 50 nM RA, luciferase activity was increased greater than threefold for the acid extract from the Calgary WWTP and by approximately fivefold for the acid extract from the Peterborough WWTP (Fig. 4). The neutral extracts prepared from the WWTP samples did not affect luciferase activities (Fig. 4).

Expression of PPARs in rt-gill-W1 cells

Semiquantitative PCR was performed to determine PPARα and PPARβ mRNA transcript levels to provide a possible basis for RA-induced increased responsiveness to bezafibrate and WWTP extracts. Under the culture and PCR conditions described, PPARα and PPARβ mRNA were slightly expressed in control cells exposed to 50 nM RA or 6 nM bezafibrate alone (Fig. 5). However, cotreatment with bezafibrate or an acidic WWTP extract with RA increased expression of both PPARα and PPARβ transcripts (Fig. 5). In contrast, there were no effects of these treatments on levels of β-actin mRNA (Fig. 5).

DISCUSSION

The data presented indicate that we have developed a highly sensitive in vitro assay using rt gill cells that can be used to...
Fig. 3. 9-cis-Retinoic acid (RA) enhances promoter activities in the presence of bezafibrate (BZ). The PGLuc-thiolase B construct was transfected into the rainbow trout (rt)-gill-W1 cell line as described in Figure 1. Data are expressed as the mean ± standard error of three independent experiments. (A) Cultures were exposed to various concentrations of bezafibrate (0.063, 0.625, 6.25, 12.5, and 25 μM) in the presence or absence of 100 nM RA with dimethyl sulfoxide (DMSO) as the control treatment. Luciferase activities were assessed after 72 h of treatment. The * indicates significant differences at \( p, 0.05 \) compared with each DMSO control group of the same treatment, BZ or BZ + RA (100 nM). (B) Relative luciferase activity was assessed in PGLuc-TB transfected rt-gill-W1 cultures at three BZ concentrations (0.006, 0.063, and 0.625 μM) in the presence of either 50 or 100 nM RA. The * indicates significant differences \( (p < 0.05) \) between 50 nM RA and 100 nM RA at the same concentration bezafibrate.

Fig. 4. Wastewater treatment plant effluent (WWTP) extracts induce PGLuc-thiolase B (TB) promoter activities. The PGLuc-TB was transfected into the rainbow trout (rt)-gill-W1 cell line and cultures were exposed to acid or neutral extracts of the final WWTP effluent from sites in Peterborough, Ontario, and Calgary, Alberta, Canada. Extracts of 6 nM bezafibrate (BZ) were applied to cell cultures with or without 50 nM 9-cis-retinoic acid (RA). Dimethyl sulfoxide (DMSO) served as control. A water control is also shown and did not differ from the DMSO control values. Luciferase activities were assessed after a 72-h exposure period. Data are expressed as mean ± standard error of three independent experiments. The * indicates a significant difference \( (p < 0.05) \) from DMSO control.

screen for PPAR agonists in extracts of environmental samples. These chemicals activate a mammalian PPRE-driven luciferase reporter gene system. Our results demonstrate that this assay can be used to detect fibrates in WWTP effluent extracts at environmentally relevant concentrations.

Cloning and sequencing of rt PPARα and PPARβ from rt-gill-W1 cells indicated that these are highly conserved sequences among vertebrates. The predicted amino acid sequence of trout PPARα is 77 to 78% identical to the human (GenBank Accession number AAA36468) and Xenopus laevis (Accession number P37232) and 83 to 88% with respect to Carassius auratus (goldfish; Accession number AAO62961) and Pleuronectes platessa (Accession number CAD62447) PPARα. Similarly, trout PPARβ is 82% identical to human (Accession number A45360) and 91 to 98% with respect to a range of fish species (Atlantic salmon, Salmo salar, Accession number CAC95232; Pleuronectes platessa, Accession number CAD62448; Japanese medaka, Oryzias latipes, Accession number AAL59881; zebrafish, Danio rerio, Accession number AAK76392) PPARβ. However, we were unable to detect expression of PPARγ in rt-gill-W1 cells. The high conservation of PPARα and PPARβ suggests that PPAR agonists active in mammalian models may interact with fish PPARs to exert biological effects [5].

Initial drug screens indicated that both PPARα and PPARβ agonists enhanced luciferase expression in gill cells transfected with a construct containing the putative PPRE of the rat liver TB gene [1,28]. In the absence of retinoic acid supplementation, we found that bezafibrate was most active, followed by gemfibrozil and WY14,643, which were similar in effect. Bezafrate is a strong PPARβ agonist, but with weak affinity for the PPARα and no affinity for PPARγ [29]. However, the PPARγ short-form transcript was increased in Atlantic salmon hepatocytes incubated with bezafibrate [30], indicating that agonist affinities may be receptor- and species-dependent. Interestingly, Atlantic salmon PPARγ cDNA sequence was used to design the PPARγ primers used in this study, adding evidence in support of the absence of this PPAR subtype in the rt-gill-W1 cell line. The PPARγ agonist rosiglitazone was not active and was not examined further. Both gemfibrozil and WY14,643 are strong PPARα agonists also having some affinity for PPARγ [29]. As originally reported [1], the PGLuc-TB construct responds to WY14,643 when transfected into mammalian COS-7 cells, suggesting that the PPRE1 of the rat thiolase B gene interacts with PPARα. However, in the same study, when a mouse PPARα was cotransfected into cells, no
have reported activations of PPARs in fish systems (see [5]). The dose-response studies we conducted in the absence of RA indicated that bezafibrate increased luciferase up to 25-fold.

Our original objective was to develop a rapid and sensitive in vitro assay for the detection of PPAR-active substances. After having established optimal transfection conditions and responses to the various PPAR agonists, we focused on increasing the sensitivity of this system. Given that many studies in various mammalian cell systems have demonstrated that PPARs interact with RXR [16,27], we reasoned that the sensitivity of our assay could be increased in the presence of the RXR agonist, RA. While a high concentration of RA (i.e., 100 nM) enhanced the response to bezafibrate, increases in basal luciferase activity appeared to reduce assay sensitivity. We found that supplementing the incubation medium with 50 nM RA permitted detection of nanomolar concentrations of bezafibrate. The likely reason for this increase in sensitivity is the induction of PPARβ, as demonstrated by an increase in PPARβ mRNA levels when cells were cotreated with RA and bezafibrate. This was also the case for PPARα, as mRNA levels also increased in rt-gill-W1 cells exposed to both RA and bezafibrate. It is not possible to exclude a role for PPARα in the increased cellular sensitivity to bezafibrate, as it does have a weak affinity for PPARα. However, a mouse PPARα does not appear to enhance the transcriptional activity of the rat TB PPRE1 [24]. Moreover, we do not know if the FBS added to L-15 medium contained RA, but perhaps it was very low and therefore, supplementing with RA is required to induce higher PPRE:RXR heterodimer activity.

The acidic extracts from WWTP effluents increased the activity of the rat thiolase B PPRE construct transfected into rt-gill-W1 cells, demonstrating that PPAR-active compounds are present in these effluent extracts. Although the degree of induction in the assay was consistent with the relative concentrations of bezafibrate and gemfibrozil in the extracts, we cannot conclude that bezafibrate and gemfibrozil were solely responsible for this induction because it is possible that other PPAR-active compounds were present in the extracts. For instance, the nonsteroidal anti-inflammatory drug ibuprofen was present in the acidic extracts from the Peterborough and Calgary WWTPs at concentrations of 9 nM and 8.4 nM, respectively ([14] and C. Metcalfe et al., unpublished data). At a concentration of 100 μM, ibuprofen was recently shown to produce a twofold increase in the activity of a rat acyl CoA oxidase PPRE-luciferase construct cotransfected with human PPARβ/δ and human RXRα1 into monkey CV-1 fibroblasts [31]. In this assay, bezafibrate and carbacyclin were also moderately active in the micromolar range, increasing luciferase activity up to fourfold. We did not study the same rat acyl CoA oxidase PPRE-luciferase construct as Kojo et al. [31] and therefore, comparisons between the assays must be indirect. However, in our assay, 100 μM bezafibrate alone induced luciferase activity by 25-fold, compared with a maximum of threefold in the study by Kojo et al. [31]. Moreover, we did not cotransfect our cells with the reporter gene, PPARs and RXRs. These differences suggest that our assay is highly responsive to bezafibrate and other PPAR-active compounds.

We have developed a sensitive reporter gene assay with a fish gill cell line that shows promise as a screening tool for PPAR agonists in environmental samples, including detection of fibrate drugs. There are few studies of the biological effects of fibrate drugs on fish, but we recently showed that doses of gemfibrozil equivalent to human therapeutic doses inhibited enhanced activity was noted. This led the authors to suggest that either another PPRE was involved or that this one in combination with other regulatory elements led to induction [24]. More recently, this same research group located a second PPRE:RXR heterodimer activity. Therefore, comparisons between the assays must be indirect.

![Image](https://example.com/image.png)

Fig. 5. Expression of peroxisome proliferator activated receptor (PPAR)β (A) and PPARα (B) in rainbow trout (rt)-gill-W1 cells. The rt-gill-W1 cells were seeded onto 100-mm plates at 1 × 10^6 cells per plate in Leibovitz’s L-15 W/L-glutamine medium with 1% fetal bovine serum. Following an overnight incubation (20°C), the cells were treated with 9-cis-retinoic acid (RA), bezafibrate (BZ), RA plus BZ, or an acid extract of a wastewater treatment plant effluent (from Peterborough, ON, Canada as in Fig. 4) for an additional 72 h. Dimethyl sulfoxide (DMSO) served as the control treatment. The cells were washed twice with cold phosphate-buffered saline and harvested using Trizol reagent to extract total RNA. The quantification of PPARα, PPARβ, and β-actin mRNA levels was carried out by reverse transcriptase polymerase chain reaction (RT-PCR), and PCR products were detected as described in the Materials and Methods section. The PCR products were subjected to gel electrophoresis on a 1% agarose gel and stained with ethidium bromide. L indicates the 1 kilobase plus DNA ladder (Invitrogen; range from 12 kilo- to 100 base pairs [bp]).
expression of growth hormone and secretogranin-II and decreased plasma triglyceride levels in the goldfish [32]. Furthermore, we have shown that environmentally relevant concentrations of waterborne gemfibrozil accumulate in the blood plasma and decrease testosterone levels in male goldfish [33]. Given the conservation of both the PPARα and PPARβ genes noted here and their physiological importance, it is reasonable to hypothesize that there will be biological effects in fish exposed to human pharmaceuticals and other PPAR agonists. Therefore, a teleost reporter gene assay for PPAR-activated substances could be used to evaluate the potential for environmental impacts in contaminated aquatic ecosystems.

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