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Molecular modifications of cholesterol metabolism in the liver and the brain after chronic contamination with cesium 137

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ABSTRACT

Twenty years after Chernobyl accident, the daily ingestion of foodstuff grown on contaminated grounds remains the main source for internal exposure to ionizing radiations, and primarily to cesium 137 (^{137}Cs). Though the effects of a long-term internal contamination with radionuclides are poorly documented, several non-cancerous pathologies have been described in this population. However, lipid metabolism was never investigated after chronic internal contamination although disturbances were observed in externally-exposed people. In this regard, we assessed the effects of a chronic ingestion of ^{137}Cs on hepatic and cerebral cholesterol metabolism. To mimic a chronically-exposed population, rats were given ^{137}Cs-supplemented water at a post-accidental dose (150 Bq/rat/day) during 9 months. The plasma profile, and brain and liver cholesterol concentrations were unchanged. A decrease of ACAT 2, Apo E, and LXR < alpha > mRNA levels was recorded in the liver. In the brain, a decrease of CYP27A1 and ACAT 1 gene expression was observed. These results clearly show that cholesterol metabolism is not disrupted by a chronic ingestion of ^{137}Cs, although several molecular alterations are observed. This work would be interestingly completed by studying the influence of ^{137}Cs in models likely more sensitive to contaminants, such as the fetus or individuals susceptible to a lipidic disease.

1. Introduction

The accident of the Chernobyl nuclear power plant on April 26th 1986 led to a major dispersion of radionuclides in the environment. Among them, cesium 137 (^{137}Cs) is the only one combining a high level of released activity (approximately 85×10^{15} Bq) and a long half-life (30 years) (IAEA, 2006). This combination makes it the prime source for a long-term exposure to ionizing radiations. Most people evacuated immediately after the explosion have soon settled back in contaminated lands, and have therefore been exposed to chronic internal radiations through food intake (Handl et al., 2003; Hoshi et al., 2000).

Twenty years after the accident, the health experts of the United Nations Chernobyl Forum, who assess Chernobyl's long-term sanitary consequences, deplore the lack of scientific knowledge concerning the health effects of a chronic internal contamination of a low dose of ionizing radiations (WHO, 2006). Indeed, the early studies addressing Chernobyl's long-term sanitary outcomes (Rozhinskaia et al., 1994; Zhavoronkova et al., 1996) were conducted on liquidators (cleanup workers on the radioactive site), who were submitted first of all to a very strong acute irradiation which biases the conclusions about chronic contamination alone. Recently, studies concerning people exposed to a chronic contamination alone have been published. Most of them mention an increase of cancers incidence (Likhtarov et al., 2005; Romanenko et al., 2003), but a number of studies focused on non-cancerous pathologies or health perturbations: birth defects (Lazjuk et al., 1997), disturbances of the immune system (DeVita et al., 2000) and an increase of cataracts (Sumner, 2007) affecting children, and cognitive decline linked with the received dose of ^{137}Cs (Gamache et al., 2005) have been described. Surprisingly, lipid metabolism was never considered although it is a crucial biological system. In order to fill this gap, an experimental study mimicking the exposure to ^{137}Cs of

Abbreviations: ^{137}Cs, Cesium 137; ABC, adenosine triphosphate binding cassette transporter; ACAT, acylCoenzymeA: cholesterol acyltransferase; ALT, alanine aminotransferase; Apo, apolipoprotein; AST, aspartate aminotransferase; CYP, cytochrome P450; FXR, farnesoid-X-receptor; 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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people living in contaminated territories was conducted on rats. The focus was set on cholesterol metabolism, for its impairments are linked to various hepatic (Kosters et al., 2003; O'Leary and Pratt, 2007) and neurological diseases (Vaya and Schipper, 2007; Vance, 2006).

Dietary ^{137}Cs is known to be efficiently absorbed (over 90%) in rats (Moore and Comar, 1962). Moreover, modifications on the metabolism of cholesterol-derived steroid hormones (Grignard et al., 2008) and vitamin D (Tissandie et al., 2006a) have been described after 9 and 3 months of ^{137}Cs ingestion, respectively. Concerning cholesterol metabolism itself, a first study was conducted with a sub-chronic internal contamination (Souidi et al., 2006), revealing subtle modulations by ^{137}Cs ingestion, notably at gene expression level.

To go further, the present work assesses the effects of a chronic (9 months, *ca.* 20 years of human lifetime) dietary contamination with ^{137}Cs on hepatic and cerebral cholesterol metabolism. Indeed, the liver is the only organ able to eliminate the excess cholesterol of the body. As for the brain, the cholesterol pool is independent from the rest of the body because the blood–brain barrier prevents any exchange of native cholesterol with the bloodstream. In both organs, synthesis, storage, catabolism, transport, and regulation pathways are analyzed.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats, aged 12 weeks, weighing 274 ± 2 g, were obtained from Charles River Laboratories (L'arbresle, France). They were housed in pairs upon arrival and allowed to recover from transportation for 2 weeks before the first day of contamination. During the experiment, they were maintained in a 12 h light/12 h dark cycle (regular cycle) at 21 ± 2 °C and 50 ± 10% humidity, with access to a standard pellet rodent diet and water ad libitum. For the experiment, the rats were divided into two groups (n = 10), control and ^{137}Cs-exposed respectively.

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 and organs removal

The contamination was carried out with ^{137}cesium chloride (^{137}CsCl, CAS number 20334-19-4) obtained from CERCA (Pierrelatte, France). The rats of the experimental group were exposed to ^{137}CsCl in their drinking water at a concentration of 6500 Bq/l (*ca.* 150 Bq/rat/day) for 9 months. This concentration is based on the top estimate of the ^{137}Cs dietary intake of the surrounding populations in the years following the Chernobyl accident (Coulon, 1994). The rats of the control group were given uncontaminated mineral water. The food and water intake of both groups was monitored on a weekly basis during the entire contamination time.

After 9 months, the rats were anesthetized by inhalation of 5% isoflurane (Abbot France, Rungis, France) and euthanized by intracardiac puncture to collect blood. Cerebral cortex and liver were immediately dissected on ice, deep-frozen in liquid nitrogen and stored at −80 °C.

2.3. Biochemical parameters assay

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 included total cholesterol, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, triglycerides, phospholipids, alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, total bilirubin and gamma-glutamyltranspeptidase (GGT).

2.4. Plasma oxysterols assay

The assay of plasma 7 < alpha > -hydroxycholesterol and 24(S)-hydroxycholesterol concentrations was performed according to Gueguen et al. (2006) on 0.5 ml of plasma samples. The oxysterols were separated and quantified by high performance liquid chromatography (Waters Symmetry, Saint-Quentin-en-Yvelines, France). 19-Hydroxy-3-acetate cholesterol (Sigma Diagnostics, L'Isle d'Abeau Chesnes, France) was added to each sample as an internal standard.

2.5. Cholesterol assay in the liver and the brain

Frozen samples (about 250 mg for liver and 30 mg for brain) were thawed and homogenized in 5 ml isopropanol using a potter with a Teflon pestle (VWR, Fontenay-sous-Bois, France). After incubation at 60 °C for 1 h 30 min and centrifugation at 3220*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) after appropriately diluting the samples. Esterified cholesterol was calculated as the difference between total and free cholesterol values.

2.6. Real-time PCR

Total RNA was extracted from 25 mg of liver samples using the RNeasy total RNA isolation Kit (Qiagen, Courtaboeuf, France) and from 30 mg brain samples using the RNEasy Lipid Tissue Mini Kit (Qiagen, Courtaboeuf, France), according to the manufacturer's respective recommendations.

Reverse transcription was performed in Sprint PowerScript PrePrimed 96 Plates (BD Biosciences Clontech, Erembodegem, Belgium) containing 1 μg of reverse transcriptase. The cDNA synthesis was conducted at 42 °C for 1 h and was ended by the inactivation of the reverse transcriptase after 10 min at 70 °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 7000 Sequence Detection System (Applied Biosystems, Courtaboeuf, France) according to the following run: incubation 2 min at 50 °C, 10 min at 95 °C for activation of the polymerase and 40 cycles of 15 s at 95 °C for denaturation and 1 min at 60 °C for annealing-extension. Results were normalized to hypoxanthine-guanine phosphoribosyltransferase (HPRT) and fold-inductions calculated relative to the control group. Sequences for the forward and reverse primers are listed in Table 1.

2.7. 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-Whithney Rank Sum Test when the equal variance test failed (determined by the SigmaStat software). Differences were considered statistically significant when $p \leqslant 0.05$.

3. Results

3.1. General health status and plasma biochemical parameters

Food and drink intake was not affected by chronic ingestion of ^{137}Cs (data not shown), and no difference was recorded in body weight (635.5 ± 16.5 g for control rats vs. 617.2 ± 15.5 g for contaminated rats) or in liver weight (22.6 ± 0.8 g *vs.* 20.8 ± 0.7 g). Macroscopic appearance of main organs (liver, lung, heart, intestines, kidney, brain and testis) from ^{137}Cs-exposed rats did not differ from those of control rats.

In the plasma, the lipid profile was unchanged (total cholesterol = 2.42 ± 0.17 mM for control rats *vs.* 2.42 ± 0.36 mM for ^{137}Cs-exposed rats, triglycerides = 2.23 ± 0.53 mM *vs.* 1.73 ± 0.18 mM, phospholipids = 1.76 ± 0.15 g/l *vs.* 1.91 ± 0.19 g/l). Blood concentrations of markers of liver integrity (ALT = 34.8 ± 5.4 U/l *vs.* 41.9 ± 5.2 U/l and AST = 71.0 ± 10.8 U/l *vs.* 71.9 ± 6.8 U/l) and liver function (GGT = 4.82 ± 0.57 U/l *vs.* 4.84 ± 0.53 U/l and total bilirubin = 3.55 ± 0.18 μM *vs.* 5.78 ± 1.84 μM) were also similar in control and ^{137}Cs-exposed rats. Finally, the plasmatic level of liver-specific 7 <alpha>- -hydroxycholesterol was unchanged between the two groups (117 ± 40 ng/ml *vs.* 109 ± 34 ng/ml), whereas the level of brain-specific 24(S)-hydroxycholesterol was under detection limit.

3.2. Gene expression study

3.2.1. Cholesterol synthesis, esterification, and catabolism

The expression of the main genes involved in cholesterol synthesis (3-hydroxy-3-methylglutaryl Coenzyme A Reductase (hmg-