Molecular biology and genetics / Biologie et génétique moléculaires

Transcriptomic effects of depleted uranium on acetylcholine and cholesterol metabolisms in Alzheimer's disease model

Effets transcriptomiques de l'uranium appauvri sur les métabolismes de l'acétylcholine et du cholestérol chez un modèle de maladie d'Alzheimer

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A R T I C L E   I N F O

A B S T R A C T

Some heavy metals, or aluminium, could participate in the development of Alzheimer disease (AD). Depleted uranium (DU), another heavy metal, modulates the cholinergic system and the cholesterol metabolism in the brain of rats, but without neurological disorders. The aim of this study was to determine what happens in organisms exposed to DU that will/are developing the AD. This study was thus performed on a transgenic mouse model for human amyloid precursor protein (APP), the Tg2576 strain. The possible effects of DU through drinking water (20 mg/L) over an 8-month period were analyzed on acetylcholine and cholesterol metabolisms at gene level in the cerebral cortex. The mRNA levels of choline acetyl transferase (ChAT) vesicular acetylcholine transporter (VAChT) and ATP-binding cassette transporter A1 (ABC A1) decreased in control Tg2576 mice in comparison with wild-type mice (respectively −89%, −86% and −44%, $p < 0.05$). Chronic exposure of Tg2576 mice to DU increased mRNA levels of ChAT (+189%, $p < 0.05$), VAChT (+120%, $p < 0.05$) and ABC A1 (+52%, $p < 0.05$) compared to control Tg2576 mice. Overall, these modifications of acetylcholine and cholesterol metabolisms did not lead to increased disturbances that are specific of AD, suggesting that chronic DU exposure did not worsen the pathology in this experimental model.

R É S U M É

Certains métaux lourds, ou l'aluminium, peuvent participer au développement de la maladie d'Alzheimer. L'uranium appauvri (UA), un autre lourd métal, module le système cholinergique et le métabolisme du cholestérol dans le cerveau de rats, mais sans troubles neurologiques. Le but de cette étude est de déterminer ce qui arrive chez des animaux exposés à l'UA et qui vont développer la maladie d'Alzheimer. Cette étude a été réalisée chez un modèle de souris transgénique pour le précurseur de la protéine amyloïde humaine (APP), la souche Tg2576. Les effets possibles de l'UA via l'eau de boisson (20 mg/ L) après huit mois de contamination ont été analysés sur les métabolismes de l'acétylcholine et du cholestérol au niveau génique dans le cortex cérébral. Les niveaux d'ARNm de la choline acétyl transferase (ChAT), du transporteur vésiculaire de

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l'acétylcholine (VAChT) et du transporteur cassette ATP-dépendant de type A1 (ABC A1) sont diminués chez la souris Tg2576 contrôle en comparaison à la souris de type sauvage (respectivement −89, −86 et −44 %, $p < 0,05$). L'exposition chronique de la souris Tg2576 à l'UA augmente le taux d'ARNm de la ChAT (+189 %, $p < 0,05$), de VAChT (+120 %, $p < 0,05$) et d'ABC A1 (+52 %, $p < 0,05$) en comparaison à des souris Tg2576 contrôles. Ces modifications sur les métabolismes de l'acétylcholine et du cholestérol n'entraînent pas d'augmentation des perturbations qui sont spécifiques de la maladie d'Alzheimer, suggérant ainsi que l'exposition chronique à l'uranium n'aggrave pas la pathologie, au moins dans ce modèle expérimental.

1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disease among senile dementia. AD brains are characterized by β-amyloid peptide (βA) accumulation. This is a consequence of the cleavage of amyloid precursor protein (APP) by α- and β-secretases. Moreover, the loss of the cholinergic system efficiency plays an important role in AD. Postmortem studies of AD patients consistently have demonstrated the loss of cortical cholinergic neurons and the decrease of the enzymatic activity of choline acetyltransferase (ChAT), the enzyme responsible for acetylcholine (ACh) synthesis [1]. This decrease of cholinergic function contributes to the severity of the cognitive and behavioral deficits, especially in the brain areas of memory and attention. In addition to this feature, numerous factors are involved in the development of AD, such as cholesterol metabolism and, in particular, the apolipoprotein E (ApoE) that plays a role in the formation of the βA burden [2,3].

A credible homologous model of AD should ideally display both the behavioural characteristics of the disease and its pathology. The Tg2576 mouse was developed by Hsiao et al. as an animal model of AD [4]. It bears the "Swedish" familial AD mutation, consisting of a double amino acid substitution in the amyloid precursor protein (APP), resulting in overproduction of βA. Studies demonstrated that these transgenic mice showed behavioural impairments, as well as pathophysiological characteristics of AD, including βA deposits, amyloid plaques, neuritic dystrophy and abnormal phosphorylation of protein tau (Tubular A Unit).

Some studies suggested that chronic contamination by metals could be responsible for the development of neurodegenerative disorders. For example, increased levels of mercury have been found in patients suffering from AD [5]. Furthermore, other studies suggested that the risk of developing AD was higher in areas where drinking water contains a high concentration of aluminium (between 0.1 and 0.2 mg/L) [6,7]. Uranium is another heavy metal, and because of its high density, it is widely used in military as well as in civilian applications and its utilization has spread almost worldwide. Uranium has a double toxicity: a radiological toxicity and a chemical toxicity [8]. In order to avoid radiological toxicity, depleted uranium (DU), with very weak radioactive activity, has been chosen in this study. Little information is available concerning the potential health hazard of chronic ingestion of low amounts of DU. Ingestion of DU may result in the manifestation of

toxicity in many parts of the body [9]. The most serious concern is DU toxicity in the kidneys [10], but more recently the central nervous system has also appeared as a sensitive target organ to DU exposure [11].

Previous studies have demonstrated that DU chronically ingested modifies the cholinergic system [12–14] and the brain cholesterol metabolism [15] in normal rats, but without neurological disorders. However, the question to know if DU could worsen the development of the pathology in organisms that will develop the Alzheimer disease is still open. The effects of a chronic ingestion of DU through drinking water (20 mg/L) over an 8-month period were analyzed on ACh and cholesterol metabolisms at gene expression level in the cerebral cortex of APP transgenic mice (Tg2576). In order to characterize the transgenic model, a comparison between wild-type and Tg2576 mice has been also made on cholinergic and cholesterol systems.

2. Materials and methods

2.1. Animals and contamination procedure

The transgenic APP mice used in this study contain the APP695 human gene, which was inserted into a hamster prion protein cosmid vector [4]. This gene with the Swedish mutation is known to be responsible for a familial form of AD [16]. The mice were supplied by Taconic US (Germantown, NY, USA).

Tg2576 ($n = 16$) male mice, aged 12–13 weeks, were housed 4 per cage for a period of 8 months. The animals were kept on a 12 h/12 h light/darkness cycle at 21 ± 2 °C. After a one-week acclimatization period, animals were distributed in two groups of 8 mice. One group of Tg2576 mice was exposed to depleted uranyl nitrate hexahydrate (98.74% ^{238}U, 0.26% ^{235}U, 0.001% ^{234}U, specific activity 1.4×10^4 Bq/g, AREVA-NC, France) at a dose of 20 mg/L (280 Bq/L) in their drinking water for 8 months. The second ("control") group of Tg2576 mice received mineral water. A third group, the wild-type mice ($n = 8$), has been constituted and drank mineral water for 8 months.

The water DU content was close to maximum uranium levels found naturally in some areas, such as southern Finland [17]. At the end of exposure, mice were 10.5 months old, corresponding to the time when behavioral impairments are just beginning to set in [18]. All groups were fed a regular rodent chow, with water and food ad libitum.

Throughout the experiment, body weight gain and food and water intake were measured weekly. After the contamination, the animals were anaesthetized and decapitated. The brain was removed and frontal cortex was dissected on ice. Samples were immediately deep-frozen in liquid nitrogen and stored at $-80\,^{\circ}$C until analysis.

All experiments were approved by the IRSN Animal Care Committee and complied with French legislation concerning animal experimentation (Ministry of Agriculture Act No. 87-848, October 19, 1987, amended May 29, 2001).

2.2. Real-time PCR

Total RNA was extracted with the RNeasy Lipid Tissue Mini-Kit (Qiagen, Courtaboeuf, France). The cDNA was produced from 1 µg of total RNA by reverse transcription with BD Sprint PowerScript PrePrimed 96 plate (BD Biosciences Clontech, Erembodegem, Belgium). Real-time PCR was performed on an AbiPrism 7900 Sequence Detection System (Applied Biosystems, Courtaboeuf, France) using SYBR Green technology, with 16 ng of template cDNA for each reaction. Thermoprofile conditions were: $50\,^{\circ}$C for 2 min, $95\,^{\circ}$C for 10 min and 40 cycles at $95\,^{\circ}$C for 15 s and $60\,^{\circ}$C for 1 min. PCR assays were done in duplicate. The comparative $\Delta\Delta C_T$-method was used for relative mRNA quantification [19]. Samples were normalized to hypoxanthine-guanine phosphoribosyltransferase (HPRT). Primer sequence of HPRT was found in the study of Ropenga et al., 2004 [20] and the other sequences were designed with PrimerExpress software and fold-inductions calculated relative to the Tg2576 control group. Sequences for the primers are indicated in Table 1.

2.3. Statistical analysis

Results are reported as means $\pm$ SEM. Statistical analyses were performed with Student's t-test. Differences were considered significant when $p < 0.05$, $p < 0.01$ or $p < 0.001$.

3. Results and discussion

The cholinergic system is among the classical neuron-modulation system of the brain. In AD, an early and progressive loss of cholinergic neurons has been described [21]. This loss has been suggested to cause the cognitive impairments observed in this neurodegenerative disease. In control Tg2576 mice, the mRNA levels of choline acetyl transferase (ChAT), the biosynthesis enzyme of ACh, and the vesicular ACh transporter (VAChT) are lowered (decrease of respectively 89 and 86%, $p < 0.01$) in comparison with the wild-type mice (Fig. 1A). Concerning ChAT, a decrease of the enzymatic activity has been previously described in AD [22]. Previous studies showed no significant difference between transgenic and wild-type mice for VAChT, but these results are not at gene expression level [23,24]. All others genes of the cholinergic system were not significantly modified in control Tg2576 mice in comparison to wild-type mice (Fig. 1A). These results suggest that ChAT and VAChT seem to be specific target genes in AD, at least in this AD model.

Conversely to the cholinergic system which appears as a consequence of the disease, cholesterol metabolism seems more and more evidently involved in the genesis of AD [25]. The cerebral cholesterol is independent from the body pool since cholesterol cannot efficiently cross the blood-brain barrier. Therefore, the brain must produce its own cholesterol endogenously and maintain its homeostasis. However, there is an intracerebral transport of cholesterol between glial and neuronal cells. Among these transporters, only the gene expression of ATP-Binding Cassette A1 (ABC A1) decreased significantly (-44%, $p < 0.001$) in Tg2576 mice in comparison to wild-type mice (Fig. 2A). The gene expression modulation of ABC A1 relates to the work of Wahrle et al. showing that an over-expression of ABC A1 protein decreases the precipitation of ßA peptides [26]. These results demonstrated that the decrease of ABC A1 is in agreement with the development

Table 1
Oligonucleotide sequences of primers used for real-time qPCR.

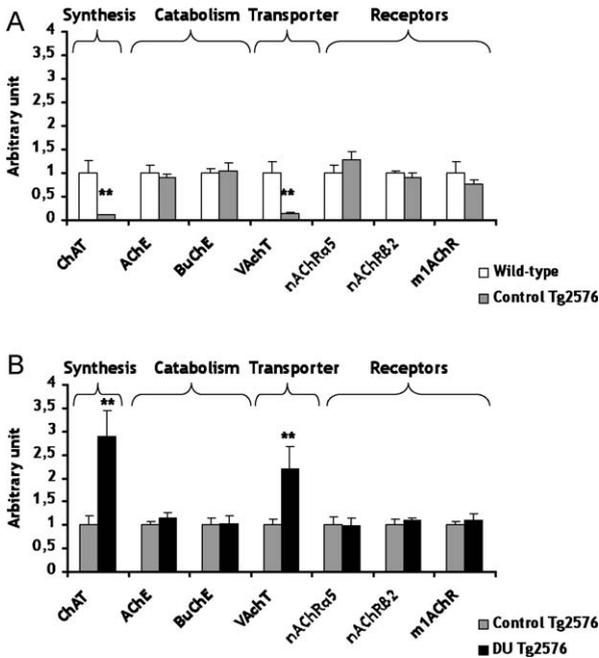| Gene	Foward	Reverse	Length (bp)	Gene accession #
HPRT	AAACTTTGCTTTCCCTGGTTA	AGGCTTTGTATTTGGCTTTTC	225	NM_013556.2
HMGR	TCGCTGGATAGCTGATCCTTCT	TTCGTCCAGACCCAAGGAAAC	73	NM_008255
HMGS	TACCTGCGGGCCTTGGAT	GAGGGTGAAAGGCTGGTTGTT	93	NM_008256
CYP46A1	CTGCATCGGCCAACAGTTT	CAATGGCTTGAGCGTAGCC	130	NM_010010
α-secretase	CGCCACAGCCCATTCAG	TCGCATGTGTCCCATTTGAT	68	NM_007399
β-secretase	CATTGCTGCCATCACTCAAT	CAGTGCCTCAGTCTGGTTGA	208	NM_011792
LXRβ	GATCGGATCCATGTCTTCTTCCCCCACAAGTTC	GATCCTCGAGTAAGATGACCACGATGTAGG	159	NM_009473
RXRα	CGCAAAGACCTGACCTACACC	TCCTCCTGCACAGCTTCCC	134	NM_011305
PPARγ	TCATGACCAGGGAGTTCCTCA	TCATCTAATTCCAGTGCATTGAACTT	103	NM_011146
SREBP 1c	CCAGAGGGTGAGCCTGACAA	CACTAAGGTGCCTACAGAGCAAGAG	116	NM_011480
ApoE	TGGCTACCAACCCCATCATC	TTGCAGGACAGGAGAAGGATACT	69	NM_009696
ABC A1	ATCTCATAGTATGGAAGAATGTGAAGCT	CGTACAACTATTGTATAACCATCTCCAAA	132	NM_013454
ABC G4	GGCCACCACTCCCAATCC	TCTATCCAGACCGAAGGCTTAGG	79	NM_138955
SR-B1	GTTGGTCACCATGGGCCA	CGTAGCCCCACAGGATCTCA	61	NM_016741
LDLr	TCTGCCCCAGGTTCTGTTTTAT	CAAGGAAATGAAGATGGGAGAAA	118	NM_010700
ChAT	GAGCGAATCGTTGGTATGACAA	AGGACGATGCCATCAAAAGG	72	NM_009891
VAChT	GGGTCGGCTCGGTCAATC	CAAATAGCACGCCTATCTTCACAT	146	NM_021712
nAChR α5	CAACATCCACCACCGCTCTT	TTTGGGAAGCTTGTGGAGAAAT	81	NM_176844
nAChR β2	TCGTCGCAAACCGCTCTT	GATGGCCAGCGAGGTGAT	69	NM_009602
m1AChR	CCCCTGGGTCACCTTCCT	TTCCCCGGGTTTCACTCTCT	100	NM_001112697
AChE	CCTGGATCCCTCGCTGAA	CCTGTGCGGGCAAAATTG	65	NM_009599
BuChE	GTTTTCTGCAGTGAGTGACAGGTATT	CAGAATCTGGATGCTGAAGTAGATAA	89	NM_009738

Fig. 1. Gene expression of biosynthesis and catabolism enzymes, transporters and receptors involved in cholinergic system within cerebral cortex of control Tg2576 mice (A) and DU Tg2576 mice (B). Results are expressed as a ratio to HPRT mRNA level. A. The levels of wild-type mice were arbitrarily set at 1. B. The levels of control Tg2576 mice were arbitrarily set at 1. Data are expressed as means ± SEM (n = 8 for each group), with **p < 0.01, significantly different from wild-type mice or control Tg2576 mice; DU: depleted uranium.

of the AD. All others genes of the cholesterol metabolism were not significantly modified in control Tg2576 mice in comparison to wild-type mice (Fig. 2A).

The development of AD could be perhaps linked to some heavy metals [5–7]. In light of this observation, we have considered to study the possible adverse consequences of a chronic exposure to DU in Tg2576 mice. Concerning the cholinergic system, the mRNA levels of ChAT and VAChT are increased (+189 and +120% respectively, p < 0.01) in Tg2576 mice exposed to DU in comparison with the control Tg2576 mice (Fig. 1B). Two enzymes participate to the catabolism of ACh, i.e. the acetylcholinesterase (AChE) and the butyrylcholinesterase (BuChE). The exposure of Tg2576 mice to DU did not induce significant modifications of mRNA levels of AChE and BuChE, but their enzymatic activities remain to be determined (Fig. 1B). Earlier studies performed on rats exposed to uranium produced apparently contradictory results on AChE. An increase of AChE activity was observed after a 1 mg/kg per day intramuscular injection of DU over a 7-day period [12], whereas a decrease of AChE activity was shown in entorhinal cortex [14] and no modification in the hippocampus [13] after chronic contamination of drinking water with DU (40 mg/L). The different contamination protocols (specific uranium salts, dose, exposure, duration, cerebral structure) used in these studies may explain this discrepancy. Concerning the gene expression of cholinergic receptors (α5nAChR, β2nAChR and m1AChR), none was significantly modified in Tg2576 exposed mice in comparison with control Tg2576 mice (Fig. 1B). Nevertheless, few investigations showed genic
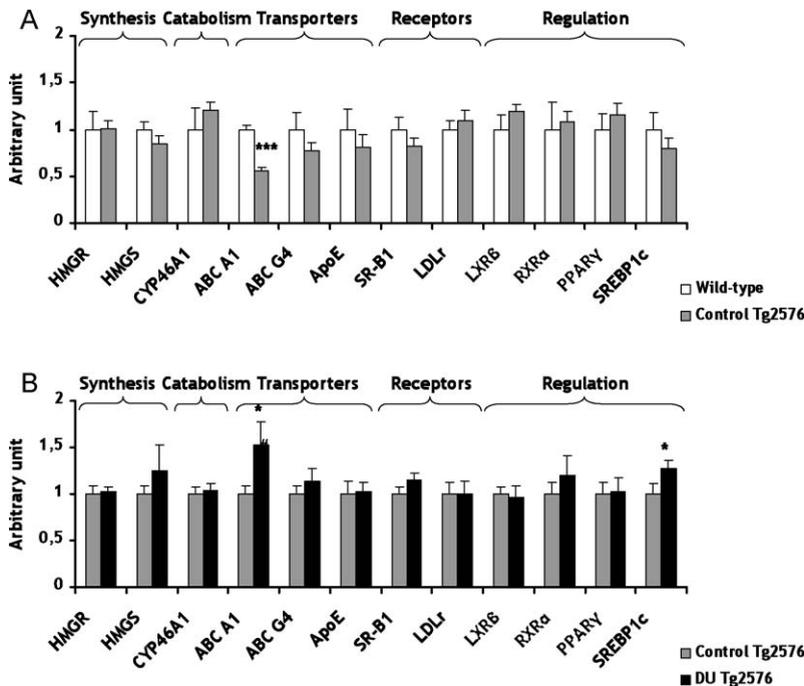

Fig. 2. mRNA levels of biosynthesis and catabolism enzymes, transporters, lipoprotein receptors and transcription factors/nuclear receptor involved in cerebral cholesterol metabolism of control Tg2576 mice (A) and DU Tg2576 mice (B). Results are expressed as a ratio to HPRT mRNA level. A. The levels of wild-type mice were arbitrarily set at 1. B. The levels of control Tg2576 mice were arbitrarily set at 1. Data are expressed as means ± SEM (n = 8 for each group), with ***p < 0.001, significantly different from wild-type mice and *p < 0.05, significantly different from control Tg2576 mice; DU: depleted uranium.

modulations of nicotinic and muscarinic receptors by heavy metals, such as mercury chloride and zinc exposures [27–29]. The results obtained in the present study suggest for the first time that chronic ingestion of DU in Tg2576 mice induces some modifications of the cholinergic pathway. This hypothesis must be confirmed in the future with other time of exposure and/or other DU doses.

Concerning cholesterol, a chronic internal contamination with DU during 8 months induced no significant effect on gene expression of two biosynthesis enzymes, 3-hydroxy-3-methylglutamyl Coenzyme A Synthase (HMGCoA Synthase) and HMGCoA Reductase (Fig. 2B). To prevent its excessive accumulation of cholesterol in the brain, cholesterol is converted into 24(S)-hydroxycholesterol by cholesterol-24-hydroxylase (CYP46A1), a brain-specific enzyme. This oxysterol being more hydrophilic than cholesterol, it can cross the blood-brain barrier more rapidly and join the general bloodstream [30]. A strong relationship between CYP46A1 and AD has been demonstrated [31]. After DU exposure, gene expression of CYP46A1 was unchanged in Tg2576 mice in comparison to control (Fig. 2B). Among proteins involved in transport of cholesterol between glial and neuronal cells, only the mRNA level of ABC A1 increased significantly (+52%, $p < 0.05$) in Tg2576 mice exposed to DU in comparison to control Tg2576 mice (Fig. 2B). This increase of ABC A1 is in agreement with a previous study performed on wild-type rats after 9 months of exposure to DU [15]. Gene expression of low-density-lipoprotein receptor (LDLr) and scavenger receptor class B type 1 (SR-B1), which both take up the cholesterol into the cell, were not modified in Tg2576 exposed mice compared to control Tg2576 mice (Fig. 2B). A previous work has demonstrated a significant increase of mRNA level of SR-B1 after chronic exposure in rat [15]. Further studies are necessary since this discrepancy between our data and those of Racine et al. cannot be explained by the only difference of species. Cholesterol metabolism is submitted to a very fine and intricate regulation at transcriptional level implying a number of nuclear receptors. In Tg2576 mice, a chronic exposure to DU increased mRNA levels of Sterol Regulatory Element-Binding Protein 1c (SREBP 1c) by 27% ($p < 0.05$) compared to non-exposed Tg2576 mice (Fig. 2B). This result is in accordance with previous studies showing that some nuclear receptors are affected by DU chronic internal exposure [32,33]. In our experimental conditions, other nuclear receptors, i.e. RXRα and PPARγ, were not significantly modified in Tg2576 exposed mice in comparison with control Tg2576 mice (Fig. 2B). All these results suggest that the global homeostasis of cholesterol in the brain is maintained in Tg2576 mice after DU exposure. Only gene expression of ABC A1 was significantly modified by DU. ABC A1 seems to be a target gene in since its expression is modified both in the pathological phenotype and after DU exposure, as for CAT and VAChT.

The homeostasis of essential metals such as copper, iron, selenium and zinc may be altered in the brain of subjects with AD [34]. The decreases of VAChT, ChAT and ABC A1 observed in Tg2576 mice in comparison to wild-type mice are reversible when Tg2576 mice were exposed to DU. A strong link between iron homeostasis and

uranium existed [35]. Previous studies have described transferrin and ferritin as uranium transporters [36]. Thus, iron and uranium are suspected to be transported by the same transporters. These studies suggest a possible competition between uranium and iron at the cellular level and could participate to the effects observed in the present study.

In conclusion, this study demonstrated for the first time that a chronic exposure by ingestion of a low dose of DU induces changes in the expression of genes implicated in the physiopathology of AD. ChAT, VAChT and ABC A1 mRNA levels are lowered in the pathological model and are modulated by DU exposure. These modulations induced by DU did not lead to the increased disturbances that are characteristic of declared AD, suggesting that chronic DU exposure did not amplified this pathology. The link between uranium and iron could perhaps participate in these effects. All these results were obtained from gene expression analyses only, and must be confirmed by assays of protein levels and enzymatic activities. Chronic ingestion of uranium did not induce any change in the size and density of senile plaques (personal data), suggesting that contamination did not worsen the pathology. In order to conclude more definitively on the harmlessness of low-dose chronic DU contamination on the development of the disease, it appears necessary to launch further experiments with longer contamination times. Finally, these results raise other issues, such as the effects of natural uranium, which is naturally present in drinking water and more radioactive than DU, on the development of the disease.

Acknowledgements

References

[1] M.M. Mesulam, The systems-level organization of cholinergic innervation in the human cerebral cortex and its alterations in Alzheimer's disease, Prog. Brain Res. 109 (1996) 285–297.

[2] J.F. Dartigues, C. Berr, C. Helmer, L. Letenneur, Épidémiologie de la maladie d'Alzheimer, Med. Sci. 18 (2002) 737–743.

[3] N.B. Chauhan, Membrane dynamics, cholesterol homeostasis, and Alzheimer's disease, J. Lipid. Res 44 (2003) 2019–2029.

[4] K. Hsiao, P. Chapman, S. Nilsen, C. Eckman, Y. Harigaya, S. Younkin, et al., Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice, Science 274 (1996) 99–102.

[5] C. Hock, G. Drasch, S. Golombowski, F. Muller-Spahn, B. Willershausen-Zonnchen, P. Schwarz, et al., Increased blood mercury levels in patients with Alzheimer's disease, J. Neural Transm. 105 (1998) 59–68.

[6] C.N. Martyn, D.J. Barker, C. Osmond, E.C. Harris, J.A. Edwardson, R.F. Lacey, Geographical relation between Alzheimer's disease and aluminum in drinking water, Lancet 14 (1989) 59–62.

[7] T.P. Flaten, Aluminium as a risk factor in Alzheimer's disease, with emphasis on drinking water, Brain Res. Bull. 55 (2001) 187–196.

[8] N.D. Priest, Toxicity of depleted uranium, Lancet 357 (2001) 244–246.

[9] E. Craft, A. Abu-Qare, M. Flaherty, M. Garofolo, H. Rincavage, M. Abou-Donia, Depleted and natural uranium: chemistry and toxicological effects, J. Toxicol. Environ. Health B Crit. Rev. 7 (2004) 297–317.

[10] R.W. Leggett, The behavior and chemical toxicity of U in the kidney: a reassessment, Health Phys. 57 (1989) 365–383.

[11] P. Lestaevel, P. Houpert, C. Bussy, B. Dhieux, P. Gourmelon, F. Paquet, The brain is a target organ after acute exposure to depleted uranium, Toxicology 212 (2005) 219–226.

[12] M.B. Abou-Donia, A.M. Dechkovskaia, L.B. Goldstein, D.U. Shah, S.L. Bullman, W.A. Khan, Uranyl acetate-induced sensorimotor deficit and increased nitric oxide generation in the central nervous system in rats, Pharmacol. Biochem. Behav. 72 (2002) 881–890.

[13] C. Bussy, P. Lestaevel, B. Dhieux, C. Amourette, F. Paquet, P. Gourmelon, et al., Chronic ingestion of uranyl nitrate perturbs acetylcholinesterase activity and monoamine metabolism in male rat brain, Neurotoxicology 27 (2006) 245–252.

[14] H. Bensoussan, L. Grandcolas, B. Dhieux-Lestaevel, O. Delissen, C.M. Vacher, I. Dublineau, P. Voisin, P. Gourmelon, M. Taouis, P. Lestaevel, Heavy metal uranium affects the brain cholinergic system in rat following sub-chronic and chronic exposure, Toxicology 261 (2009) 59–67.

[15] R. Racine, Y. Gueguen, P. Gourmelon, G. Veyssiere, M. Souidi, Modifications of the expression of genes involved in cerebral cholesterol metabolism in the rat following chronic ingestion of depleted uranium, J. Mol. Neurosci. 38 (2009) 159–165.

[16] M.N. Rossor, S. Newman, R.S. Frackowiak, P. Lantos, A.M. Kennedy, Alzheimer's disease families with amyloid precursor protein mutations, Ann. N. Y. Acad. Sci. 695 (1993) 198–202.

[17] R. Juntunen, Etelä-Suomen kallioporakaivojen uraani-ja radontukimukset (Uranium and radon in wells drilled into bedrock in southern Finland. Report of Investigation, Geological Survey of Finland 98, 1991.

[18] D.L. King, G.W. Arendash, Behavioral characterization of the Tg2576 transgenic model of Alzheimer's disease through 19 months, Physiol. Behav. 75 (2002) 627–642.

[19] N. Brink, M. Szamel, A.R. Young, K.P. Wittern, J. Bergemann, Comparative quantification of IL-1ß, Il-10, IL-10r, TNF-α and IL-7 mRNA levels in UV-irradiated human skin in vivo, Inflamm. Res. 49 (2000) 290–296.

[20] A. Ropenga, A. Chapel, M. Vandamme, N.M. Griffiths, Use of reference gene expression in rat distal colon after radiation exposure: a caveat, Radiat. Res. 161 (2004) 597–602.

[21] L.M. Bierer, V. Haroutunian, S. Gabriel, P.J. Knott, L.S. Carlin, D.P. Purohit, et al., Neurochemical correlates of dementia severity in Alzheimer's disease: relative importance of the cholinergic deficits, J. Neurochem. 64 (1995) 749–760.

[22] R. Quirion, Cholinergic markers in Alzheimer disease and the auto-regulation of acetylcholine release, J. Psychiatry Neurosci. 18 (1993) 226–234.

[23] J.T. Gau, M.L. Steinhilb, T.C. Kao, C.J. D'Amato, J.R. Gaut, K.A. Frey, et al., Stable beta-secretase activity and presynaptic cholinergic markers during progressive central nervous system amyloidogenesis in Tg2576 mice, Am. J. Pathol. 160 (2002) 409–411.

[24] M. Klingner, J. Apelt, A. Kumar, D. Sorger, O. Sabri, J. Steinbach, et al., Alterations in cholinergic and non-cholinergic neurotransmitter receptor densities in transgenic Tg2576 mouse brain with beta-amyloid plaque pathology, Int. J. Dev. Neurosci. 21 (2003) 357–369.

[25] L.A. Shobab, G.Y. Hsiung, H.H. Feldman, Cholesterol in Alzheimer's disease, Lancet Neurol. 4 (2005) 841–852.

[26] S.E. Wahrle, H. Jiang, M. Parsadanian, J. Kim, A. Li, A. Knoten, et al., Overexpression of ABCA1 reduces amyloid deposition in the PDAPP mouse model of Alzheimer disease, J. Clin. Invest. 118 (2008) 671–682.

[27] B. Hsiao, D. Dweck, C.W. Luetje, Subunit-dependent modulation of neuronal nicotinic receptors by zinc, J. Neurosci. 21 (2001) 1848–1856.

[28] A. Mirzoian, C.W. Luetje, Modulation of neuronal nicotinic acetylcholine receptors by mercury, J. Pharmacol. Exp. Ther. 302 (2002) 560–567.

[29] R. Bonfante-Cabarcas, I. Bravo, C. Nello, E. Gutiérrez-Reyes, N.E. Loureiro Dos Santos, et al., Pharmacological doses of Zn2+ induce a muscarinic cholinergic supersensitivity, J. Biomed. Sci. 9 (2002) 639–644.

[30] I. Bjorkhem, Crossing the barrier: oxysterols as cholesterol transporters and metabolic modulators in the brain, J. Intern. Med 260 (2006) 493–508.

[31] N. Bogdanovic, L. Bretillon, E.G. Lund, U. Diczfalusy, L. Lannfelt, B. Winblad, et al., On the turnover of brain cholesterol in patients with Alzheimer's disease. Abnormal induction of the cholesterol-catabolic enzyme CYP46 in glial cells, Neurosci. Lett. 314 (2001) 45–48.

[32] M. Souidi, Y. Gueguen, C. Linard, N. Dudoignon, S. Grison, C. Baudelin, et al., In vivo effects of chronic contamination with depleted uranium on CYP3A and associated nuclear receptors PXR and CAR in the rat, Toxicology 214 (2005) 113–122.

[33] E. Tissandié, Y. Guéguen, J.M. Lobaccaro, L. Grandcolas, P. Voisin, J. Aigueperse, et al., In vivo effects of chronic contamination with depleted uranium on vitamin D3 metabolism in rat, Biochim. Biophys. Acta. 1770 (2007) 266–272.

[34] L. Gerhardsson, T. Lundh, L. Minthon, E. Londos, Metal concentrations in plasma and cerebrospinal fluid in patients with Alzheimer's disease, Dement Geriatr. Cogn. Disord. 25 (2008) 508–515.

[35] H. Berradi, J.M. Bertho, N. Dodoignon, A. Mazur, L. Grandcolas, C. Baudelin, S. Grison, P. Voisin, P. Gourmelon, I. Dublineau, Renal anemia induced by chronic ingestion of depleted uranium in rats, Toxicol. Sci. 103 (2008) 397–408.

[36] J.R. Cooper, G.N. Stradling, H. Smith, S.E. Ham, The behaviour of uranium-233 oxide and uranyl-233 nitrate in rats, Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med. 41 (1982) 421–433.