Gene expression and functional analysis of toxicologically important ABC transporters in rainbow trout (*Oncorhynchus* mykiss) tissues and cell lines

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Introduction

The important role of ABC transporters in tissue defense is reflected by their tissue distribution. Numerous studies revealed the highest expression of transporters from the ABCB, ABCC and ABCG subfamily in important physiological and/or pharmacological barriers of mammals[1-3]. On the contrary, detailed studies of ABC transporter genes expression and function in aquatic organisms are scarce[4]. Consequently, the main goals of our study were: (1) gene expression analysis of ABC transporters (abcb1, abcb11, abcc1-5 and abcg2) implicated in disposition of various xenobiotics in rainbow trout tissues (brain, gills, liver, kidney, gonads, distal and proximal intestine); (2) gene expression and functional analysis of target ABC transporters in rainbow trout cell lines originating from different tissues (liver: RTL-W1, R1; hepatoma: RTH-149; gonad: RTG-2; gill: RTgill-W1; gut: RTgut and brain: RTbrain); and (3) obtaining the first insights into transcriptional regulation of target ABC transporters in liver cell line (R1) after 24 h exposure to various xenobiotics.
Gene expression of target ABC transporters was measured using relative qPCR with SYBR green. Expression of target genes was normalized to the housekeeping gene (EF1α and 18S rRNA) using q-Gene[5] and Pfaffl[6] equations. Functional assays in trout cell lines were preformed using various model inhibitors and fluorescent substrates.

Experimental procedure

Total RNA was isolated from whole trout liver, gonads, proximal and distal gut, brain, gills and kidney and 7 trout cell lines: RTL-W1, R1, RTH-149, RTG-2, RTgill-W1, RTgut, RTbrain and reverse transcribed to cDNA.

Gene sequences were cloned in our labs. Specific primers were designed for real time PCR absolute quantification using ABI primer express 2.0 software.

Gene expression was quantified using Sybr green absolute quantification. Data analysis was done with ABI SDS 2.0 software and GraphPad 5.0 software. Folds were calculated according to qGene and Pfaffl equations.

Functional assays were done on all cell lines using model fluorescent substrates and inhibitors.

Induction experiments R1 cell line was chosen as the most suitable cell line. The range of chemicals was picked according to literature, 24 h toxicity was established and the max nontoxic concentration (10-100μM range) chosen for 24h exposure period with cell seeding density of 0.8×10^5 cells/cm².

Results

a) Tissue RNA distribution (Figure 1)

- Constitutive expression of al tested transporters throughout the tissues with high expression of abcb1, abcc3 and abcg2
- Abcb11 expression is tissue specific for liver
- Low transporters expression in gills

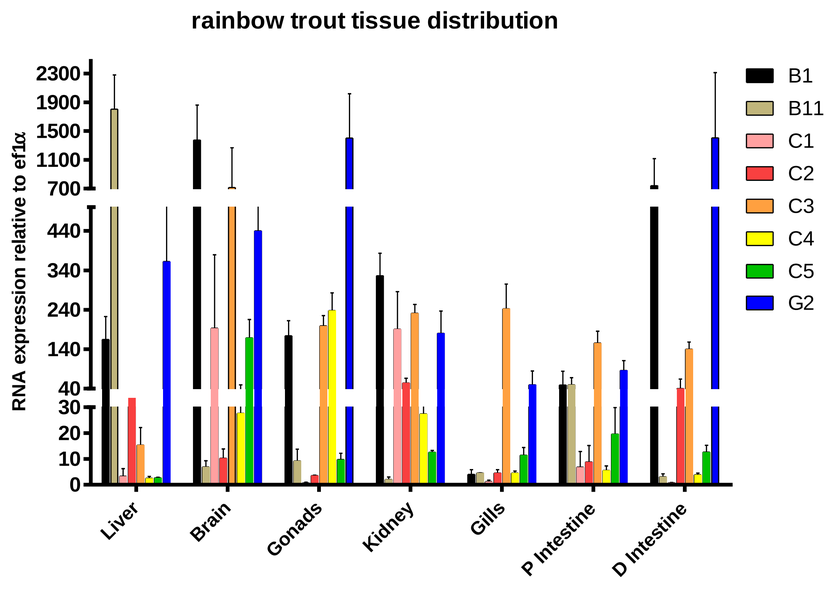

Figure 1. ***Tissue RNA distribution*** *Relative expression levels of ABC transporters abcb1, abcb11, abcc 1-5 and abcg2 in trout tissues. Expression is presented relative to the housekeeping gene ef1a RNA as mean ± SD obtained from 3 independent RNA isolations of each cell line. Ef1a RNA (ef1a) is set to 10,000 in all cell lines.*

b) Cell line RNA distribution (Figure 2)

- Constitutive expression of all tested transporters with high expression of abcc1-3 and extremely low expression of abcb1, abcb11 and abcg2

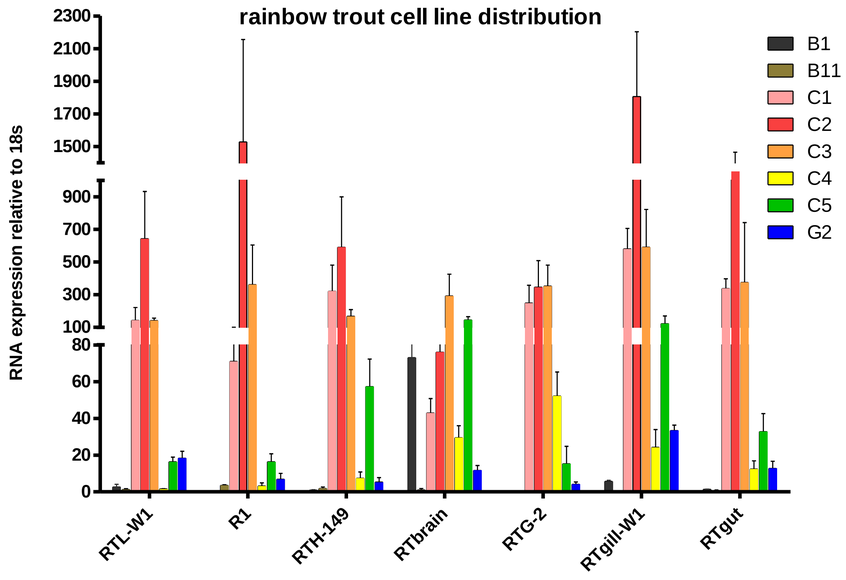

Figure 2. ***Cell line RNA distribution*** *Relative expression levels of ABC transporter genes abcb1, abcb11, abcc 1-5 and abcg2 in permanent rainbow trout cell lines RTgill-W1, RTL-W1, R1, RTgut, RTbrain, RTH-149 and RTG-2. Expression is presented relative to the housekeeping gene 18s RNA as mean ± SD obtained from 3 independent RNA isolations of each cell line. 18s RNA (18s) is set to 10,000 in all cell lines.*

c) Cell line functional assays (Table 1)

- Accumulation of fluorescent substrate proves functional activity of transporters from B and C subfamilies
- Inhibitor specificity follows the RNA expression pattern considering abcb1 and abcc 1-3 RNA expression

Table 1. ***MXR assay*** *– data are presented as ratios of maximal inhibitor effect (substrate accumulation) relative to control with Calcein-AM as fluorescent substrate*

	Calcein-AM			
	REV205	PSC 833	CsA	MK 571
RTL-W1	1.59	2.30	2.48	2.35
R1	1.74	2.45	3.00	3.31
RTH-149	1.70	2.17	2.35	2.04
RTbrain	1.84	2.09	3.08	3.58
RTG-2	2.31	2.66	2.89	2.87
RTgill-W1	1.65	2.04	2.19	2.14
RTgut	1.73	2.78	2.71	3.05

d) R1 cell line induction (Table 2)

- Short term exposures to wide range of known transporter substrates and model nuclear receptors ligands show differential effect on mRNA expression of ABC transporters with B1 being the most inducible one
- abcg2 was the only transporter which expression was not modulated by any of the tested compounds

Table 2. ***R1 cell line induction*** *– data are presented as fold increases relative to control. Folds were calculated according to Pfaffl equation.*

inducer / gene	B1	B11	C1	C2	C3	C4	C5	G2
Doxorubicine	20.46	0.25		0.18	0.18		0.18	
As2O3	8.68	0.15		3.05				
Bilirubin								
Cisplatinum	2.67							
Clofibrate	2.42			2.21	3.53	2.11		
TCDC	2.10	4.59	3.34		0.49	2.01		
Oltipraz	2.33	5.19	2.43		0.49	2.51		
Taurocholate	3.16	4.37						
Clotrimazol	5.38	2.69						
tBHQ	20.17		0.48	5.58		2.59		
Carbamazepine								
PCN	3.55							
Dexamethasone								
Rifampicin	4.45	3.31		0.45	2.72			
Vinblastine	4.16		0.25	0.19			0.32	

FOLD	Regulation	Strength
< 0.2	down	strong
0.2 - 0.5		midium
0.5 - 2		no effect
2 - 3	up	weak
3 - 5		midium
5 - 10		strong
>10		extremely strong

Conclusions

1. Significant decrease in RNA expression ratio of abcb1 and abcg2 compared to abcc's in cell lines compared to respective tissues.
2. Functional assays show correlation between inhibitor specificity, protein activity and gene expression for abcb1 and abcc1-3.
3. R1 cell line induction experiments show that abcb1 is transporter with highest induction potential.

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