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COMPARATIVE ANALYSIS OF GENE EXPRESSION IN BRAIN, LIVER, SKELETAL MUSCLES, AND GILLS OF ZEBRAFISH (*DANIO RERIO*) EXPOSED TO ENVIRONMENTALLY RELEVANT WATERBORNE URANIUM CONCENTRATIONS

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Abstract—The effects of waterborne uranium (U) exposure on gene expression were examined in four organs (brain, liver, skeletal muscles, and gills) of the zebrafish (*Danio rerio*). Adult male fish were exposed to three treatments: No added uranium (control), 23 ± 6 μg U/L, and 130 ± 34 μg U/L. After 3, 10, 21, and 28 d of exposure and an 8-d depuration period, gene expression and uranium bioaccumulation were analyzed. Bioaccumulation decreased significantly in liver during the depuration phase, and genes involved in detoxification, apoptotic mechanism, and immune response were strongly induced. Among these genes, *abcb3l1,* which belongs to the adenosine triphosphate (ATP)–binding cassette transporter family, was induced 4- and 24-fold in organisms previously exposed to 23 ± 6 and 130 ± 34 μg U/L, respectively. These results highlight the role of liver in detoxification mechanisms. In gills, at the highest uranium concentration, *gpx1a, cat, sod1*, and *sod2* genes were up-regulated at day 21, indicating the onset of an oxidative stress. Mitochondrial metabolism and DNA integrity also were affected, because *coxI, atp5f1*, and *rad51* genes were up-regulated at day 21 and during the depuration phase. In skeletal muscles, *coxI, atp5f1*, and *cat* were induced at day 3, suggesting an impact on the mitochondrial metabolism and production of reactive oxygen species. In brain, *glsI* also was induced at day 3, suggesting a need in the glutamate synthesis involved with neuron transmission. No changes in gene expression were observed in brain and skeletal muscles at days 21 and 28, although bioaccumulation increased. During the depuration phase, uranium excretion was inefficient in brain and skeletal muscles, and expression of most of the tissue-specific genes was repressed or unchanged.

Keywords—Uranium *Danio rerio* Gene expression Bioaccumulation

INTRODUCTION

Uranium (U) is a naturally occurring heavy metal and a member of the actinide series. Its concentration in ecosystems can increase according to various anthropogenic contributions, originating from the nuclear fuel cycle, farming, research laboratories, and military use of depleted uranium. It is found in aquatic systems at concentrations varying from 0.01 μg/L up to 2 mg/L, depending on the geological background [1]. The major forms are U(VI) in oxic water and U(IV) in anoxic water [2].

Mechanisms of uranium toxicity have not been studied extensively in nonhuman species, and most of the studies that have been performed relate to acute lethality data [3]. Acute toxicity assays conducted with zebrafish (*Danio rerio*) exposed to waterbone uranium showed that the 96-h median lethal concentration was 3.05 mg/L [4]. Several studies have examined uranium effects on the central nervous system. In rats, uranium crosses the blood–brain barrier and accumulates in brain [5,6], which is a target organ after acute exposure to depleted uranium [7]. Electrophysiological changes were reported in vitro in rat hippocampal slices embedded with depleted uranium fragments [8]. A recent study demonstrated that in the mammal brain, after inhalation exposure, the highest uranium concentration was found in the olfactory bulb [9]. In fish, where an intact nervous system is required to mediate relevant behaviors, such as food search, predator recognition, communication, and orientation [10,11], such high metal accumulation in the olfactory system might have dramatic consequences. In a recent study with zebrafish exposed to 100 μg/L, uranium induced a significant increase in acetylcholinesterase activity, an enzyme playing a key role in neurotransmission [12].

Additionally, like any heavy metal, uranium is able to enhance the production of free radical species [13,14]. In rainbow trout (*Oncorhynchus mykiss*) and zebrafish [12], uranium decreases the activities of enzymes involved in antioxidant defenses, such as superoxide dismutase, catalase, and gluthatione peroxidase, for fish exposed to 20 and 100 μg/L. Genotoxicity assays revealed a significant effect of waterborne uranium on DNA integrity of erythrocytes in zebrafish exposed to the same concentration range [15]. Uranyl ion (UO_2^{2+}) is able to inhibit adenosine triphosphate (ATP) and the activity of enzymes involved in energetic metabolism [16,17]. In mammals, exposure to uranium might affect xenobiotic detoxification by altering some cytochrome P450 enzyme activities [18]. Furthemore, it has been demonstrated that the multixenobiotic resistance protein was induced in gills of the freshwater clam (*Corbicula fluminea*) exposed to 80 μg/L of uranium [19].

Gene expression measurements present a relevant endpoint to reveal the initial effects of uranium on organisms at low concentrations, well below those causing adverse effects. Changes in gene response are among the most sensitive indicators of exposure to a toxicant in organisms. To our knowl-

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edge, no study of adult fish transcriptional responses to uranium contamination has been reported. The zebrafish, whose genome is entirely sequenced, is a model for gene function, developmental biology, and toxicology. The main objective of the present study was to address the question of whether uranium at low doses (set around environmental concentrations found close to uranium mining sites) is likely to modify and disturb gene expression. To achieve such a goal, 20 genes involved in several cellular toxicity mechanisms and adaptive responses were selected. The effects of uranium on neuronal mechanisms were explored through monitoring variations in the expression of genes involved in neuroinflammation (*gfap* and *cd11b*) or in the cholinergic system (*ache, vchat*, and *chat*) or the glutamatergic system (*glsI*). Genes encoding antioxidant defenses (*cat, sod1, sod2, gpx1a, gstp1*, and *hspb1*) were selected. Genotoxicity was investigated through the expression of two genes that are known to be expressed in DNA repair (*gadd45b* and *rad51*). The mitochondrial metabolism also was investigated using *coxI* and *atp5f1*. Other selected genes are involved in detoxification mechanisms (*cyp3c1l2* and *abcb3l1*) and apoptotic mechanisms (*bax*). Inflammation was considered with *il1*. The expression levels of these 20 genes were investigated in four organs after 3, 10, 21, and 28 d of exposure followed by an 8-d depuration period. The possible effects of three uranium exposure treatments were tested: No added uranium (control), 23 μg/L (97 nM), and 130 μg/L (546 nM) (referred to C1 and C2, respectively). These two exposure conditions are within the range of environmental concentrations found close to mining sites [20] or in drilled wells [21]. Furthermore, the first concentration is close to the provisional drinking water guideline value of the World Health Organization (15 μg/L) [22].

MATERIALS AND METHODS

Exposure conditions

Adult zebrafish were obtained from a French supplier (Exomarc) and acclimated to laboratory conditions for two weeks before the experiment. Male fish (body wt, 0.70 ± 0.08 g wet wt; standard length, 3.33 ± 0.14 cm) were randomly placed in 18 cages distributed in three continuous-flow-through tanks at a density of 20 fish/cage. One cage was sampled at each time point to reduce fish stress during collecting times (including time zero [i.e., the onset of the experiment]). Female fish were excluded to avoid any interference caused by reproductive processes. The system was alimented by artificial water (Ca^{2+}, 8.7 mg/L; Mg^{2+}, 1.4 mg/L; Na^{+}, 5.1 mg/L; K^{+}, 1 mg/L; Cl^{-}, 13.4 mg/L; SO_4^{2-}, 12.5 mg/L; NO_3^{-}, 9.2 mg/L; CO_3^{2-}, 0.45 mg/L). Major anion concentrations were analyzed in water samples by ionic chromatography (DX-120; Dionex); cation and uranium concentrations were measured after 2% (v/v) HNO_3 acidification by inductively coupled plasma–atomic emission spectrometry (Optima 4300DV; PerkinElmer). Throughout the experiment, water temperature was maintained at 24.5 ± 0.5°C, and pH was adjusted continuously to maintain 6.4 ± 0.2 (pH regulators; Consort R301) to optimize uranium bioavailable form concentration. Tank bottoms were cleaned every day to eliminate fish feces and food remains. Fish in each cage were fed once a day with a quantity of standard fish pellets corresponding to 2% of the fish body weight. Control fish were maintained in a noncontaminated tank. In exposure tanks, fish were contaminated with uranium by the direct route at C1 (23 ± 6 μg/L; 97 ± 25 nM) and C2 (130 ± 34 μg/L; 546 ± 143 nM). Water contamination was maintained by daily addition of uranyl nitrate solution ($UO_2(NO_3)_2{\cdot}6H_2O$; Sigma). The quantity of added uranium was adapted to compensate for the decrease in metal water concentrations over the 24-h cycle. Twenty fish per condition were removed after 3, 10, 21, and 28 d of exposure and an 8-d depuration phase. They were killed by immersion in melting ice. For each organism, the brain, liver, skeletal muscles, and gills were dissected and frozen at −80°C in RNAlater (Qiagen). For each organ, five replicates were prepared by pooling four fish. Each pool was homogenized using sterilized scalpels. Each replicate was split into two parts for genetic analysis and determination of uranium concentration.

Uranium analysis

For each replicate, the subpool dedicated to uranium analyses was dried at 50°C during 48 h and weighed (dry wt) using a microbalance (SE2; Sartorius). Samples were digested by nitric acid (1 ml of 65% HNO_3) in polypropylene tubes at 100°C for 3 h. After acidic dilution of the digests, uranium concentrations were determined by an inductively coupled plasma–mass spectrometer (quantification limit, 1 ng/L; model 810; Varian) according to French standards.

Quantitative real-time polymerase chain reaction

Total RNAs were extracted from 40 mg of fresh tissue using the Absolutely RNA real-time polymerase chain reaction (PCR) miniprep kit (Stratagene) according to the manufacturer's instructions. The quality of RNA produced was evaluated by electrophoresis on a 1% agarose–formaldehyde gel. For each exposure condition and each organ, samples were analyzed in five replicates.

First-strand cDNA was synthesized from 5 μg of total RNA using the AffinityScript™ Multiple Temperature cDNA Synthesis kit (Stratagene) according to the manufacturer's instructions. The cDNA mixture was conserved at −20°C until use in real-time PCR reactions.

The accession numbers of the 20 genes used in the present study are reported in Table 1. For each gene, specific primer pairs were determined using the LightCycler probe design software (Ver 1.0; Roche).

The amplification of cDNA was monitored using the DNA intercalating dye SyberGreen I (Roche). Real-time PCR reactions were performed in a LightCycler (Roche) and Mx3000P QPCR System (Stratagene) according to the manufacturer's instructions (LightCycler: One cycle at 95°C for 10 min and then 50 amplification cycles at 95°C for 5 s, 60°C for 5 s, and 72°C for 20 s; Mx300P QPCR System: One cycle at 95°C for 10 min and then 50 amplification cycles at 95°C for 1 min, 60°C for 1 min, and 72°C for 1 min). Each 20-μl reaction contained 2 μl of reverse-transcribed product template (0.5 μg of the retrotranscribed RNA), 1 μl of master mix including the SyberGreen I fluorescent dye (Roche), and the gene-specific primer pair at a final concentration of 300 nM for each primer.

The reaction specificity was determined for each reaction from the dissociation curve of the PCR product. This dissociation curve was obtained by following the SyberGreen fluorescence level during a gradual heating of the PCR products from 60 to 95°C. The β-actin gene was chosen as the reference gene because of its stability over time and treatment in the experiment. Relative quantification of each gene expression level was normalized to the β-actin gene expression and calculated by the delta-delta threshold cycle (C_t) method [23].

Table 1. Gene names, abbreviations, accession numbers, and specific primer pairs for the 20 genes from *Danio rerio* used in the present study

Gene name	Abbreviation	Accession no.	Primer (5′-3′)[a]
Acetylcholinesterase	*ache*	NM_131846.1	F: GTGGCAACTCGCATGGT R: AGTGCGGGCGAAATTAGC
Choline acetyltransferase	*chat*	NM_001130719.1	F: GGACTGCCATAAAAGCCCAA R: TTGGGACGACTGGACCAT
Vesicular acetylcholine transporter	*vchat*	NM_201107.1	F: TCCGTTTGGCGGAATCCT R: ACGGCGATGTACGGGTC
Glial fibrillary acidic protein	*gfap*	NM_131373.2	F: AGTACCAGGACCTGCTCAA R: ACAGTTCGCACAACTATGCT
Integrin alpha-M precursor	*cd11b*	XM_687072.3	F: ACGTGACGCTGTTTGTCG R: GCCAGCAGCACAAGTCC
Glutaminase, like	*glsl*	NM_001045044.1	F: AGGCCATGCTGAGGTTG R: CTGCCGTCTCTTTTTCGCT
β-Actin 1	*bactin*	NM_131031	F: AAGTGCGACGTGGACA R: GTTTAGGTTGGTCGTTCGTTTGA
Adenosine triphosphate (ATP)–binding cassette, subfamily B, member 3 like 1	*abcb3l1*	NM_001006594.1	F: GCAAGATAGCGATGGTGGG R: CGTTGGCTTTGCTTGCAG
Cytochrome P450, family 3, subfamily c, polypeptide 1 like 2	*cyp3c1l2*	NM_001007400.1	F: GGGTCTGTAAGAAGACTGTAGAGC R: CGAGGCCCGAGTCCAA
Heat shock protein, alpha-crystallin–related 1	*hspb1*	NM_001008615.1	F: CAGCACACACTGGCCG R: GTGATTGACATCTAGGCTGATCTT
Glutathione peroxidase 1a	*gpx1a*	NM_001007281.2	F: CACCCTCTGTTTGCGTTCC R: CTCTTTAATATCAGCATCAATGTCGATGG
Glutathione-*S*-transferase pi	*gstp1*	NM_131734.3	F: CGGATTCCTGGTTGGCG R: TGCCATTGATGGGCAGTTT
Catalase	*cat*	NM_130912.1	F: CCTGTGGGGCGTTTTG R: CGGTACGGGCAGTTGAC
Superoxide dismutase 1, soluble	*sod1*	BC055516.1	F: TGAGACACGTCGGAGACC R: TGCCGATCACTCCACAGG
Superoxide dismutase 2, mitochondrial	*sod2*	CB923500.1	F: TTCAGGGCTCAGGCTGG R: ATGGCTTTAACATAGTCCGGT
bcl2-Associated X protein	*bax*	AF231015.1	F: GGCTATTTCAACCAGGGTTCC R: TGCGAATCACCAATGCTGT
Growth arrest and DNA-damage-inducible, beta	*gadd45b*	BC059472.1	F: GCTTGTTCGTGTCTTCTGTGG R: CTTCCCGCATTCAGCGAT
RAD51 homologue	*rad51*	BC062849.1	F: TGCTGCGTCTCGCTGA R: GCCTCGGCCTCTGGTAA
Cytochrome *c* oxidase subunit I	*coxI*	NC_002333.2	F: GGAATACCACGACGGTACTCT R: AGGGCAGCCGTGTAAT
ATP synthase, H^+ transporting mitochondrial, F0 complex, subunit b, isoform 1	*atp5f1*	BC083308.1	F: GTGTGACAGGGCCTTATATGC R: CTGAGCCTTTGCTATTTTATCCGC
Interleukin-1β	*il1b*	NM_212844.1	F: GGTTGCGCAGCGGCGGATCTC R: GACCCGCTGATCTCCTTGAGT

[a] F = forward primer; R = reverse primer.

For each gene expression level, the mean value and the associated standard deviation ($n = 5$) were determined (*Supporting Information,* Table S1 [http://dx.doi.org/10.1897/08-357.S1], Table S2 [http://dx.doi.org/10.1897/08-357.S2], Table S3 [http://dx.doi.org/10.1897/08-357.S3], and Table S4 [http://dx.doi.org/10.1897/08-357.S4]). Induction factors of each gene were obtained by comparing each mean value observed in the exposed condition with that of the control condition.

Statistical analysis

Because normality (Shapiro–Wilk test) and independence assumptions for valid parametric analyses were not satisfied, significant variations in gene expression levels in the four organs were determined using the nonparametric Kruskall–Wallis test ($p < 0.05$). The statistically significant changes in expression level as compared to control are presented in Table 2. The same test was used to assess any significant effects of time and water concentration on tissue uranium data ($p < 0.05$).

RESULTS

Experimental conditions

No mortality was observed during the acclimation and metal exposure periods. Furthermore, biological (fish body wet wt) and physicochemical parameters, such as water composition, water uranium concentration, temperature, and pH, remained stable (data not shown).

Uranium bioaccumulation

Total uranium concentrations were determined in brain, liver, skeletal muscle, and gills after each sampling time for the three exposure treatments (Fig. 1). For the control fish, these concentrations were less than the detection limit in brain and skeletal muscles but reached 10 and 100 ng/g for gills and liver, respectively. Those values were found at the beginning of the experiment and remained constant until the end.

Uranium accumulation levels were 10-fold higher in liver and gills than in brain and skeletal muscles (for fish exposed to C1 and C2, respectively).

Table 2. Significant ($p < 0.05$, $n = 5$) variations in gene expression as compared to control in brain, skeletal muscles, liver, and gills from *Danio rerio* after 3, 10, 21, and 28 d of exposure to uranium at 23 ± 6 μg/L (C1) and 130 ± 34 μg/L (C2) and after an 8-d depuration phase (8d)[a]

		C1					C2				
Function	Gene	3	10	21	28	8d	3	10	21	28	8d
Brain											
Detoxification	*abcb3l1*										÷4
	cyp3c1l2	×6	×4				×5				
Oxidative stress	*hspb1*			÷3							
	gpx1a		÷4					÷3			
	gstp1		÷3								÷20
	cat		×10					×5			
	sod1										
	sod2	÷3									
Apoptosis	*bax*										
DNA repair	*gadd*		×2			÷4					÷3
	rad51										
Mitochondrial metabolism	*coxI*										
	atp5f1										
Inflammatory process	*il1b*		÷8	÷7	÷4		÷10				
Neuronal response	*ache*							×2			
	chat										
	vchat	×7									
	gfap										
	cd11b										÷100
	glsl	×19					×11				
Skeletal muscles											
Detoxification	*abcb3l1*	÷8			÷20		÷33				
	cyp3c1l2		÷4				÷6				
Oxidative stress	*hspb1*		×3								
	gpx1a										
	gstp1										
	cat	×4				÷2	×4				
	sod1										
	sod2					÷4					
Apoptosis	*bax*	÷16	÷20	÷25	÷9		÷16	÷5	÷9		
DNA repair	*gadd*										
	rad51						÷8				
Mitochondrial metabolism	*coxI*	×13	×4			÷3	×22				
	atp5f1	×3					×4				
Inflammatory process	*il1b*	÷7	÷12			÷14	÷30	÷6	÷9		
Liver											
Detoxification	*abcb3l1*					×4		×2			×24
	cyp3c1l2	÷3									×2
Oxidative stress	*hspb1*										×35
	gpx1a	÷8	÷8		×17						
	gstp1		÷100		×8		÷3				
	cat	÷4	÷12		×7				÷14		
	sod1				×5						
	sod2				×11						
Apoptosis	*bax*					×4					×10
DNA repair	*gadd*	÷8	×6		×4						
	rad51										
Mitochondrial metabolism	*coxI*		×4		×5		÷3				
	atp5f1	÷7			×4						
Inflammatory process	*il1b*					×4					×45
Gills											
Detoxification	*abcb3l1*										
	cyp3c1l2								×5		
Oxidative stress	*hspb1*										
	gpx1a			×3					×5	×3	
	gstp1										
	cat								×3		
	sod1								×3		
	sod2			×2					×7		
Apoptosis	*bax*						÷4				
DNA repair	*gadd*										
	rad51								×3	×2	×5
Mitochondrial metabolism	*coxI*								×4		×4
	atp5f1									×2	
Inflammatory process	*il1b*									×3	

[a] Empty cells are not statistically significant. × = fold-induction; ÷ = repression factor.

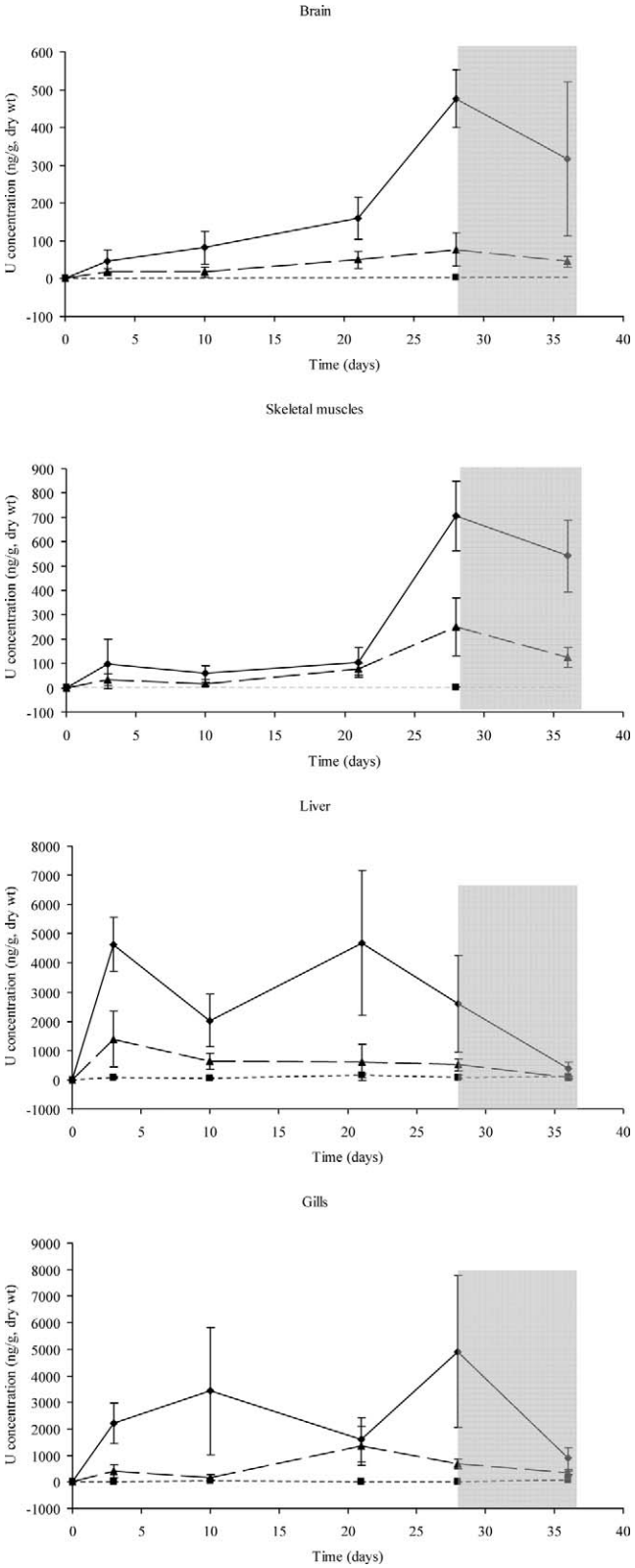

Fig. 1. Average uranium concentration (ng/g dry wt; mean ± standard deviation, $n = 5$) in the brain, skeletal muscles, liver, and gills of *Danio rerio* after 3, 10, 21, and 28 d of three exposure treatments: No added U (C0; ■), 23 ± 6 μg U/L (C1; ▲) and 130 ± 34 μg U/L (C2; ◆) and after an 8-d depuration phase (in gray).

A threefold significant increase in uranium accumulation was observed in the brain between days 10 and 21 for the C1 group (i.e., 18–49 ng/g dry wt) and between days 21 and 28 for the C2 group (i.e., 160–476 ng/g dry wt) ($p < 0.05$). A sevenfold significant increase was observed (i.e., 105–707 ng/g dry wt) between day 21 and 28 in skeletal muscles of fish exposed to C2 ($p < 0.05$). Depuration did not lead to any significant decrease in uranium concentration in these organs ($p > 0.05$).

In gills, between days 3 and 10 in the C1 group, no significant increase of bioaccumulation was measured ($p > 0.05$), whereas a significant increase was observed between days 10 and 21 ($p < 0.05$), with an eightfold increase (i.e., 173–1,345 ng/g dry wt). During the C2 exposure, no significant trend of bioaccumulation was observed ($p > 0.05$). At the depuration time, internal concentrations of uranium significantly decreased by a factor of 2 and 5.5 in organisms preliminarily exposed to C1 and C2, respectively ($p < 0.05$).

In the liver, a twofold early significant decrease appeared at day 10 for fish exposed to C2 ($p < 0.05$), followed by a stagnation until day 28. Between days 3 and 28 in the C1 group, no significant change of uranium bioaccumulation was measured ($p > 0.05$). Depuration had an impact on this organ, in which tissue concentrations of uranium decreased significantly, by a factor of six (i.e., 512 to 81 ng/g dry wt) and seven (i.e., 2,599 to 371 ng/g dry wt) for fish preliminarily exposed to C1 and C2, respectively ($p < 0.05$).

Gene expression levels

The genetic analysis evidenced a tissue-specific basal expression rate. For instance, the *abcb3/1* gene showed a higher basal expression rate in liver (*Supporting Information,* Table S3; http://dx.doi.org/10.1897/08-357.S3) and skeletal muscles (*Supporting Information,* Table S2; http://dx.doi.org/10.1897/08-357.S2) than in gills (*Supporting Information,* Table S4; http://dx.doi.org/10.1897/08-357.S4) or brain (*Supporting Information,* Table S1; http://dx.doi.org/10.1897/08-357.S1). Higher basal expression levels were found in liver as compared to the other organs, particularly for genes involved in antioxidant defenses (*sod1*). In the four tissues, *cox1* showed the highest basal expression rate. Besides these basal gene expressions, marked differences in the transcriptional response onset and intensity in response to uranium exposures appeared between organs (Table 2).

In the gills, the effects of an increase in uranium concentration were obvious, because at day 21, only *gpx1a* and *sod2* genes were up-regulated in the C1 group, whereas *cyp3c1l2, gpx1a, cat, sod1, sod2, rad51*, and *cox1* genes were up-regulated in the C2 group ($p < 0.05$). This differential gene regulation, however, is promoted by equivalent uranium concentrations within gills (i.e., 1,345 and 1,587 ng/g at day 21 for fish exposed to C1 and C2, respectively) ($p > 0.05$).

In the liver, an inverse situation occurred in which many genes (*gpx1a, gstp1, cat, sod1, sod2, gadd45b, cox1*, and *atp5f1*) were up-regulated at day 28 in the C1 group ($p < 0.05$), whereas none of them displayed differential expression in the C2 group ($p > 0.05$). At day 28, however, the liver contained fivefold more uranium in the C2 group than in the C1 group (2,600 vs 512 ng/g). In the C2 group, a strong transcriptional response could be observed only during the depuration phase. At this stage, *abcb3l1, cyp3c1l2, hspb1, bax*, and *il1* genes were up-regulated; meanwhile, uranium accumulation in the liver was similar to that observed in the C1

group at day 28 (371 vs 512 ng U/g). In the C1 group, *abcb3l1, bax*, and *il1* also were up-regulated during the depuration phase, but to a lower extent than in the C2 group (4- vs 24-fold for *abcb3l1*, 4- vs 10-fold for *bax*, and 4- vs 45-fold for *il1*) ($p < 0.05$), presumably because the uranium concentration in this tissue after 8 d of depuration had declined to 81 ng/g under C1 group versus 371 ng/g under C2 ($p < 0.05$). At early times of exposure in the liver, many genes were down-regulated in the C1 group, whereas their expression remained equal to that in the control tissue in the C2 group.

In skeletal muscles, a 3-d waterborne uranium exposure triggered an up-regulation of *cat, coxI*, and *atpb* genes and a down-regulation of *abcb3l1, bax*, and *il1* genes, whatever the contamination pressure ($p < 0.05$). At day 10, *hspb1* and *coxI* genes were up-regulated in C1 muscles but not in C2 muscles, although C2 muscles accumulated fivefold more uranium than C1 muscles (10 vs 50 ng/g). At this date, the levels of down-regulation were higher for *cyp3c1l2, bax*, and *il1* genes in C1 muscles than in C2 muscles.

In the brain, effects of uranium on gene expression were observed as early as 3 and 10 d after exposure. Brain-specific genes, such as *glsI* and *vacht*, were strongly up-regulated after 3 d, indicating an impact of uranium on the cholinergic pathway and glutamate synthesis. At day 3, *vacht* and *glsI* gene responses were higher in the C1 group than in the C2 group (sevenfold up-regulation in C1 vs basal level expression in C2 for *vacht*, and 19-fold up-regulation in C1 vs 11-fold up-regulation in C2 for *glsI*). At that time, however, C2 brains accumulated twice as much uranium than C1 brains (45 vs 18 ng/g). At day 10, the same trend was verified. *Cyp3c1l2, cat*, and *gadd45b* were up-regulated 4-, 10-, and 2-fold, respectively, in the C1 group. In the C2 group, *Cyp3c1l2* and *gadd45b* gene responses were similar to their basal expression levels and the *cat* gene was fivefold induced. The *Gstp1* and *il1* genes were down-regulated three- and eightfold, respectively, in the C1 group, compared with basal level expressions for both genes in the C2 group. At that time, however, C2 brains accumulated fivefold more uranium compared with C1 brains (82 vs 18 ng U/g) ($p < 0.05$).

DISCUSSION

During the present study, no mortality was observed, and swimming velocity seemed to be unchanged. This observation could be correlated to the middle-range water uranium concentrations. Indeed, behavioral changes were observed during acute toxicity experiments in which fish exposed to 3.05 mg/L of uranium swam in a disorientated way [4].

That the highest uranium accumulations are found in the liver and gills had been shown already by others studies [12]. This heterogeneous distribution throughout the body revealed the physiological role of organs in the uptake and transport of uranium in zebrafish.

Gills represent the main uptake route for waterborne exposures, and they could be an effective barrier to uptake by trapping uranium within the protective mucous layer, as shown previously for other divalent metals in fish [24]. Furthermore, UO_2^{2+} is able to exchange with Ca^{2+} at the surface of bone mineral crystals [25] and can concentrate in gill arches, which are rich in calcium. This could explain the higher uranium accumulation in this tissue as compared to brain or skeletal muscle during the exposure period. After a depuration phase, a significant decrease of uranium concentration in gills highlights the efficiency of uranium excretion in this tissue.

In the liver, no gene expression response could be observed above a tissue uranium concentration of 2,031 ng/g, as if high uranium concentrations inhibited liver transcriptional response, whereas a gene response was observed with only 81 ng/g at day 8 of depuration in the C1 group. The occurrence of hepatic and biliary histopathologies in lake whitefish exposed to dietary uranium could be linked to a possible biliary excretion [26]. This is consistent with the prominent role of the bile in the excretion of other metals administrated orally to fish [27,28]. Indeed, in the present study, depuration resulted in a decreased uranium concentration in the liver of fish previously exposed to both the C1 and C2 conditions. The pattern of gene expression observed during the depuration phase indicates that in this organ, several molecular pathways, such as oxidative stress, apoptosis, immune response, and detoxification, are impacted. Among genes involved in detoxification, *abcb3l1* is up-regulated 4- and 24-fold in liver of fish previously exposed to C1 and C2, respectively. The ATP-binding cassette (ABC), subfamily B, member 3 like 1, is the closest zebrafish counterpart of the mammalian multidrug resistance member, a protein using ATP as an energy source to extrude xenobiotics and metals [29]. Other members of the ABC transporter superfamily, such as the multidrug resistance–associated protein [30], the *Leishmania* LtpgpA transporter [31], and the yeast cadmium resistance factor 1 [32], are xenobiotic pumps and also are involved in metal detoxification. A recent study on the effects of waterborne cadmium exposure on gene expression in zebrafish drew a parallel between the 100-fold increase in *abcb3l1* expression and the decrease in cadmium burden in gills and a three-fold increase in *abcb3l1* expression and a stagnation of cadmium loading in muscles [33]. A gene involved in inflammatory response, *il1*, also was found to overexpress by 4- and 45-fold in the liver of fish previously exposed to C1 and C2, respectively. Interleukin (IL)-1ß is an important inflammatory cytokine and a good marker of inflammatory response [34]. It plays an important role in the host response to tissue injury and immunological reactions and is produced mainly by blood monocytes and tissue macrophages [35]. Its strong induction in fish previously exposed to C2 highlights the effects of uranium on the immune response. In the literature, data concerning uranium immunotoxicity are scarce. Nevertheless, a serial analysis of gene expression of the mouse kidney revealed that exposure to uranyl nitrate leads to an up-regulation of immunity gene expression levels [36], and single inhalation exposure to uranium significantly increased the mRNA levels of IL-8 and IL-10 in rat lung tissues [37]. Interleukin-17 may activate pro-apoptotic members and, hence, initiate the apoptotic process associated with an increased translocation of *Bax* to the mitochondria and a decrease of the mitochondrial transmembrane potential [38]. The costimulation of IL-4 and IL-10 also induced mast cell apoptosis [39]. In the present study, *bax* gene was up-regulated 4- and 10-fold in fish previously exposed to C1 and C2, respectively. After 28 d of exposure to C1, many genes involved in oxidative stress, DNA repair, and mitochondrial metabolism were induced. The disruption of the mitochondrial chain transport may lead to the formation of free radicals and DNA damage. This is in keeping with the results of recent studies on hepatic cells and erythrocytes of zebrafish exposed to uranium at 100 μg/L during a 20-d exposure period, showing the effects of uranium on antioxidant enzyme activities and DNA integrity [12,15].

In the brain, early transcriptional responses were observed

with gene inductions involved in detoxification, oxidative stress, DNA repair, and neuronal response concerning cholinergic system and glutamate synthesis, in agreement with the increase of acetylcholinesterase activity measured in the zebrafish brain after 20 d of exposure to uranium at 100 μg/L [12].

In the brain and skeletal muscles, early inductions of the *cat* gene involved in oxidative balance also occurred, suggesting a production of reactive oxygen species. Early genetic inductions may indicate that cells are still able to establish defense mechanisms. At later exposure times, repression or lack of variation in gene expression were observed for fish exposed to C2. This may be interpreted as an incapacity to mount an efficient transcriptional response considering our gene selection. For instance, in the brain, after the depuration period in fish previously exposed to C2, some genes were strongly repressed (*cd11b* gene was repressed 100-fold).

The early inductions or late repressions that we observe in the brain or skeletal muscles relative to the liver and gills occur despite the fact that brain and skeletal muscles accumulate 10-fold less uranium compared with these other tissues. Consequently, the brain and muscles appear to display a higher sensitivity than the liver and gills. Indeed, a gene expression response was observed in the brain and in the muscles as soon as after 3 d of exposure when uranium concentrations within these tissues had reached 18 and 32 ng/g, respectively. Thus, uranium concentrations as low as 0.08 and 0.13 nmol/g dry weight are able to trigger a gene response within the brain and muscles, respectively. As a means of comparison, after zebrafish were exposed to waterborne cadmium, the observed minimal tissue cadmium concentrations necessary to trigger transcriptional responses in the brain and muscles were equal to 3.9 and 0.4 nmol/g wet weight, respectively (or 16 and 2 nmol/g dry weight, respectively) [33]. Therefore, the zebrafish brain and muscles are supposed to be much more sensitive to uranium than cadmium contamination.

The present study showed that uranium at low doses (set around the environmental concentrations close to uranium mining sites) is likely to modify and disturb gene expression. The study of the selected genes and the link with internal uranium concentrations demonstrated that the effects of uranium exposure on the molecular pathways considered are time-, concentration-, and tissue-dependent. We cannot, however, exclude that other molecular pathways may be affected by uranium. These results will be completed by a set of new data obtained by DNA microarray.

SUPPORTING INFORMATION

Table S1. Gene expression as compared to actin (mean ± standard deviation, $n = 5$) in the brain of zebrafish (*Danio rerio*) exposed to three exposure treatments: No added U (C0), 23 ± 6 μg U/L (C1), and 130 ± 34 μg U/L (C2) and after an 8-d depuration phase.

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Table S2. Gene expression as compared to actin (mean ± standard deviation, $n = 5$) in the skeletal muscles of zebrafish (*Danio rerio*) exposed to three exposure treatments: No added U (C0), 23 ± 6 μg U/L (C1), and 130 ± 34 μg U/L (C2) and after an 8-d depuration phase.

Found at DOI: 10.1897/08-357.S2 (86 KB PDF).

Table S3. Gene expression as compared to actin (mean ± standard deviation, $n = 5$) in the liver of zebrafish (*Danio rerio*) exposed to three exposure treatments: No added U (C0), 23 ± 6 μg U/L (C1), and 130 ± 34 μg U/L (C2) and after an 8-d depuration phase.

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Table S4. Gene expression as compared to actin (mean ± standard deviation, $n = 5$) in the gills of zebrafish (*Danio rerio*) exposed to three exposure treatments: No added U (C0), 23 ± 6 μg U/L (C1), and 130 ± 34 μg U/L (C2) and after an 8-d depuration phase.

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