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Hepatic Cholesterol Metabolism Following a Chronic Ingestion of Cesium-137 Starting at Fetal Stage in Rats

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Cesium-137/Cholesterol metabolism/Chronic contamination/Liver/Low dose.

The Chernobyl accident released many radionuclides in the environment. Some are still contaminating the ground and thus the people through dietary intake. The long-term sanitary consequences of this disaster are still unclear and several biological systems remain to be investigated. Cholesterol metabolism is of particular interest, with regard to the link established between atherosclerosis and exposure to high-dose ionizing radiations. This study assesses the effect of cesium-137 on cholesterol metabolism in rats, after a chronic exposure since fetal life. To achieve this, rat dams were contaminated with cesium-137-supplemented water from two weeks before mating until the weaning of the pups. Thereafter, the weaned rats were given direct access to the contaminated drinking water until the age of 9 months. After the sacrifice, cholesterol metabolism was investigated in the liver at gene expression and protein level. The cholesterolemia was preserved, as well as the cholesterol concentration in the liver. At molecular level, the gene expressions of ACAT 2 (a cholesterol storage enzyme), of Apolipoprotein A-I and of RXR (a nuclear receptor involved in cholesterol metabolism) were significantly decreased. In addition, the enzymatic activity of CYP27A1, which catabolizes cholesterol, was increased. The results indicate that the rats seem to adapt to the cesium-137 contamination and display modifications of hepatic cholesterol metabolism only at molecular level and within physiological range.

INTRODUCTION

The explosion of the nuclear reactor at the Chernobyl Power Plant in 1986 led to the spread and deposition of various radionuclides in forests, in the soil and in many bodies of water. Some of these radionuclides have long half-lives, especially cesium-137 (^{137}Cs). Therefore, they still generate a direct environmental contamination[1)] threatening the surrounding wildlife and crops, which in turn induces the dietary contamination of the populations through the food chain. These populations have become very important in the framework of the research on Chernobyl's long-term sanitary consequences, especially with regard to their contamination pattern: internal exposure to low levels of ionizing radiations over a long stretch of time. Within this group, children are of particular importance because of their enhanced sensitivity towards many contaminants including ionizing radiations,[2)] and because of their lifespan, making them the prime target for long-term effects.

Some health effects of ionizing radiations are already observed during childhood. Besides thyroid cancer and leukemia, various non-cancerous effects have been studied. To date, only focal lens defects[3)] and cataracts[4)] have been directly attributed to ionizing radiations. Other non-cancerous effects require greater statistical power or further investigation. Despite the link established between high-dose external irradiation and atherosclerosis,[5)] cardiovascular symptoms stand among the less documented areas. In the only easily available study on cardiovascular symptoms in the "Chernobyl children", the authors describe abnormal cardiac features.[6)] Besides, another work on children reports modifications in the levels of plasmatic markers of hepatic structure and function.[7)] In this regard, cholesterol metabolism

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appears as a prime target for studies. Indeed, it is an important risk factor for cardiovascular complications (as are high levels of ionizing radiations) and it is mainly catabolized in the liver.

Disruptions of cholesterol homeostasis are linked with various diseases, among which cardiovascular diseases like atherosclerosis[8] or neurodegenerative pathologies such as Alzheimer's disease.[9] The metabolism of cholesterol in the liver is crucial, since it is the only organ able to eliminate the excess body cholesterol by converting it into bile acids. Bile acids also have an important role in the intestinal absorption of liposoluble vitamins and in the regulation of cholesterol homeostasis.[10] If hepatic cholesterol metabolism is disrupted, many pathologies such as cholestasis, jaundice or gallstones can occur,[11,12] possibly leading to a perturbation of the hepatic function at a larger scale. Moreover, the intricate crosstalks in the regulation of cholesterol metabolism and that of other metabolic pathways (fatty acids, glucose…) may induce wide repercussions if one of these systems is perturbed. Thus, cholesterol metabolism seems an important system to study in order to evaluate certain non-cancerous effects of ionizing radiations. This is supported by the work of Wong *et al*. reporting an increase of serum cholesterol levels in atomic bomb survivors,[13] which appeared to be linked with the radiation level. Moreover, cholesterol metabolism is of prime importance in the development of the fetus because of its multiple roles in membrane structure, membrane protein activity, and as a precursor for steroids involved in metabolic regulation.[14]

Thus, the present experimental work addresses two requests of the United Nations Scientific Committee on the Effects of Atomic Radiation[15] and the World Health Organization[16]: studying the impact of a chronic low dose of ionizing radiations on a biological system (cholesterol metabolism) related to cardiovascular diseases and studying the long-term consequences of an exposure pattern covering the childhood. As recreating the exact mixture of the remaining radiations sources would be very difficult, the focus was set on ^{137}Cs alone, which is mainly a beta-gamma emitter with a decay period of 30 years and therefore the main contributor to the current level of ionizing radiations resulting from Chernobyl accident. The exposure started at embryo stage in order to mimic in the closest way the exposure of children living on contaminated land. This also allows studying the effects of ^{137}Cs chronic ingestion on the fetus, whose sensitivity towards many contaminants makes it a reference model in toxicology. Thus, the aim of this study is to investigate the effects of a chronic ingestion of a low level of ^{137}Cs on the five main pathways of hepatic cholesterol metabolism (biosynthesis, catabolism, storage as esters, transport and transcriptional regulation) in rats exposed since fetal life.

MATERIALS AND METHODS

Animals and ^{137}Cs administration

The study included male Sprague-Dawley rats divided into ^{137}Cs-exposed and control groups. Each group consisted of 10 animals. The parents of the rats in the experimental group were exposed to ^{137}CsCl (CERCA, Pierrelatte, France) at a concentration of 6.5 kBq/l (approximately 610 Bq/kg/day) in their drinking water for two weeks before mating. Then, pregnant females were administered ^{137}Cs throughout the pregnancy and until the weaning, so that the pups were contaminated *via* their dam's milk. After weaning, the pups were directly exposed to ^{137}Cs-supplemented water until they were 9 months old.

This pattern of exposure was chosen in order to mimic the children born to people living on contaminated land after Chernobyl accident and daily ingesting contaminated foodstuff. The concentration of ^{137}Cs is based on the top estimate of their dietary intake in the years following the accident.[17] This level of ^{137}Cs, which establish the reality of the food contamination, leads nevertheless to a low-dose internal exposure at organ level, as will be confirmed by the measurement of ^{137}Cs in the liver of the treated rats.

The animals were housed in pairs and were maintained in a 12 h light/12 h dark cycle. Food and water were freely accessible and their intakes were monitored on a weekly basis. The animals were weighed once a week and right before the sacrifice.

At the end of the period of exposure to ^{137}Cs, blood was collected by intracardiac puncture under gaseous anesthesia and the liver was dissected on ice. All samples were deep-frozen in liquid nitrogen. All experimental procedures were approved by the Animal Care Committee of the Institute (IRSN) and complied with French regulations for animal experimentation (Ministry of Agriculture Act No. 87-848, October 19, 1987, modified May 29, 2001).

Plasma parameters assessment

Routine plasma biochemical parameters were measured using a Konelab 20 automated spectrophotometer (Thermo Electron Corporation, Cergy-Pontoise, France) and the manufacturer's reagents. The parameters measured in plasma included total cholesterol, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, triglycerides, phospholipids, alanine aminotransferase (ALT), aspartate aminotransferase (AST), direct and total bilirubin, gamma-glutamyltranspeptidase (γGT), creatinine and urea.

Plasma 7α-hydroxycholesterol assay

The assay of 7α-hydroxycholesterol 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 per-

formance liquid chromatography as an internal standard for quantification of the peak.

Hepatic ^{137}Cs assay

^{137}Cs measurement in liver samples was conducted using gamma spectrometry with a Packard Cobra II gamma counter (PerkinElmer, Courtaboeuf, France). In order to obtain a conservative, upper estimate of the dose, the activity measured in the liver at the autopsy was assumed to have been constant from the start of the experiment. The resulting number of nuclear transformations of ^{137}Cs and Barium-137 m was therefore obtained by multiplying this activity by the duration of the experiment, expressed in seconds. Each nuclear transformation is associated with the photon and electron emissions described in ICRP publication 107.[19] The resulting liver absorbed dose was derived from the number of nuclear transformations and energy spectrum by applying the absorbed fraction of energy calculated by Stabin *et al*.[20] for photons and electrons in a rat computational model, and dividing by the mass of the liver.

Hepatic cholesterol assay

Cholesterol was extracted from 250 mg liver samples as described by Boelher *et al*..[21] Total and free cholesterol were assessed using the Amplex Red Cholesterol Assay kit (Invitrogen-Life technologies, Cergy-Pontoise, France) according to the manufacturer's instructions. Esterified cholesterol was calculated as the difference between total and free cholesterol values.

Real-time PCR

Total RNA of liver samples was extracted using RNeasy total RNA isolation Kit (Qiagen, Courtaboeuf, France) according to the manufacturer's instruction. Reverse transcription was performed with BD Sprint PowerScript PrePrimed 96 Plate (BD Biosciences Clontech, Erembodegem, Belgium). Real–time PCR was then carried out on an AbiPrism 7000 Sequence Detection System (Applied Biosystems,

Table 1. Oligonucleotide sequences of primers used in real-time quantitative PCR

GENE NAME	ACCESSION #	FORWARD PRIMER	REVERSE PRIMER	LENGTH (bp)	REF
hprt	NM_012583	gctcgagatgtcatgaaggaga	tcagcgctttaatgtaatccagc	109	(22)
hmgcoa-s	NM_173094	ggcgggtcctgcaagtg	gcaggtgagcgggtgaga	150	(23)
hmgcoa-r	X55286	ggtggtgggaccaaccttct	cacgccccttgaacaccta	70	(24)
cyp27a1	NM_178847	ggaaggtgccccagaacaa	gcgcagggtctccttaatca	65	(25)
cyp7a1	NM_012942	ccaagtcaagtgtccccctcta	gactctcagccgccaagtg	60	
cyp7b1	NM_019138	tcagatgcaaagacggtcaga	ttcatgcccgtagtatttttttcag	71	(23)
acat 2	NM_153728	gccccagccgacatttt	gtgcagtgtgaagccttgactt	80	
nceh	L46791	agcaagagtttggctggatcat	agagggatttggctgttttctg	87	
ldlr	NM_175762	cagccgatgcattcctgact	agttcatccgagccattttcac	63	(23)
sr-b1	NM_031541	gttggtcaccatgggcca	cgtagccccacaggatctca	134	(23)
abc a1	NM_178095	atctcatagtatggaagaatgtgaagc	cgtacaactattgtataaccatctccaaa	99	(23)
abc g5	NM_053754	cgcaggaaccgcattgtaa	tgtcgaagtggtggaagagct	69	(23)
abc g8	NM_130414	gatgctggctatcatagggagc	tctctgcctgtgataacgtcga	51	
apo a-1	NM_012738	aatgggacagggtgaagga	tgaacccagagtgtcccagtt	58	
apo b	NM_019287	tcctaacatcattgtgccttcat	ccttgaaatctgggagggaaaact	51	
lxrα	NM_031627	agcaacagtgtaacaggcgct	gtgcaatgggccaaggc	132	(26)
rxr	NM_012805	cgcaaagacctgacctacacc	tcctcctgcacggcttccc	69	(27)
fxr	NM_021745	tgacaaagaagccgcgaat	tgtaatggtacccagaggccc	65	(18)
pparα	NM_013196	tctcttcccaaaactccttca	gcacgagctgcgcatgctc	67	(25)
hnf1α	NM_012669	acacctggtacgtccgcaag	cgtgggtgaattgctgagc	146	(28)
hnf4α	NM_022180	tggcaaacactacggagcct	ctgaagaatcccttgcagcc	106	(28)
srebp 2	XM_216989	agctggcaaatcagaaaaacaag	cgatcttcaagtccacatcactgt	69	(24)

The primers sequences are given in the 5'-3' orientation. The primers without reference have been designed for this study by the authors with the PrimerExpress software.

Courtaboeuf, France) using SYBR Green technology with 0.4 µg/ml cDNA and 0.3 nmol/ml primers for each reaction. Samples were normalized to hypoxanthine-guanine phosphoribosyltransferase (HPRT) and fold-inductions were calculated relative to the control group. Sequences for the primers are listed in Table 1.[18,22–28)]

Western Blot

Assays were carried out on liver homogenate of five animals of each group (randomly selected). Proteins were separated on a 12% SDS polyacrylamide gel electrophoresis and transferred onto a nitrocellulose membrane. The membranes were blocked for one hour at room temperature in 5% non-fat dry milk reconstituted in TBS. A rabbit anti-retinoid-X-receptor (RXR) polyclonal antibody (Santa Cruz Biotechnology Inc., Heidelberg, Germany) and a rabbit anti-Apolipoprotein A-I (Apo A-I) polyclonal antibody (Abcam, Cambridge, United Kingdom) were diluted in 2% non-fat dry milk in TBS and incubated overnight at 4°C with the membranes. Secondary antibody coupled to horseradish peroxidase was incubated with the membranes in 2% non-fat milk for one hour at room temperature. Samples were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and beta-actin respectively. Signal was detected using ECL technology (Immobilon Western, Millipore, Saint-Quentin-en-Yvelines, France). The reaction intensity was quantified by computer-assisted densitometry (Fuji Las3000, Fujifilm, Courbevoie, France).

Enzymes specific activities assay

The specific activities of cytochrome P450 (CYP) 27A1 and CYP7A1 were assessed on hepatic mitochondrial and microsomal fractions respectively using a radioisotopic method described previously.[29)] The assay was conducted on six animals of each group, randomly selected.

Statistics

Significance was assessed using Student's t-test or Mann-Whitney Rank Sum Test when Student's test failed (determined by Sigmastat software). Differences were considered significant when $p < 0.05$. Results are expressed as means ± SEM.

RESULTS

Accumulation of ^{137}Cs in the liver of contaminated rats

The measured level of hepatic ^{137}Cs in the exposed rats was 5.48 ± 0.57 Bq/g liver whereas the level in the control rats was below detection limit ($p = 0.029$). The radiation level in the liver was calculated as 60 cGy over 10 months (*cf.* Material and Methods).

General and plasma parameters

The contaminated rats were in a good general status: their food and water intakes, as well as the final body weight and liver weight were similar to those of the control group.

The plasma lipids profile (including levels of total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, and phospholipids) was statistically similar in both groups. This was also the case for the plasmatic markers of liver integrity (ALT and AST) and liver function (direct and total bilirubin and γGT) as shown in Table 1. Plasma levels of creatinine and urea were also unchanged after ^{137}Cs chronic administration.

The plasma level of 7α-hydroxycholesterol was not significantly different between the two groupsas as reported in Table 1.

RT-qPCR

The gene expression of two key enzymes of cholesterol synthesis, 3-hydroxy-3-methylglutaryl Coenzyme A (HMG-CoA)-Synthase and HMGCoA-Reductase, were unchanged between ^{137}Cs-exposed and control rats, as illustrated in Fig. 1. Likewise, the main enzymes involved in cholesterol catabolism into bile acids in the liver (CYP7A1, CYP27A1, and CYP7B1) displayed statistically similar mRNA levels in

Table 2. General and plasma biochemical parameters after chronic ingestion of ^{137}Cs

	Control	^{137}Cs-exposed
Final body weight (g)	558 ± 26	572 ± 19
Final liver weight (g)	18.5 ± 0.9	16.4 ± 0.7
Plasma		
Cholesterol (mM)	2.71 ± 0.19	2.66 ± 0.17
HDL Cholesterol (mM)	2.23 ± 0.2	2.14 ± 0.11
LDL Cholesterol (mM)	0.4 ± 0.05	0.36 ± 0.04
Triglycerids (mM)	1.48 ± 0.27	1.65 ± 0.16
Phospholipids (g/l)	1.96 ± 0.09	2.02 ± 0.09
ALT (U/l)	60.5 ± 9.6	49.2 ± 4.3
AST (U/l)	156 ± 21	146 ± 15
Direct Bilirubin (µM)	4.88 ± 0.27	4.65 ± 0.31
Total Bilirubin (µM)	5.19 ± 0.46	5.15 ± 0.4
γGT (U/l)	2.27 ± 0.42	2.87 ± 0.29
Creatinine (µM)	49.6 ± 1.7	52.9 ± 2.1
Urea (mM)	6.01 ± 0.17	6.17 ± 0.17
7α-hydroxycholesterol (ng/ml)	257 ± 61	576 ± 144

Data are expressed as means SEM (n = 10, except 7α-hydroxycholesterol: control n = 5, ^{137}Cs-exposed n = 9). HDL: high-density lipoprotein, LDL: low-density lipoprotein, ALT: alanine aminotransferase, AST: aspartate aminotransferase, gamma-GT: gamma glutamyltranspeptidase.

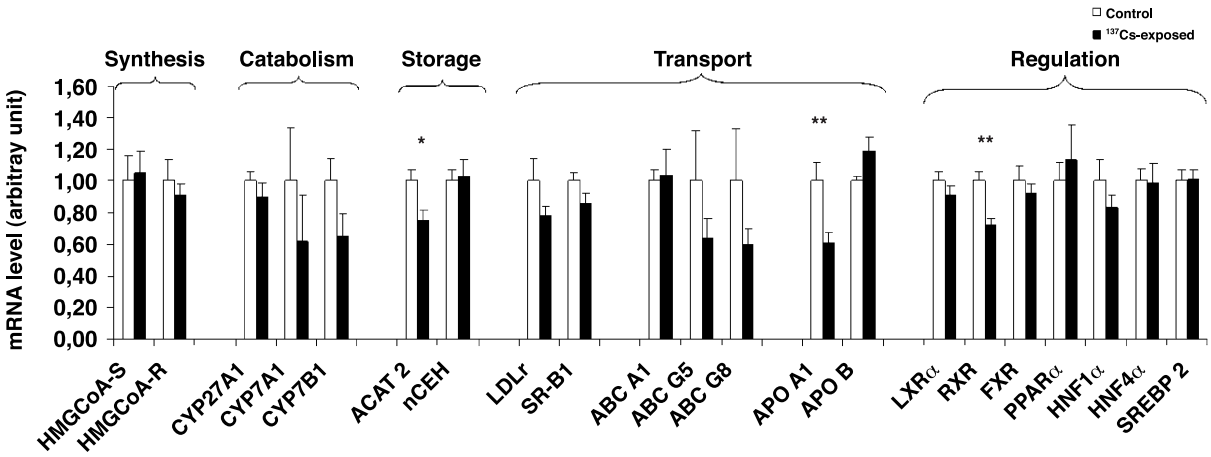

Fig. 1. Gene expression of the molecular actors of cholesterol metabolism in the liver after chronic contamination with ^{137}Cs. Results are expressed as a ratio to HPRT mRNA level. The mRNA levels of control rats were arbitrarily set at 1. Data are expressed as means ± SEM (n = 10). * and ** mark $p < 0.05$ and $p < 0.01$ respectively. HMG-S: 3-hydroxy-3-methylglutaryl Coenzyme A Synthase, HMG-R: 3-hydroxy-3-methylglutamyl Coenzyme A Reductase, ACAT 2: acylCoenzymeA: cholesterol acyltransferase, nCEH: hepatic neutral cholesteryl ester hydrolase, LDLr: low-density lipoprotein receptor, SR-B1: scavenger receptor class B type 1, ABC: adenosine triphosphate binding cassette transporter, APO: apolipoprotein, LXRα: liver-X-receptor alpha, RXR: retinoid-X-receptor, FXR: farnesoid-X-receptor, PPARα: peroxisome proliferator-activated receptor alpha, HNF 1α/4α: hepatocyte nuclear factor 1alpha/4alpha, SREBP 2: sterol regulatory element binding protein 2.

both groups.

The gene expression of AcylCoenzymeA: cholesterol acyltransferase 2 (ACAT 2), which esterifies free cholesterol into its storage form, statistically decreased of 25% ($p = 0.023$). Conversely, the hepatic neutral cholesteryl ester hydrolase (nCEH), which catalyses the reverse reaction, did not exhibit any significant modification of its mRNA level after ^{137}Cs exposure.

Lipoprotein receptors LDL-receptor (LDLr) and Scavenger Receptor type B class 1 (SR-B1), as well as adenosine triphosphate binding cassette transporters (ABC) A1, ABC G5 and ABC G8 had statistically similar mRNA level in ^{137}Cs-exposed and control groups. So did apolipoprotein (Apo) B, which is involved in LDL formation, whereas Apo A-I (involved in HDL formation) had its gene expression decreased of 39% ($p = 0.006$) after chronic administration of ^{137}Cs, as shown in Fig. 1.

Finally, concerning the studied transcription factors, retinoid-X-receptor (RXR) gene expression decreased significantly of 28% ($p = 0.001$). Conversely, liver-X-receptor α (LXRα), farnesoid-X-receptor (FXR), peroxisome proliferator-activated receptor α (PPARα), hepatocyte nuclear factor 1α (HNF 1α), HNF 4α and the sterol regulatory element binding protein 2 (SREBP 2) mRNA levels were not statistically different between the experimental and the control group.

Western Blot

The relative protein levels of hepatic RXR and Apo A-I were assessed by Western Blot (*cf.* Fig. 2). No significant difference was seen for either RXR (1.92 ± 0.19 for the ^{137}Cs-exposed rats *vs.* 2.53 ± 0.61 for the control rats) or Apo A-I (0.41 ± 0.11 and. 0.62 ± 0.12 for the experimental and the control groups respectively).

Hepatic cholesterol assay

The assessment of cholesterol hepatic concentration is represented in Fig. 3. The total cholesterol was measured (2.16 ± 0.31 mg/g liver for the ^{137}Cs-exposed vs. 2.02 ± 0.23 for the control rats) as well as the free cholesterol (1.82 ± 0.31 and 1.52 ± 0.16 mg/g liver for the experimental and control groups respectively). The esterified fraction corresponds to the difference between total and free cholesterol: the means for cholesteryl ester concentration in the liver are 0.35 ± 0.11 mg/g liver in the ^{137}Cs-treated group and 0.50 ± 0.10 in the sham-treated group. None of these values indicate a significant difference in cholesterol hepatic content after ^{137}Cs administration.

CYP7A1 and CYP27A1 specific activities

The specific activities for CYP7A1 and CYP27A1 (initiating both pathways for cholesterol catabolism into bile acids in the liver) are reported in Fig. 4. For CYP7A1, the ^{137}Cs-exposed rats displayed a specific activity of 8.76 ± 0.69 pmol/min/mg proteins *vs.* 10.19 ± 2.23 in the control rats, leading to no significant difference between both groups. Conversely, the specific activity of CYP27A1 displayed a significant increase of 66% after a chronic internal exposure to ^{137}Cs (22.36 ± 3.91 pmol/min/mg proteins and 13.43 ± 1.56 in ^{137}Cs-exposed and control rats respectively), as shown in Fig. 4.

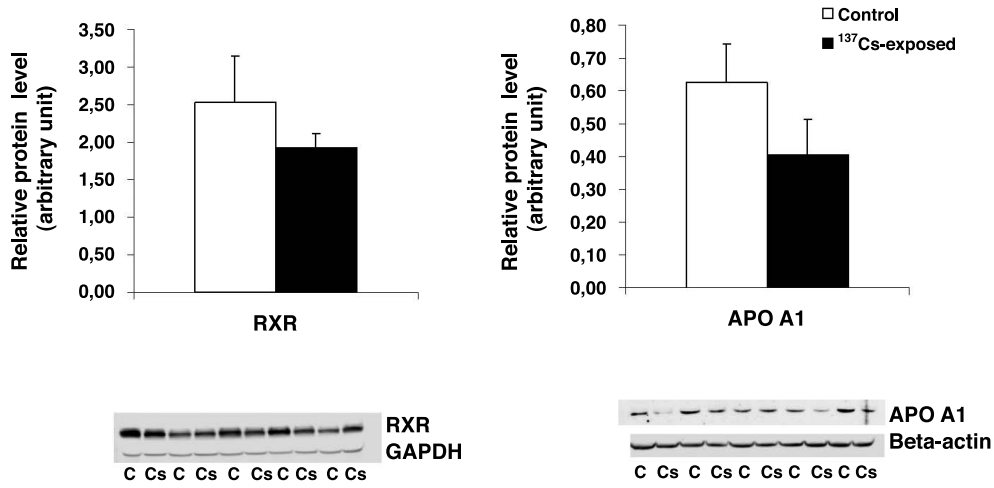

Fig. 2. Relative protein level of RXR and Apo A-I in the liver following exposure to ^{137}Cs. Results are expressed as a ratio to GAPDH protein level for RXR and to beta-actin protein level for Apo A-I. Data are expressed as means ± SEM (n = 5). C: Control, Cs: ^{137}Cs-exposed.

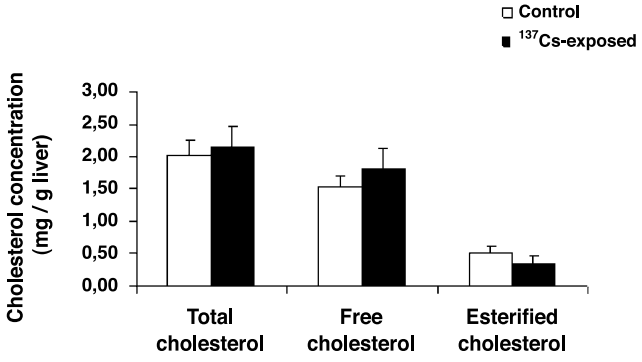

Fig. 3. Total, free and esterified cholesterol concentrations in the liver after ^{137}Cs chronic ingestion. Results are expressed in mg cholesterol/g liver. Data are expressed as means ± SEM (n = 7–9).

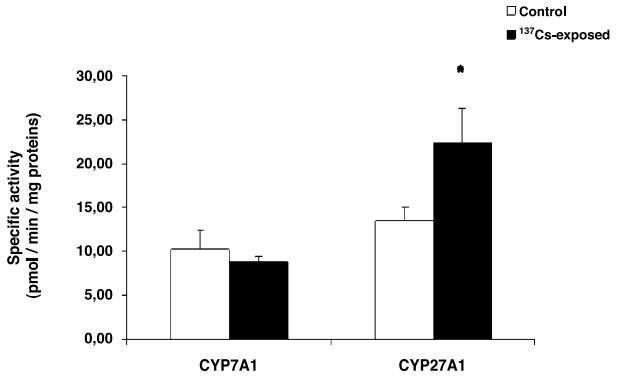

Fig. 4. CYP7A1 and CYP27A1 specific activities in the liver after chronic ingestion of ^{137}Cs. Results are expressed in pmol substrate/ min/mg of microsomal or mitochondrial proteins for CYP7A1 and CYP27A1 respectively. Data are expressed as means ± SEM (n = 6). * indicates a significant difference with $p < 0.05$.

DISCUSSION

There is a gap in the knowledge concerning the long-term sanitary consequences (especially the non-cancerous effects) of an internal exposure since fetal life to long-lived radionuclides, as generated by the Chernobyl accident. To address this, an experimental model was developed: rat pups were administered ^{137}Cs *in utero* and until the age of 9 months (approximately corresponding to 20 years of human life). The continuity of the contamination is ensured by the fact that ^{137}Cs crosses the placental barrier[30] and is transmitted to the pup through the dam's milk.[31] This is supported by the amount of ^{137}Cs in the livers of the exposed animals (5 Bq/g liver, *i.e.* 60 cGy for the whole liver in 10 months), whereas the ^{137}Cs hepatic concentration was under detection limit in the control rats. This value confirms the fact that the liver of the contaminated animals was exposed to a low level of ionizing radiations in this study.

Despite the accumulation of ^{137}Cs in the exposed rats, they were in a good general status: their food and water intakes, and their final body and liver weights did not differ from those of the untreated animals. This is noteworthy since a decrease in food intake and body weight gain is induced by a high-dose whole body gamma irradiation (*e.g.* at 1, 3, 5, and 8 Gy).[32,33] This external evaluation was confirmed by the levels of routine biochemical parameters: the plasma lipids profile and the hepatic profile were at similar levels with or without ^{137}Cs chronic internal administration. These results indicate a preservation of the lipid homeostasis and no declared hepatic toxicity, as opposed to the consequences of an external high dose gamma irradiation in rats: Pradeep *et al.* reported a decrease of plasma levels of ALT, AST, γGT as well as lactate dehydrogenase and alkaline

phosphatase at 1, 3 and 5 Gy and in correlation with the radiation level.[32)]

7α-hydroxycholesterol has been proposed as an early marker of liver impairment after external irradiation.[34)] In this work, the authors hypothesize that a change in 7α-hydroxycholesterol plasma level may foreshadow a hepatic alteration that is not yet detectable with the ALT and AST tests. Levels of plasmatic 7α-hydroxycholesterol in our study were not significantly different between the two groups, implying that no predictable liver alteration is induced in these experimental conditions.

Thus, the absence of significant difference in the plasma profile between control and exposed rats suggests that the chronic ingestion of a post-accidental level of ^{137}Cs has no effect on the lipid homeostasis at body level. Still, ^{137}Cs may induce modifications at organ level. Therefore, the five pathways of cholesterol hepatic metabolism (biosynthesis, storage, transport, catabolism and regulation) have been studied.

First of all, a gene expression study was conducted (*cf.* Fig. 1). Three of the main actors of cholesterol metabolism had decreased gene expression levels: ACAT 2, which esterifies free cholesterol into its storage form, Apo A-I, involved in HDL formation and cholesterol excretion, and retinoid-X-receptor (RXR), a heterodimer partner for many nuclear receptors regulating cholesterol metabolism and other systems.

The protein levels of Apo A-I and of RXR were not significantly different in the livers of ^{137}Cs-administered and control rats (*cf.* Fig. 2). This suggests that the modifications at gene level are of mild importance since they are not reflected on the protein level. It is however possible that the non-significance of this result is due to a limited statistical power. Indeed, the protein expression of Apo A-I and RXR decrease by 34% and 24% respectively, which is close to the 39% and 28% respective decrease in their gene expression. Nevertheless, even admitting that the protein and mRNA levels decrease coordinately, the expression of no other gene involved in RXR or Apo A-I functions is modified. If these changes had led to non-physiological modifications of a metabolic pathway, heterodimer partners to RXR (LXRα, FXR, PPARα, or others) and interacting partners of Apo A-I (such as ABC A1) would have been expected to undergo matching changes.

Concerning ACAT 2, the repercussion of its mRNA level decrease was assessed by the measurement of its product: esterified cholesterol. The hepatic concentration of esterified cholesterol was similar in both groups, as was the concentration of the free cholesterol fraction (*cf.* Fig. 3). Therefore, it is inferred that the decrease in the gene expression of ACAT 2 has no consequence on the global hepatic cholesterol homeostasis. Moreover, this conservation of the total cholesterol rate (corresponding to the sum of the free and esterified fractions) is in accordance with the absence of modification in the biosynthesis pathway and in the gene expression of nCEH, which produces free cholesterol from the esterified form.

A 66% increase of CYP27A1 specific activity was observed (*cf.* Fig. 4). Concurrently, no significant modification of CYP27A1 gene expression was recorded (*cf.* Fig. 1). Although there is no certainty about this, at least two hypotheses can be formulated. The first one would regard the possibility of a post-translational modification. The phosphorylation status has been described to influence the activity of cytochromes P450 (CYPs).[35)] Even if most studies concern xenobiotics-metabolizing CYPs, the modulation of cholesterol-catabolizing CYP7A1 activity by phosphorylation/dephosphorylation has been demonstrated in vitro.[36)] Moreover, Ghazarian *et al.* have shown that CYP27A1 activity is decreased after phosphorylation in a study using the mitochondria of chicken kidney.[37)] A second hypothesis to explain the discrepancy between the increased CYP27A1 activity and the unchanged mRNA level could lie in an interference with the enzyme degradation system, leading to a higher activity global level than needed. This could be triggered by an action at the proteasome level, or in the ubiquitination of the enzymes to be degraded. However, although ubiquitination is a known process for degradation of some xenobiotics-metabolizing CYPs,[35)] no study concerning CYP27A1 degradation is available in this regard.

Whatever the mechanism lying behind this discrepancy between the effect of ^{137}Cs chronic ingestion on CYP27A1 gene expression and specific activity, the issue of the biological significance of these results remains. Indeed, the specific activity of CYP27A1 (which initiates the alternative pathway for cholesterol catabolism into bile acids) is enhanced after a chronic ingestion of ^{137}Cs but that of CYP7A1 (initiating the classical pathway) is not. The combination of these two results suggest that the increase in CYP27A1 specific activity is probably a physiological adjustment, since no other changes in this metabolism support a direct effect of ^{137}Cs intake. Indeed, the alternative pathway is usually enhanced when the classical pathway is disrupted,[38)] which is not the case here. Moreover, it has been shown in a previous study that a whole body external gamma irradiation of 8 Gy in rats modifies the specific activities of several CYPs of the bile acids synthesis, including CYP7A1 but not CYP27A1.[39)]

In conclusion, this study assessed for the first time the effects of a chronic ingestion of a low level of ^{137}Cs since fetal life in rats. After 10 months of continuous internal exposure at 6.500 Bq/l, ^{137}Cs does not appear to induce hepatic toxicity and nor affect cholesterol hepatic metabolism in rats, neither at organ nor at body level. The observed molecular changes are of physiological range, and the rats seem to adapt to the lasting contamination. This long-term exposure to ^{137}Cs was started on a sensitive model: rat embryo. Yet, the effects of this type of exposure on more

sensitive individuals (already developing a lipidic pathology like hypercholesterolemia) are still unknown and would be helpful to ascertain the harmlessness of a chronic ingestion of ^{137}Cs on cholesterol hepatic metabolism.

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