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Cholesterol 7α-hydroxylase (CYP7A1) activity is modified after chronic ingestion of depleted uranium in the rat

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ABSTRACT

Depleted uranium (DU) is a radioactive heavy metal derived from the nuclear energy production. Its wide use in civilian and military items increases the risk of its environmental dissemination, and thus the risk of internal contamination of populations living in such contaminated territories. Previous studies have shown that vitamin D and cerebral cholesterol metabolisms were affected following chronic ingestion of DU. Even more than the brain, the liver is a crucial organ in cholesterol homeostasis since it regulates cholesterol distribution and elimination at body level. The aim of this work was to assess the impact of a low-level chronic ingestion of DU on hepatic cholesterol metabolism. Rats were contaminated with DU in their drinking water at a concentration of 40 mg/l for 9 months. The major effect induced by DU was a decrease of CYP7A1 specific activity (−60%) correlated with a matching decrease of its product 7α-hydroxycholesterol in the plasma. Hepatic gene expression of transporters ABC A1, ABC G5, ABC G8 and of nuclear receptor RXR was increased, whereas that of catabolism enzyme CYP7B1 was decreased. Thus, after a chronic ingestion of DU, rats experience a modulation of cholesterol catabolism but overcome it, since their cholesterolemia is preserved and no pathology is declared.

1. Introduction

Natural uranium is a radioactive heavy metal that is enriched in fissile isotope to produce energy in nuclear power plants. The waste product of this process is depleted uranium (DU), which is 40% less radioactive than natural uranium but retains its chemical properties [1]. DU is commonly used in industry (for example as counterweight or shielding), but also in military items such as armor-plate for vehicles or aircrafts and in various ammunition including armor-piercing rockets. These uses of DU contribute to its dispersion in the environment and therefore increase the risk of human contamination. The major exposure route for the public would be through ingestion of contaminated food and drink [2]. Moreover, the 4 billiard-years' radiological half-life of DU makes it a perennial threat in contaminated territories.

Although DU is poorly absorbed in the gastrointestinal tract (ca. 1% in the rat), many experimental studies report biological alterations following a chronic internal contamination. These data mainly describe modifications in the physiology of the kidney [3–5], the bone [6,7] and the central nervous system [8–11]. However, modifications of several metabolisms have also been observed after a long-term internal exposure to DU. In particular, vitamin D and xenobiotics metabolisms are modulated after such a contamination [12,13]. Both metabolisms require the action of cytochrome P450 (CYP) enzymes and nuclear receptors, and both of these classes of molecules were affected by DU exposure. Following these results, we tested in a previous study the impact of a chronic ingestion of DU on cerebral cholesterol metabolism, another metabolism involving CYPs and nuclear receptors. We found that the gene expression of many actors involved in cholesterol synthesis, catabolism, and transport was modified, as well as that of nuclear receptors regulating these pathways [14], suggesting that cholesterol metabolism might be a target for DU. Because of the blood–brain barrier, cerebral cholesterol metabolism is independent from cholesterol metabolism in the rest of the body [15,16]. Thus, the impact of a chronic internal contamination of DU on cholesterol metabolism

Abbreviations: ABC, adenosine triphosphate binding cassette transporter; ACAT, acylCoenzymeA: cholesterol acyltransferase; ALT, alanine aminotransferase; Apo, apolipoprotein; AST, aspartate aminotransferase; CYP, cytochrome P450; DU, depleted uranium; FXR, farnesoid-X-receptor; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GGT, gamma-glutamyltranspeptidase; HDL, high-density lipoprotein; HMGCoA R/S, 3-hydroxy-3-methylglutaryl Coenzyme A Reductase/Synthase; HPRT, hypoxanthine-guanine phosphoribosyltransferase; HNF, hepatocyte nuclear factor; LDL, low-density lipoprotein; LDLr, low-density lipoprotein receptor; LRH-1, liver receptor homolog-1; LXR, liver-X-receptor; PPAR, peroxisome proliferator-activated receptor; RXR, retinoid-X-receptor; SHP, small heterodimer partner; SR-B1, scavenger receptor class B type 1; SREBP, sterol regulatory element binding protein.

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from the general pool is still unknown. The present study was conducted in order to address this issue. The focus was set on the liver because of its high metabolic activity and of its importance in maintaining cholesterol homeostasis at organ and body level. Indeed, the liver synthesizes high amounts of cholesterol, it has a central role in cholesterol fluxes at body level through lipoprotein metabolism and it is the only organ able to massively discard the body excess cholesterol through the biosynthesis of bile acids.

The aim of the present work is thus to assess the effects of a chronic ingestion of a low level of DU on hepatic cholesterol metabolism. In this regard, rats were contaminated with DU-supplemented drinking water for 9 months. Following this exposure, the impact of the contamination was assessed on various actors of the four pathways of cholesterol metabolism in the liver, namely biosynthesis, transport, storage as cholesterol esters and catabolism into bile acids. In addition, the transcriptional regulation controlling these pathways was also studied.

2. Materials and methods

2.1. Animals

20 male Sprague–Dawley rats (Charles River, L'Arbresle, France) aged 12 weeks were divided into two groups ($n = 10$), control and DU-exposed respectively. During the experiment, they were housed in pairs, and maintained in a 12 h light/12 h dark cycle (regular cycle) at $21 \pm 2\,^{\circ}C$ and $50 \pm 10\%$ humidity. The animals had access to a standard pellet rodent diet and water *ad libitum* throughout the experiment.

All experimental procedures were approved by the Animal Care Committee of the Institute of Radioprotection and Nuclear Safety, and complied with French regulation for animal experimentation (Ministry of Agriculture Act No. 87-848, October 19th 1987, modified May 20th 2001).

2.2. Contamination procedure

Depleted uranyl nitrate hexahydrate ($UO_2(NO_3)_2{\cdot}6H_2O$, AREVA-NC, France) was dissolved at a concentration of 40 mg/l (approximately 2 mg/kg/day or 14 Bq/rat/day) in mineral water, which is twice the highest concentration of uranium naturally found on Earth, in drilled wells in Finland [17]. Specific activity of DU was 1.4×10^4 Bq/g and its isotopic composition is $^{238}U = 99.74\%$, $^{235}U = 0.255\%$ and $^{234}U = 0.0055\%$. Rats of the experimental group were given the DU-supplemented water as the only drink for 9 months. Control rats received mineral water. After 9 months, the animals were anesthetized by inhalation of 5% isoflurane (Abbot France, Rungis, France) and euthanized by intracardiac puncture to collect blood. Livers were immediately dissected, deep-frozen in liquid nitrogen and stored at $-80\,^{\circ}C$ until analysis.

2.3. Plasmatic parameters assay

2.3.1. Biochemical parameters

Biochemical parameters were measured in plasma samples with an automated spectrophotometric system (Konelab 20 from Thermo Electron Corporation, Cergy-Pontoise, France), using the manufacturer's biological chemistry reagents. The parameters measured in plasma include total cholesterol, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, triglycerides, phospholipids, alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, total bilirubin, gamma-glutamyltranspeptidase (GGT), creatinine and urea.

2.3.2. Plasma 7α-hydroxycholesterol assay

The assay of 7α-hydroxycholesterol (a marker of cholesterol hepatic catabolism) plasma level was performed as described elsewhere [18] on 0.5 ml plasma. 19-Hydroxy-3-acetate cholesterol (Sigma Diagnostics, Isle d'Abeau Chesnes, France) was added to each sample before high performance liquid chromatography as an internal standard for quantification of the peak.

2.4. Hepatic cholesterol assay

Frozen liver samples (about 250 mg) were thawed and homogenized in 5 ml isopropanol using a potter with a Teflon pestle (VWR, Fontenay-sous-Bois, France). After incubation at $60\,^{\circ}C$ for 1 h:30 and centrifugation at $3220 \times g$ for 10 min, the supernatant was collected and the pellet was re-extracted in 5 ml isopropanol (same procedure but with a 1-h incubation). The supernatant of this second extraction was added to the first one and the total volume was adjusted to the precise weight of 10 ml isopropanol for further calculations. Total and free cholesterol were then assayed using the Amplex Red Cholesterol Assay kit (Invitrogen-Life technologies, Cergy-Pontoise, France) according to the manufacturer's instructions. Before the assay, 100 μl of the samples (isopropanol extracts) were dried down under nitrogen flow and diluted to 1/12 in the reaction buffer of the kit. Esterified cholesterol was calculated as the difference between total and free cholesterol values.

2.5. Real-time PCR

Total RNA was extracted from 25 mg of thawed liver samples using the RNeasy total RNA isolation Kit (Qiagen, Courtaboeuf, France) according to the manufacturer's respective recommendations.

Reverse transcription was performed on 1 μg total mRNA using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Courtaboeuf, France) according to the manufacturer's instructions. The cDNA synthesis was conducted at $37\,^{\circ}C$ for 2 h and was ended by the inactivation of the reverse transcriptase after 5 min at $85\,^{\circ}C$.

Real-time PCR was then carried out on 0.4 ng/μl cDNA and 0.3 pmol/μl primers for each reaction, using the SYBR® Green technology (Power SYBR® Green PCR Master Mix, Applied Biosystems, Courtaboeuf, France) according to the manufacturer's instructions. The plates were analyzed on an AbiPrism 7900 Sequence Detection System (Applied Biosystems, Courtaboeuf, France) according to the following run: incubation 2 min at $50\,^{\circ}C$, 10 min at $95\,^{\circ}C$ for activation of the polymerase and 40 cycles of 15 s at $95\,^{\circ}C$ for denaturation and 1 min at $60\,^{\circ}C$ for annealing–extension. Results were normalized to hypoxanthine–guanine phosphoribosyltransferase (HPRT) and fold-inductions calculated relatively to the control group. Sequences for the forward and reverse primers are listed in Table 1 [14,19–26].

2.6. Western blot

All antibodies were purchased from Tebu-bio (Le Perray-en-Yvelines, France).

Proteins from liver homogenate (liver microsomes for CYP7A1) were loaded, separated by 10% SDS polyacrylamide gel electrophoresis and transferred onto nitrocellulose membrane. The membranes were blocked for 1 h in 5% non-fat dry milk in TBS. The blots were incubated overnight at $4\,^{\circ}C$ with primary antibodies anti-ABC A1 (sc-53569), anti-ABC G5 (sc-18204), anti-ABC G8 (sc-30111), anti-RXR (sc-553) or anti-CYP7A1 (sc-25536) diluted to the appropriate concentration in 2% non-fat dry milk in TBS. Immune complexes were revealed using anti-goat IgG (sc-2768), anti-mouse IgG (sc-2005) or anti-rabbit IgG (sc-2004)

Table 1
Oligonucleotide sequences of primers used in real-time quantitative PCR. The primers sequences are given in the 5′–3′ orientation. The primer without reference has been designed by the authors with the PrimerExpress software.

Gene	Forward	Reverse	Length (bp)	Gene accession #	Ref.
hprt	gctcgagatgtcatgaaggaga	tcagcgctttaatgtaatccagc	109	NM_012583	[19]
cyp27a1	ggaaggtgccccagaacaa	gcgcagggtctccttaatca	71	NM_178847	[20]
cyp7a1	ccaagtcaagtgtccccctcta	gactctcagccgccaagtg	150	NM_012942	[25]
cyp8b1	gtacacatggaccccgacatc	gggtgccatcagggttgag	76	AB009686	[21]
cyp7b1	tcagatgcaaagacggtcaga	ttcatgcccgtagtatttttcag	70	U36992	[14]
cyp51	ctggtcatcctgggtcctaaga	cagaggccatgaaggatgct	81	NM_012941	[14]
hmgcoa r	ggcgggtcctgcaagtg	gcaggtgagcgggtgaga	60	NM_173094	[25]
hmgcoa s	ggtggtgggaccaaccttct	cacgccccttgaacaccta	65	X55286	[21]
acat 2	gccccagccgacatttt	gtgcagtgtgaagccttgactt	80	NM_153728	[23]
ldl-r	cagccgatgcattcctgact	agttcatccgagccattttcac	69	NM_175762	[25]
sr-b1	gttggtcaccatgggcca	cgtagccccacaggatctca	65	NM_031541	[25]
abc a1	atctcatagtatggaagaatgtgaagct	cgtacaactattgtataaccatctccaaa	132	NM_78095	[25]
abc g1	aggtctcagccttctaaagttcctc	tctctcgaagtgaatgaaatttatcg	85	NM_053502	[14]
abc g5	cgcaggaaccgcattgtaa	tgtcgaagtggtggaagagct	67	NM_053754	[25]
apo a1	aatgggacagggtgaagga	tgaacccagagtgtcccagtt	146	NM_012738	[25]
apo b	tcctaacatcattgtgccttcat	ccttgaaatctgggagggaaaact	106	NM_019287	
apo e	tgggtgcagacgctttctg	ttgtatgcctttacttcagtcatagt	110	NM_138828	[25]
lxrα	agcaacagtgtaacaggcgct	gtgcaatgggccaaggc	63	NM_031627	[22]
lxrβ	cttcccccacaagttc	cctactcgtgga	151	NM_031626	[14]
rxrα	cgcaaagacctgacctacacc	tcctcctgcacggcttccc	134	NM_012805	[25]
fxr	tgacaaagaagccgcgaat	tgtaatggtacccagaggccc	99	NM_021745	[25]
shp	cctggagcagccctcgt	aacactgtatgcaaaccgagg	64	NM_057133	[18]
lrh-1	gcacctgcaccaggatcag	cccggtgtttttcctcaagtt	58	NM_021742	[22]
pparα	tctcttcccaaaactccttca	gcacgagctgcgcatgctc	69	NM_013196	[20]
pparγ	tcatgaccagggagttcctca	tcatctaattccagtgcattgaactt	103	NM_013124	[20]
hnf 1α	acacctggtacgtccgcaag	cgtgggtgaattgctgagc	51	NM_012669	[24]
hnf 4α	tggcaaacactacggagcct	ctgaagaatcccttgcagcc	51	NM_022180	[24]
srebp 2	agctggcaaatcagaaaaacaag	cgatcttcaagtccacatcactgt	58	XM_216989	[22]

antibodies coupled to horseradish peroxidase and the luminol derivative of Immobilon Western (Millipore, Billerica, USA). Samples were normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) which was detected using a rabbit anti-rat primary antibody (sc-25778) or to β-actin using a mouse anti-rat primary antibody (sc-47778). Reaction intensity was determined by computer-assisted densitometry using a LAS-3000 analyser (Fujifilm, Courbevoie, France).

2.7. Assay of CYP7A1, CYP27A1, CYP7B1 and CYP8B1 specific activities

The specific activities of CYP7A1 and CYP27A1 were assessed on hepatic microsomal and mitochondrial fractions respectively using a radioisotopic method described previously [27]. The specific activities of CYP7B1 and CYP8B1 were conducted on hepatic microsomes according to Souidi et al. [28,29]. The assays were conducted on six animals of each group, randomly selected.

2.8. Statistical analysis

Results are expressed as mean ± SEM. Unpaired Student's *t*-test was routinely performed for statistical analysis of the data, but was replaced by Mann–Whitney Rank Sum Test when the equal variance test failed (determined by the SigmaStat software). Differences were considered statistically significant when $p < 0.05$.

3. Results

3.1. General health status and plasma biochemical parameters

Food and drink intakes were not affected by chronic ingestion of DU (data not shown), and no difference was recorded in body weight or in liver weight at the end of the contamination (Table 2). Macroscopic appearance of main organs (liver, lung, heart, intestines, kidney, brain, testis) from DU-exposed rats did not differ from that of control rats (data not shown).

In the plasma, the lipid profile was unchanged after DU chronic ingestion. Blood concentrations of markers of liver integrity (ALT and AST) and liver function (GGT and total bilirubin) were also similar in control and DU-exposed rats.

Finally, the plasmatic level of 7α-hydroxycholesterol (a liver-specific biomarker of the classical pathway of cholesterol catabolism) displayed a 59% decrease ($p < 0.05$) in the DU-exposed rats compared to the controls.

3.2. Tissue cholesterol assay

Levels of total cholesterol and free cholesterol were measured in the liver from animals of both groups, and esterified cholesterol was

Table 2
General health and biochemical parameters in control and DU-exposed animals. Values are expressed as mean ± SEM ($n = 10$) $^{*}p < 0.05$. ALT = alanine aminotransferase, AST = aspartate aminotransferase, GGT = gamma-glutamyltranspeptidase, HDL = high-density lipoprotein, LDL = low-density lipoprotein.

Parameters	Control	DU-exposed
Final body weight (g)	595 ± 11	578 ± 21
Final liver weight (g)	19.3 ± 0.7	18.7 ± 0.9
Plasma profile		
Cholesterol (mM)	2.76 ± 0.17	2.53 ± 0.13
HDL-cholesterol (mM)	1.65 ± 0.10	1.62 ± 0.10
LDL-cholesterol (mM)	0.48 ± 0.07	0.46 ± 0.04
Triglycerides (mM)	1.78 ± 0.20	1.52 ± 0.13
Phospholipids (g/l)	1.93 ± 0.08	1.79 ± 0.06
ALT (U/l)	51.0 ± 6.5	55.2 ± 6.5
AST (U/l)	122 ± 12	114 ± 8
GGT (U/l)	4.29 ± 1.35	2.73 ± 0.29
Total bilirubin (μM)	5.96 ± 0.65	4.89 ± 0.44
Creatinine (μM)	56.4 ± 2.8	58.1 ± 3.2
Urea (mM)	5.9 ± 0.5	5.7 ± 0.2
7α-Hydroxycholesterol (ng/ml)	69.0 ± 11.3	28.3 ± 4.0 *
Hepatic cholesterol		
Total cholesterol (mg/g liver)	2.14 ± 0.12	2.41 ± 0.26
Free cholesterol (mg/g liver)	1.59 ± 0.05	1.78 ± 0.26
Esterified cholesterol (mg/g liver)	0.54 ± 0.15	0.62 ± 0.16

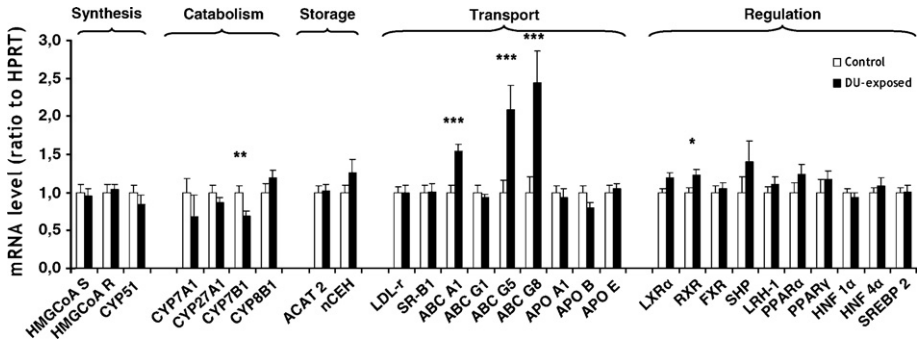

Fig. 1. mRNA levels of major actors involved in cholesterol metabolism in the liver following chronic DU ingestion. Results are expressed as a ratio to HPRT mRNA level. The levels of non-contaminated control rats were arbitrarily set at 1. Data are expressed as mean ± SEM ($n = 10$). $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$.

calculated as their difference. These values are reported in Table 2. No difference was observed in the levels of total, free or esterified hepatic cholesterol between DU-exposed and control rats.

3.3. Gene expression

The mRNA levels of the major actors of cholesterol synthesis, catabolism, storage, transport and of transcription factors regulating these proteins are reported in Fig. 1. The studied genes were involved in (i) cholesterol synthesis (3-hydroxy-3-methylglutaryl Coenzyme A Synthase (HMGCoA S), 3-hydroxy-3-methylglutaryl Coenzyme A Reductase (HMGCoA R) and cyp51), (ii) cholesterol catabolism (CYP7A1, CYP27A1, CYP7B1, and CYP8B1), (iii) cholesterol storage as esters (acylCoenzymeA: cholesterol acyltransferase 2 (ACAT 2) which esterifies cholesterol and neutral hepatic cholesterol ester hydrolase (nCEH) which catalyzes the reverse reaction), (iv) hepatic cholesterol fluxes (LDL-receptor (LDL-r), scavenger receptor class b type 1 (SR-B1), ATP binding cassette (ABC) transporter A1 (ABC A1), ABC G1, ABC G5, ABC G8), and apolipoproteins (APO) A1, APO B and APO E), and finally (v) major nuclear receptors and transcription factors involved in the transcriptional regulation of the metabolic pathways cited above (liver-X-receptor α (LXRα), retinoid-X-receptor α (RXR), farnesoid-X-receptor (FXR), small heterodimer partner (SHP), liver-receptor homolog-1 (LRH-1), peroxisome proliferator-activated receptor α (PPARα), PPARγ, hepatocyte nuclear factor 1α (HNF 1α), HNF 4α, and sterol regulatory element binding protein (SREBP) 2).

The gene expression of the enzymes involved in cholesterol synthesis and storage was unchanged after chronic contamination with DU.

The hepatic gene expression of CYP7B1, involved in cholesterol catabolism, was significantly decreased (−31%, $p < 0.01$) in DU-exposed animals compared to the control animals.

The mRNA level of the nuclear receptor RXR was increased (+23%, $p < 0.05$) in the contaminated rats compared to the control rats.

Concerning the actors involved in cholesterol transport, the gene expression of three ABC transporters was increased in the experimental group compared to the control group: ABC A1 (+54%, $p < 0.001$), ABC G5 (+109%, $p < 0.001$), and ABC G8 (+144%, $p < 0.001$).

3.4. Specific activities of catabolism enzymes (CYP7A1, CYP27A1, CYP7B1, and CYP8B1)

The specific activities of hepatic CYP7A1, CYP27A1, CYP7B1, and CYP8B1 are reported in Fig. 2. For CYP7A1, the DU-exposed rats displayed a specific activity of 2.97 ± 0.78 pmol/min/mg proteins *vs.* 7.46 ± 1.67 pmol/min/mg proteins in the control rats, leading to a significant decrease in the exposed group (−60%, $p < 0.05$). Conversely, the specific activities of CYP27A1

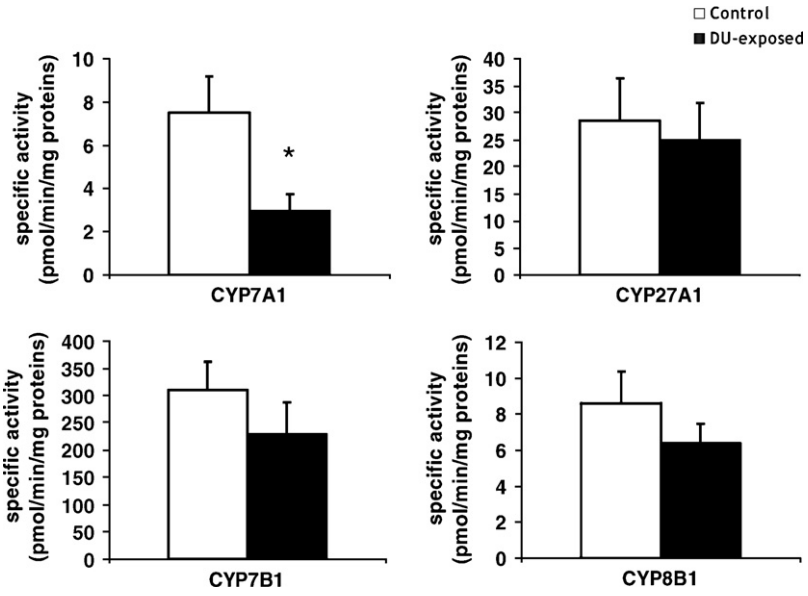

Fig. 2. Specific activities of hepatic CYP7A1, CYP27A1, CYP7B1, and CYP8B1 following chronic DU ingestion. Data are expressed as means ± SEM ($n = 6$). $^{*}p < 0.05$.

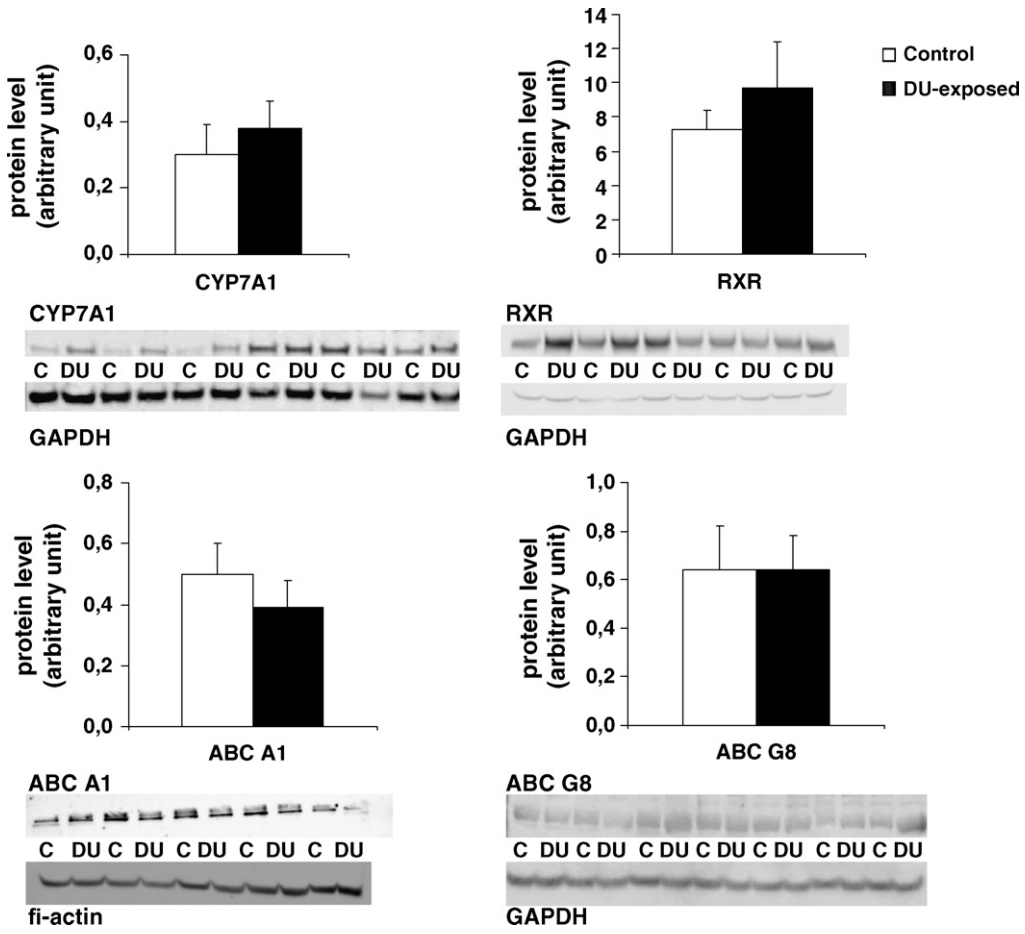

Fig. 3. Protein levels of hepatic CYP7A1, ABC A1, ABC G8 and RXR following chronic DU ingestion. Results are expressed as a ratio to GAPDH or β-actin protein level. Data are expressed as means ± SEM (n = 5–7).

(28.47 ± 7.91 pmol/min/mg proteins *vs*. 24.88 ± 6.88 pmol/min/mg proteins in the control rats), CYP7B1 (310.13 ± 53.23 pmol/min/mg proteins *vs*. 228.80 ± 59.65 pmol/min/mg proteins in the control rats), and CYP8B1 (8.6 ± 1.8 pmol/min/mg proteins *vs*. 6.40 ± 1.1 pmol/min/mg proteins in the control rats) did not differ significantly between both groups.

3.5. Protein expression

The relative protein levels of hepatic ABC A1, ABC G5, ABC G8, RXR and CYP7A1 were assessed by Western blot and the values are reported in Fig. 3.

The various antibodies tested for the assay of ABC G5 protein level did not yield a sufficient signal to be considered specific; therefore no data is presented for ABC G5 in this report.

No significant difference between both groups was observed for either ABC A1 (0.5 ± 0.1 for the DU-exposed rats *vs*. 0.39 ± 0.09 for the control rats), ABC G8 (0.64 ± 0.18 and 0.64 ± 0.14 for the experimental and the control groups respectively), RXR (7.30 ± 1.11 for the DU-exposed rats and 9.69 ± 2.69 for the control rats) or CYP7A1 (0.30 ± 0.09 for the DU-exposed rats and 0.38 ± 0.08 for the control rats).

4. Discussion

The aim of the present work was to ascertain whether cholesterol metabolism could be altered following a chronic internal exposure to DU. In this regard we assessed the consequences of such a contamination on cholesterol metabolism in rats given DU in their drinking water for 9 months. This study focuses on the liver, in which DU is known to accumulate to some extent [3,4,30].

During the whole contamination time, the general health parameters (food and drink intakes, body weight gain) were preserved in the animals of the experimental group (data not shown). The plasma level of biomarkers of hepatic integrity (ALT, AST) and function (GGT, bilirubin) as well as those of biomarkers of renal function (creatinine, urea) were unchanged following DU exposure. The latter results suggest that the level of DU in the contamination solution is neither hepatotoxic nor nephrotoxic. The plasma lipid profile was also unaffected after the contamination, including total cholesterol, HDL- and LDL-cholesterol concentrations, indicating no perturbation of lipid metabolism at body level.

The plasma level of 7α-hydroxycholesterol displayed a 59% decrease in rats chronically ingesting DU compared to the control rats. This oxysterol is synthesized from cholesterol by the liver-specific enzyme cholesterol 7α-hydroxylase (CYP7A1), which initiates the classical pathway of the catabolism of cholesterol into bile acid. The level of circulating 7α-hydroxycholesterol has been previously correlated with the specific activity of CYP7A1 [31–33]. Interestingly, 7α-hydroxycholesterol plasma level was also reported to decrease 3 days after a unique subcutaneous injection of a high dose of DU in rats [18]. Moreover, two human studies reported variations of 7α-hydroxycholesterol plasma levels after accidental exposure to a high dose of ionizing radiations. In one case, it was reported to decrease [34], whereas in the other case the authors showed an increase of 7α-hydroxycholesterol blood level [35]. In both cases the variation in 7α-hydroxycholesterol preceded

a matching decrease/increase of ALT and AST levels in the blood of a man accidentally exposed to β radiations. The discrepancy in the variations observed in the different studies may be due to the difference in the exposure pattern or in the nature of the radiations (DU emits α radiations). Although these discrepancies are not yet understood, the variations of 7α-hydroxycholesterol plasma levels reported in these studies and the present work draw attention to a possible sensitivity of this metabolite to ionizing radiations exposure. Moreover, 7α-hydroxycholesterol was recently proposed as a potential biomarker for the evaluation of liver alteration after external high dose irradiation [35].

As mentioned earlier, the level of circulating 7α-hydroxycholesterol is linked to the specific activity of CYP7A1 in the liver. Thus, this activity was assessed and was found to decrease by 60% in the DU-exposed rats compared to the control group. This result perfectly matches the decrease of the plasma level of 7α-hydroxycholesterol and confirms the direct link between the level of the circulating product and the hepatic enzymatic activity.

Since the decrease of the specific activity of CYP7A1 is likely to induce a perturbation of the catabolism of cholesterol into bile acids, the specific activities of other major enzymes of this metabolic pathway were assessed. Surprisingly, the alternative pathway of bile acids synthesis did not seem to be affected, as shown by the unchanged activities of CYP27A1 and CYP7B1. Thus, these two results suggest that although the classical pathway of bile acids synthesis was decreased in the animals chronically ingesting DU, the alternative pathway did not compensate by increasing its rate.

To assess the repercussions of these results on cholesterol homeostasis in the liver, the hepatic cholesterol concentration was measured. The liver concentration of total cholesterol was unaffected after DU-exposure, as were the free and esterified fractions. Thus, the observed decrease in cholesterol hepatic catabolism did not lead to a perturbation of cholesterol homeostasis at organ level in contaminated animals.

At this point three questions arose: (1) is the effect observed on CYP7A1 activity due to an action at protein level or is it a consequence of a change in the transcriptional regulation system? (2) is this decrease compensating a modification of another metabolic pathway such as cholesterol synthesis or uptake by the liver? and (3) are there other "targets" of DU-exposure in cholesterol metabolism? To answer these questions, the gene expression of the major actors involved in cholesterol metabolism was assessed.

The answer to the first question was given by the mRNA level of CYP7A1, which did not statistically differ between DU-exposed and control rats. Thus, the action of DU is not taking place at gene expression level. This was confirmed by the absence of modification in the mRNA levels of many nuclear receptors and transcription factors regulating CYP7A1. In particular, LXRα, LRH-1, HNF 4α, FXR, SHP and PPARα are involved in CYP7A1 transcriptional regulation [15,36–38] and displayed similar mRNA levels in both groups. The protein level of CYP7A1 in hepatic microsomes was also unaffected after the contamination. This invalidates the hypothesis of an action of DU on the protein's synthesis or degradation rates. Consequently, it suggests that the decrease in CYP7A1 activity rather results from an alteration of a post-translational feature known to influence CYP7A1 activity (for instance its phosphorylation status [39–42]).

The questions of the impact of DU on other pathways than catabolism and of other possible targets were answered by a PCR study covering cholesterol synthesis, storage, transport, and the associated nuclear receptors. Most of these genes had an unaltered expression, suggesting that the decrease in CYP7A1 activity was not triggered by a perturbation of synthesis or storage processes, nor did it respond to a modification in cholesterol uptake. In contrast, the mRNA levels of three ABC transporters involved in cholesterol efflux were increased: ABC A1 (which is involved in the secretion of native HDLs with Apo A-I), and ABC G5 and ABC G8, which heterodimerize and export free cholesterol directly into the bile. This concurrent increase in the transcription of ABC G5 and ABC G8 is noteworthy, since it is required for the synthesis of a functional heterodimer. In addition to the ABC transporters, a decrease in the transcription of CYP7B1 and an increase in that of RXR were observed in the DU-exposed rats. The lack of impact of all these modifications at organ level was confirmed by the absence of alteration of the corresponding enzymatic activity (for CYP7B1) or protein levels (despite the lack of data for ABC G5, the fact that ABC G8 translation was not activated is sufficient to infer that no increase in the functional heterodimer was triggered).

Thus, every pathway of cholesterol hepatic metabolism seem unaltered after DU exposure except for the matching decreases of hepatic CYP7A1 activity and 7α-hydroxycholesterol circulating level. This decrease of the classical pathway of bile acids biosynthesis is unlikely to seriously alter the bile acid pool. Indeed, an impaired pool of bile acids would have triggered changes in cholesterol intestinal absorption, thus in hepatic uptake and possibly in cholesterolemia. Moreover, bile acids are potent activators of nuclear receptors like FXR or PXR, which regulate their synthesis and transport. These nuclear receptors and their target genes would have been expected to undergo major modifications in case of a disruption in bile acid synthesis. Thus, this decrease of CYP7A1 activity is difficult to explain since cholesterol metabolism seems balanced. Further studies are needed to assess more precisely the impact of these effects on bile acid metabolism.

In any case, the results reported in this study differ entirely from those obtained in the cerebral cholesterol study after the same contamination [14]. Indeed, many genes involved in cholesterol synthesis, transport, catabolism and regulation pathways were modified in the brain after DU-exposure. Of these, only ABC A1 and RXR are in common with the present work. This is in accordance with the fact that cholesterol homeostasis is maintained independently in the brain and the liver. Moreover, the difference in DU accumulation in these organs may also be responsible for this discrepancy.

In conclusion, this work reports a 60% decrease in CYP7A1 specific activity and 7α-hydroxycholesterol blood level in rats after a daily ingestion of a low concentration of DU for 9 months. Further studies are required to elucidate the mechanism (possibly post-translational) behind this variation. Despite this modification, cholesterol homeostasis was preserved at liver and body level. The impact of this type of contamination on an already unbalanced system (like animals prone to hypercholesterolemia or cholelithiasis) would be of interest to ascertain the influence of DU on cholesterol metabolism.

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