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PAPER

Protection and safety of a repeated dosage of KI for iodine thyroid blocking during pregnancy

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Abstract

In case of nuclear power plant accidents resulting in the release of radioactive iodine (^{131}I) in large amounts, a single intake of stable iodine is recommended in order to prevent ^{131}I fixation to the thyroid gland. However, in situations of prolonged exposure to ^{131}I (e.g. Fukushima-Daiichi natural and nuclear disaster), repetitive administration of iodine may be necessary to ensure adequate protection, with acceptable safety in vulnerable populations including pregnant women. Here we conducted toxicological studies on adult rats progeny following prolonged exposure to potassium iodide (KI) *in utero*. Pregnant Wistar rats were treated with 1 mg kg d^{-1} KI or saline water for 2 or 4 d either between gestation days gestational day (GD) GD 9–12, or GD13–16. Plasma samples from the progeny were tested 30 d post-weaning for clinical biochemistry, thyroid hormones, and anti-thyroid antibody levels. Thyroid and brain were collected for gene expression analysis. The hormonal status was similar for the mothers in all experimental conditions. In the offspring, while thyroid-stimulating hormone and anti-thyroid peroxidase (anti-TPO) antibody levels were similar in all groups, a significant increase of FT3 and FT4 levels was observed in GD9–GD10 and in GD13–GD14 animals treated for 2 d, respectively. In addition, FT4 levels were mildly decreased in 4 d treated GD13–16 individuals. Moreover, a significant decrease in the expression level of thyroid genes involved in iodide metabolism, TPO and apical iodide transporter, was observed in GD13–GD14 animals treated for 2 d. We conclude that repeated KI administration for 2–4 d during gestation did not induce strong thyroid toxicity.

1. Introduction

In the event of a nuclear accident, radioactive iodine (I^{131}) may be released in a plume, or cloud, contaminating the environment, thus resulting in external exposure. Inhalation of contaminated air and ingestion of contaminated food and drinking water may lead to internal radiation exposure and uptake of radioactive iodine mainly by the thyroid. In the absence of appropriate radiation protection countermeasures, this exposure may bring about thyroid cancer, especially during childhood or foetal development [1–4]. Indeed, a dramatic increase in the incidence of thyroid cancer (mainly papillary type) has been observed in the territories most contaminated by the fallout from the Chernobyl accident in Belarus, Ukraine and Russia [5]. A large majority of individuals who developed thyroid cancer (90%) were under five years old, or exposed *in utero* at the time of the disaster [6]. To prevent thyroid cancer, a single dose of 130 mg of potassium iodine (KI) is recommended to saturate the thyroid gland and block I^{131} uptake, while a second dose is possible in adult, but not in children and pregnant women [7]. However, in a situation of prolonged I^{131} release or difficulties to evacuate, as in Fukushima-Daiichi natural and nuclear disaster, repeated KI intake may be necessary for adequate protection of the thyroid gland [4, 8]. Studies on the safety of repeated KI administration still lacking. Numerous studies, have been published addressing the safety of a

single dose KI administration in several preclinical models [9–11]. Scientific evidence base on repetitive iodine thyroid blocking (ITB), showed no adverse effects following repeated treatment with sodium iodide 100 mg for 12 d on adult men and women [12]. A recent study in a preclinical model showed that repeated prophylaxis of 1 mg kg d^{-1} KI over 8 d had no adverse effects in adult male rats [13]. In these preclinical settings, the repeated KI treatment did not impact thyroid hormone metabolism and thyroid expression of key regulators of the Wolff–Chaikoff effects. Based on these preclinical results and complementary good laboratory practices (GLP) studies that have been necessary for (that were necessary to), the modification of the marketing authorisation (MA) for KI was approved very recently by national health medical authority (ANSM) (Agence nationale de sécurité du médicament et des produits de santé) for a repeated prophylaxis of daily 130 mg up to 7 d for adults and adolescents over 12 years [14]. To date, we do not have sufficient knowledge of repeated stable iodine prophylaxis in sensitive population especially pregnant women and foetus.

Iodine is essential for the synthesis of thyroid hormone, including during pregnancy [15, 16]. Maternal thyroid hormones have essential roles in foetal brain development, regulating both morphological and biochemical changes before the onset of foetal thyroid function [17]. Moderate and severe iodine deficiency during pregnancy may lead to insufficient maternal thyroid hormone, subsequently causing potential adverse effects on the neurological and cognitive functions of the offspring [16]. In the other side, one consequence of excessive iodine intake is the blocking of thyroid hormone synthesis (Wolff–Chaikoff effect), which may lead to hypothyroidism [18]. However, iodine excess does not necessary translate in deleterious effects in animal experimentation, and there are very few studies that treats repeated prophylaxis at KI. Moreover, study on non-human primates showed that repeated sodium iodide treatment of 6.5 mg kg d^{-1} during pregnancy over 11 d, had no impact on the endocrinological parameters of the mothers and their progeny [19]. By contrast, a repeated prophylaxis of 1 mg kg d^{-1} KI over 8 d on pregnant female rat induced congenital hypothyroidism and neurotoxicity of their progeny [20]. May be the duration of the treatment was not appropriate, or the choice of foetal development window was not adequate (mid-gestation/late gestation). This study propose to explore the potential effects in terms of thyroid dysfunction of the progeny exposed to repeated KI treatment during gestation at different foetal developmental windows, and different treatment time (2 or 4 d) through biochemical, hormonal and genic parameters in rats (during pregnancy/post weaning) and their progeny.

2. Material and methods

2.1. Experimental procedure

2.1.1. Ethical approval

Animal experimentation was approved by the Animal Care Committee of the Institute of Radiation Protection and Nuclear Safety and conforms to French regulations (Ministry of Agriculture Act No.87–848, 19 October 1987, modified 29 May 2001).

2.1.2. Animals and treatments

The 0.9% NaCl (pH 7.4) and potassium iodine solution 0.35 g l^{-1} were manufactured and provided by the Central Pharmacy of Armed Forces (Orleans, France).

Animals were housed individually upon arrival and allowed to recover from transportation for one week. Rats were kept in regular light/dark schedule (12 h/12 h), at 21 ± 2 °C and 50 ± 10% humidity. Food (0.3 mg l kg^{-1} of pellet; A04-10 SAFE, Augy, France) and water were freely accessible.

This study included 64 pregnant *Wistar* rats (Charles River laboratories) divided in four groups (16 rats/group). Two periods of prophylaxis are tested. The first one, between the 9th and the 12th gestational day (GD) including a first group (G1) treated by gavage with either KI at 1 mg kg d^{-1} or saline water for two days and a second group (G2) treated with either KI or saline water for four days. The second period is between 13th and the 16th GD with a group (G3) treated with either KI or saline water for two days and a group (G4) treated with either KI or saline water for four days (figure 1).

After weaning, male progeny were divided into two groups for each prophylactic design (24 male/group): progeny not exposed *in utero* to KI and progeny exposed *in utero* to KI. Male progeny are kept in regular light/dark schedule (12 h/12 h), at 21 ± 2 °C and 50 ± 10% humidity. Food (0.3 mg l kg^{-1} of pellet; A04-10 SAFE, Augy, France) and water were freely accessible.

2.1.3. Samples collection

Two days after treatment pregnant rats were transferred to individual metabolic cages for 12 h, getting access to diet and water ad libitum. The progeny are placed under same conditions 30 d after weaning. Urine was collected and stored at −80 °C.

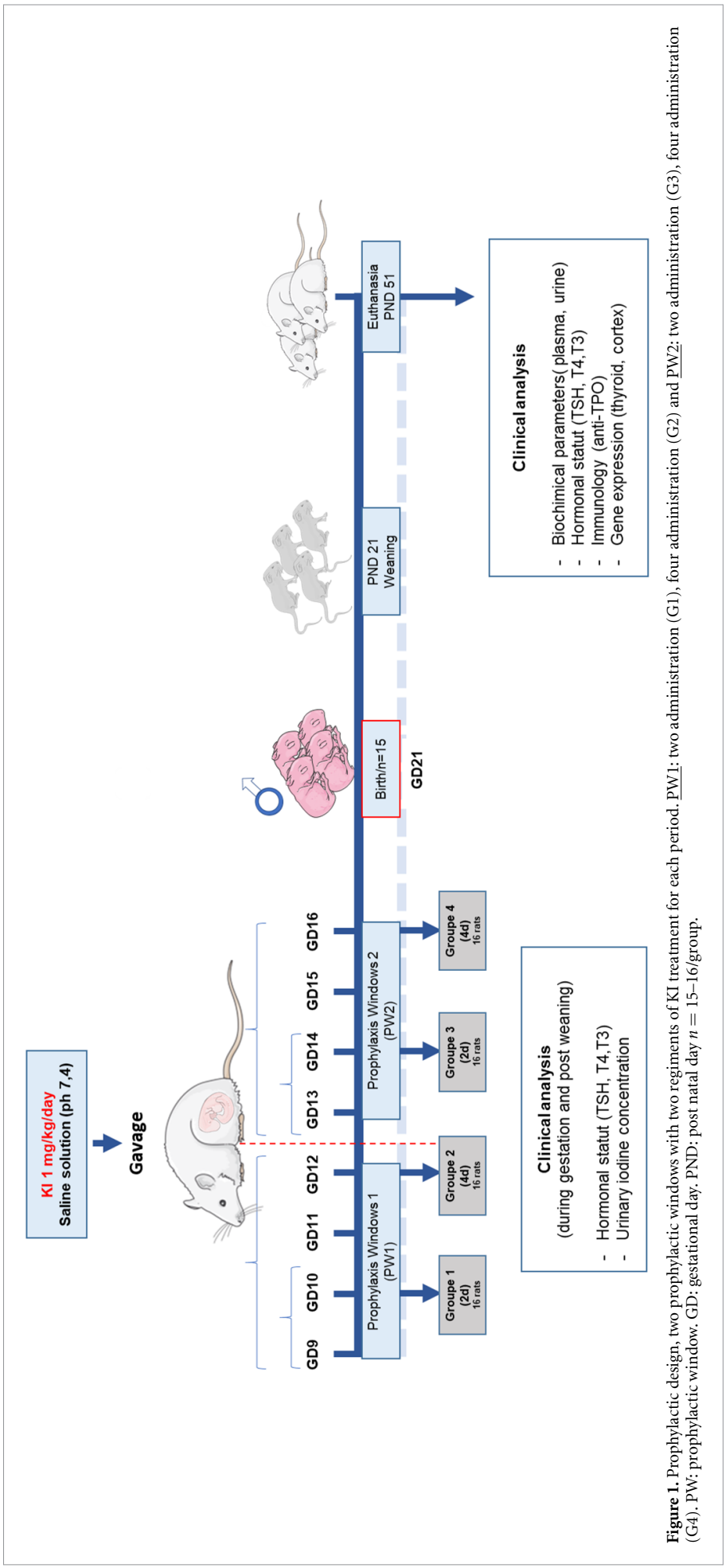

Figure 1. Prophylactic design, two prophylactic windows with two regiments of KI treatment for each period. PW1: two administration (G1), four administration (G2) and PW2: two administration (G3), four administration (G4). PW: prophylactic window. GD: gestational day. PND: post natal day $n = 15–16$/group.

During pregnancy, blood was collected by intravenous puncture in tail vein under isoflurane anaesthesia and then by intracardiac puncture at endpoint for rats and their progeny. After centrifugation 3000 rpm 10 mn, plasma is collected and stored at −80 °C.

Thyroid and brain from progeny were harvested, instantly deep-frozen in liquid nitrogen and stored at −80 °C.

2.2. Technical procedure

2.2.1. Urinary iodine concentration measurement

From 600 μl sample, urinary iodine measurements are based on colorimetric ceric-arsenic assays (Sandell and Kolthoff method). This assay is based on the catalytic effect of iodide in the redox reaction between yellowcerium (IV) and arsenic (III), to yield the colourless cerium (III) and arsenic (V). The reduction in the yellow cerium is measured spectrophotometrically at 410 nm. IODOLAB, MARCY l'ETOILE, France.

2.2.2. Biochemical assays

Plasma and urine biochemical parameters were measured with a spectrophotometric system (Konelab20i; Thermo Fischer Scientific, France) using calibrators and reagents recommended by manufacturer (Thermo Fischer Scientific, France).

The biomarkers measured in plasma were aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) for hepatic function, creatinine, urea and total proteins for renal function, iron, glucose, total cholesterol, total bilirubin for metabolism; and calcium/potassium (electrolytes).

The biomarkers measured in urine were glucose, total proteins, creatinine, urea, uric acid, calcium and potassium for renal function and metabolism.

2.2.3. Hormonal parameters assays

Plasma thyroid-stimulating hormone (TSH) was determined with the TSH rat ELISA kit for rats (MP Biomedicals, Illkirch-Graffenstaden, France). Plasma free triiodothyronine (FT3) and free thyroxine (FT4) levels were determined by immunoassay on an IMMULITE 2000 system from Siemens (Saint-Denis, France), VEBIO laboratory (Arcueil France).

The analytical sensitivities of TSH, FT3, and FT4 are 0.1 ng ml^{-1}, 2.83 pmol l^{-1}, and 1.5 pmol l^{-1} respectively.

2.2.4. Thyroid autoimmunity marker

Plasma anti-Thyroid antibodies was determined with anti-thyroid peroxidase antibody (TPO-AB) bioassay TM ELISA kit (Rat) (Bluegene E02T0531). The analytical sensitivities of anti-TPO are 1 ng ml^{-1} respectively.

2.2.5. Real time polymerase chain reaction (PCR) analysis

Total ribonucleic acid (RNA) was extracted from both thyroid lobes, an average 25 mg of cortex ($N = 12$/group), using mirVana™ miRNA Isolation Kit (Ambion, Cat. No. 1560). The 1 μg of total RNA was reversely transcribed using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Courtaboeuf, France) according to the manufacturer's instructions. Real-time qPCR was performed with quantStudio 12 K Flex Real-Time PCR System (Thermo Fisher scientific, Cergy Pontoise, France). Taqman primers (applied biosystems, Courtaboeuf, France) were used to analyse the mRNA levels in thyroid [13], cortex and cerebellum (table 1). PCR cycles used for cDNA amplification are designed as described: hold stage 95 °C, PCR stage: step 1; 95 °C; 1 s step 2; 60 °C; 20 s for 40 cycles. Relative changes of genes mRNA expression in KI exposed samples were calculated using 2–ΔΔCt method; GAPDH and ACTB were used as housekeeping genes. Results are expressed as mean ± standard error of the mean (SEM).

2.3. Statistical analysis

Results were expressed as mean ± SEM. One-way analysis of variance was used to compare each parameter across the different groups. All p-values in the different statistical analysis sections were corrected for multiple testing using Benjamini and Hochberg procedure [21]. A value of $p < 0.05$ was considered significant.

3. Results

3.1. Mother's results

As shown in table 2, urinary iodine concentration was similar between the groups treated and not treated with KI, suggesting the absence of impact of *in utero* KI exposure on iodine metabolism.

In addition, repeated iodine administration resulted in non-significant modulations of blood TSH levels in all groups (table 3). Similarly, no significant variations were observed for circulating FT4 and FT3 during

Table 1. Taqman primers used for genes expression analysis through real-time PCR.

Genes/brain/cortex	Assay ID number	Function
Tbr2 T-box transcription factor Eomes 2.	Rn01746545_m1	Plays a role in brain development. Functions in trophoblast differentiation and later in gastrulation [32].
MBP Myelin basic protein	Rn01399619_m1	Has a role in formation and stabilisation of the myelin membrane in the CNS [33].
MOBP Myelin-associated oligodendrocytic	Rn01445606_m1	Plays a role in compacting or stabilising the myelin sheath [34].
MAG Myelin-associated glycoprotein	Rn01457782_m1	Adhesion molecule that mediates interactions between myelinating cells and neurons [35].
PLP1 Proteolipid protein 1	Rn01410490_m1	Plays an important role in the formation or maintenance of the multilamellar structure of myelin [36].
ARC Activity regulated cytoskeleton associated protein	Rn00571208_m1	Regulates synaptic plasticity by promoting endocytosis of AMPA receptors (AMPARs) in response to synaptic activity [37].
Kcna1 Potassium voltage-gated channel subfamily a member	Rn00597355_m1	Mediates transmembrane potassium transport in excitable membranes, primarily in the brain and the central nervous system [38].
APRC5 Actin protein related complex subunit 5	Rn01759260_m1	Component of the Arp2/3 complex The Arp2/3 complex mediates the formation of branched actin networks in the cytoplasm, providing the force for cell motility [39].
CamkIV Calcium/calmodulin- dependent protein kinase	Rn00664802_m1	Mediates the control of a large number of enzymes, ion channels and other proteins by Ca2 [40].
Genes/thyroid	**Assay ID number**	**Function**
GAPDH: glyceraldehyde-3-phosphate dehydrogenase	Rn01775763_g1	Housekeeping genes [41]
B actine: actine, beta	Rn00667869_m1	Housekeeping genes [41]
NIS (Slc5a5): sodium/iodine symporter	Rn00583900_m1	Iodide uptake [42]
TPO: thyroid peroxidase	Rn00571159_m1	Key enzyme in thyroid hormone biosynthesis. It catalyses both iodination and coupling of iodotyrosine residues in TG [43].
Tg: thyroglobulin	Rn00667257_g1	Substrate for the synthesis of the thyroid hormones [44].
AIT (SLC5a8): apical iodide transporter	Rn01503812_m1	Iodide efflux [45]
DUOX2: dual oxidase 2	Rn00666512_m1	Generates hydrogen peroxide which is required for the activity of thyroid peroxidase/TPO [46].
MCT8 (Slc16a2): monocarboxylate transporter 8	Rn00596041_m1	Thyroid hormones transport and release [47].
TSH-R: thyrotropin receptor	Rn00563612_m1	Activates all functional aspects of the thyroid cell [48].
DIO2: iodothyronine deiodinase 2	Rn00581867_m1	Responsible for the deiodination of T4 (3,5,3′,5′-tetraiodothyronine) [49].

pregnancy and post-weaning, except a significant increase of FT4 (20%) and significant decrease of FT3 (26%) for treated individuals in G4 (table 3). These results showed the limited impact of repeated KI administration on the pituitary thyroid axis in pregnant adults' rats.

3.2. Progeny's results

3.2.1. General health parameters and biochemical status

As shown in table 4, body weight of male progeny at post natal day 51(PND 51) was affected by the *in utero* exposure to KI only for G1 animals in comparison to control group (+9.5%, $p < 0.05$). Thyroid organ coefficient, defined as thyroid weight/body weight, as well as brain organ coefficient, defined as brain weight/body weight, were not significantly different between individuals exposed and not exposed to KI from all groups. While plasma markers of liver were not affected for all groups exposed to KI in comparison to controls, we observed a significant increase for some metabolic markers in KI-treated individuals of G2 (glucose +18%, calcium +13%) and G4 (glucose +15%, total proteins +9%), ($p < 0.05$) (table 4). Urine markers analysis revealed an increase of potassium (+36%) and uric acid levels (+49%) in treated

Table 2. Urinary iodine concentration 30 d post treatment in female rats PW1: early two administration (G1), early four administration (G2) and PW2: late two administration (G3), late four administration (G4). Data are expressed as mean ± SEM, $n = 10$/group.

Group	Treatment	Urinary inorganic iodine $\mu g\,l^{-1}$
G1	Control	500.6 ± 214.17
	KI	414.3 ± 85.58
	P-value	0.39
G2	Control	384.9 ± 110.51
	KI	391.1 ± 69.88
	P-value	0.91
G3	Control	287.5 ± 104.02
	KI	323.3 ± 101.42
	P-value	0.58
G4	Control	207.3 ± 28.02
	KI	220.1 ± 49.12
	P-value	0.59

individuals of G1 (table 4). Overall, marginal modifications were found for a limited number of biochemical parameters in the progeny after repeated *in utero* exposure to KI.

3.2.2. Evaluation of the hormonal status

As shown in figure 2 repeated KI administration during foetal life resulted in non-significant modulation of blood TSH levels for all four groups in comparison to controls. Nonetheless, we find that *in utero* repeated exposure to KI resulted in a significant increase of circulating FT3 (+41%) and FT4 levels (+13%) in G1 and G3, respectively, as well as a significant decrease of FT4 in G4 individuals (−11%), in comparison to controls (figure 3) ($p < 0.05$).

3.2.3. Assessment of autoimmunity

Data presented in figure 4 shows that *in utero* repeated KI intake resulted in non-significant variations of TPO antibody level for all groups in comparison to non-exposed control animals, suggesting the absence of autoimmune reaction.

3.2.4. Thyroid gene expression analysis

The expression level of genes involved in thyroid hormones synthesis was not significantly different between the progeny exposed and non-exposed to KI during pregnancy (figure 5). However, we observed a significant decrease in the expression level of genes involved in iodide metabolism for the individuals of the G3, TPO (−47%) and AIT (−60%) ($P < 0.05$, figure 5).

3.2.5. Brain gene expression analysis

The expression of genes involved in transcriptional regulation (Tbr2), myelination (MBP—MOBP—MAG and PLP1), synaptogenesis (ARC—Kcna1 and APRC5), and maturation (CamkIV) in the cortex was similar between the progeny exposed and non-exposed to KI during pregnancy (figure 6).

4. Discussion

Knowledges on the toxicity of a repeated ITB in pregnant women or the offspring are very scarce. Recent preclinical studies conducted in our laboratory have demonstrated that KI prophylaxis at 1 mg kg d^{-1} for 8 consecutive days in pregnant rats, resulted in cognitive impairment in PND 51 offspring, associated with discreet changes in TSH and FT4 levels and specific metabolites and lipids [20, 22]. This work suggests that changes in thyroid hormone (TH) levels induced by repeated exposure to KI for 8 d *in utero* could have an impact on central nervous system (CNS) development, which could result in behavioural disorders in the offspring. Based on these observations, we wished to investigate more in detail the modalities of repeated KI administration regarding the duration of the prophylaxis and the period of foetal development considered, in order to define an optimal repeated prophylaxis during pregnancy. Thus, we studied different KI treatment durations of 2 or 4 consecutive days, covering the foetal development windows GD9–GD12 and GD13–GD16. We first studied the impact of this treatment in mothers, on TSH, which is the most sensitive and earliest parameter to be examined for primary thyroid disorders [19], and on thyroid hormones, which are used to assess thyroid function. We did not observe any changes in TSH levels during gestation and at weaning in the mothers under any experimental conditions, suggesting that repeated treatment with 1 mg KI over a short period (2–4 d) during gestation does not induce a central response involving TSH regulation. On

Table 3. Pregnant rats endocrinological profile during gestation and post weaning PW1: early two administration (G1), early four administration (G2) and PW2: late two administration (G3), late four administration (G4). Data are expressed as mean ± SEM. *: $P < 0.05$, $n = 10$/group.

		During pregnancy			Post weaning		
Group	Treatment	TSH ng ml^{-1}	FT4 pmol l^{-1}	FT3 pmol l^{-1}	TSH ng ml^{-1}	FT4 pmol l^{-1}	FT3 pmol l^{-1}
G1	Control	0.78 ± 0.15	18.84 ± 2.22	4.31 ± 0.28	1.72 ± 0.72	19.0 ± 2.66	4.27 ± 1.12
	KI	0.75 ± 0.16	19.19 ± 1.89	4.64 ± 0.85	2.05 ± 0.85	20.79 ± 4.49	4.11 ± 1.03
	P-value	0.35	0.36	0.24	0.21	0.65	0.82
G2	Control	0.91 ± 0.19	15.8 ± 1.91	3.84 ± 0.57	1.42 ± 0.26	19.5 ± 2.88	3.75 ± 1.14
	KI	0.82 ± 0.15	16.8 ± 2.59	4.2 ± 0.78	1.98 ± 0.83	18.6 ± 1.73	4.15 ± 1.00
	P-value	0.18	0.25	0.24	0.06	0.65	0.65
G3	Control	0.73 ± 0.13	14.45 ± 1.79	5.59 ± 0.35	1.54 ± 0.74	18.6 ± 1.45	7.23 ± 1.46
	KI	0.82 ± 0.21	15.94 ± 1.78	5.24 ± 0.34	1.08 ± 0.33	17.8 ± 1.47	6.98 ± 2.62
	P-value	0.15	0.13	0.09	0.15	0.65	0.82
G4	Control	1.34 ± 0.19	12.35 ± 1.97	5.38 ± 1.08	1.79 ± 0.49	17.99 ± 1.03	5.43 ± 1.84
	KI	1.38 ± 0.19	14.84 ± 1.46	3.97 ± 0.54	1.94 ± 0.48	15.87 ± 2.06	4.33 ± 2.09
	P-value	0.37	**0.04***	**0.04***	0.58	0.44	0.65

Table 4. Progeny's general and biochemical parameters. TW: thyroid weight, CW: cortex weight PW1: early two administration (G1), early four administration (G2) and PW2: late two administration (G3), late four administration (G4). Data are expressed as mean ± SEM. *: $P < 0.05$, $n = 15$/group.

		F1/G1		F1/G2		F1/G3		F1/G4	
Function	Parameters	Control	KI	Control	KI	Control	KI	Control	KI
(a) General indicators	Final body weight (g)	230 ± 14.76	**252 ± 17.91***	227 ± 14.8	233 ± 27.1	260 ± 14.9	267 ± 24.3	277 ± 24.1	265 ± 16.4
	TW/BW ratio	$1.9 \times 10^{-04} \pm 2.6 \times 10^{-04}$	$7.51 \times 10^{-05} \pm 4.5 \times 10^{-05}$	$6.9 \times 10^{-05} \pm 1.5 \times 10^{-05}$	$8.7 \times 10^{-05} \pm 7.7 \times 10^{-05}$	$9.2 \times 10^{-05} \pm 1.2 \times 10^{-04}$	$4.8 \times 10^{-05} \pm 2.3 \times 10^{-05}$	$6.82 \times 10^{-05} \pm 1.8 \times 10^{-05}$	$6.49 \times 10^{-05} \pm 1.88 \times 10^{-05}$
	CW/BW ratio	$6.5 \times 10^{-04} \pm 1.2 \times 10^{-04}$	$6.5 \times 10^{-04} \pm 2.5 \times 10^{-04}$	$9.4 \times 10^{-04} \pm 2.1 \times 10^{-04}$	$1.0 \times 10^{-03} \pm 3.3 \times 10^{-04}$	$9.9 \times 10^{-04} \pm 1.6 \times 10^{-04}$	$8.7 \times 10^{-04} \pm 2.6 \times 10^{-04}$	$9.9 \times 10^{-04} \pm 1.88 \times 10^{-04}$	$1.25 \times 10^{-03} \pm 2.16 \times 10^{-04}$
(b) Plasma biomarkers									
1. Metabolic markers	Cholesterol (mmol l^{-1})	1.56 ± 0.20	1.62 ± 0.17	1.58 ± 0.20	1.75 ± 0.27	1.52 ± 0.23	1.57 ± 0.23	1.53 ± 0.17	1.55 ± 0.17
	Total proteins (g l^{-1})	55.36 ± 4.51	57.25 ± 2.46	56.74 ± 2.59	57.37 ± 4.50	55.8 ± 3.61	52.8 ± 3.38	50.44 ± 3.32	**55.09 ± 2.63***
	Glucose (mM)	11.93 ± 2.09	11.25 ± 0.80	11.34 ± 1.93	**13.37 ± 0.96***	11.4 ± 1.14	12.2 ± 1.72	10.19 ± 1.12	**11.76 ± 0.95***
	Bilirubin T (μl)	7.63 ± 3.87	5.59 ± 2.25	7.30 ± 4.76	5.03 ± 1.74	6.3 ± 2.42	5.5 ± 1.46	7.76 ± 4.13	7.81 ± 4.12
	Iron (μM)	39.67 ± 5.83	45.18 ± 8.65	42.17 ± 6.93	42.29 ± 6.65	37.9 ± 5.33	33.9 ± 6.22	34.45 ± 7.33	37.39 ± 7.43
2. Electrolytes	Calcium (mmol l^{-1})	2.55 ± 0.30	2.53 ± 0.29	2.45 ± 0.27	**2.77 ± 0.20***	2.62 ± 0.23	2.5 ± 0.25	2.32 ± 0.24	2.51 ± 0.18
	Potassium (mmol l^{-1})	4.88 ± 0.34	5.08 ± 0.81	6.28 ± 0.54	5.74 ± 0.60	6.15 ± 1.02	5.15 ± 0.80	6.45 ± 1.02	6.02 ± 1.29
3. Liver markers	ALAT (U/L)	27.83 ± 7.21	23.67 ± 2.35	30.24 ± 12.41	26.04 ± 3.08	25.8 ± 4.61	23.06 ± 6.16	20.19 ± 5.26	28.26 ± 11.57
	ASAT (U/L)	124.95 ± 47.69	102.46 ± 27.15	125.29 ± 87.26	103.48 ± 32.55	97.7 ± 23.81	87.7 ± 19.10	78.34 ± 18.77	86.20 ± 19.11
	ASAT/ALAT ratio	4.47 ± 1.08	4.21 ± 1.27	3.91 ± 0.88	3.94 ± 1.12	3.96 ± 0.95	4.14 ± 1.75	3.75 ± 1.01	3.21 ± 0.97
4. Kidney markers	Creatinine (μM)	43.42 ± 4.15	40.66 ± 3.79	41.94 ± 4.33	45.20 ± 2.86	43.03 ± 3.66	43.2 ± 1.97	40.31 ± 3.57	38.99 ± 8.39
	Urea (Mm)	4.31 ± 0.42	3.88 ± 0.77	3.99 ± 0.67	4.27 ± 0.43	4.14 ± 0.66	3.61 ± 0.52	3.71 ± 0.42	3.98 ± 0.61

(Continued.)

Table 4. (Continued.)

Function	Parameters	F1/G1		F1/G2		F1/G3		F1/G4	
		Control	KI	Control	KI	Control	KI	Control	KI
(c) Urine biomarkers									
1. Electrolytes	Potassium mmol/24 h)	0.98 ± 0.36	**1.34 ± 0.26***	1.21 ± 0.41	1.45 ± 0.45	0.88 ± 0.19	0.89 ± 0.22	1.05 ± 0.29	0.97 ± 0.26
	Calcium (μmol/24 h)	27.1 ± 18.59	26.29 ± 14.77	38.38 ± 22.05	45.27 ± 23.19	27.09 ± 18.59	26.29 ± 14.77	50.38 ± 30.89	47.11 ± 25.51
2. Miscellaneous	Urinary proteins mg/24 h	8.59 ± 3.4	10.24 ± 3.52	15.33 ± 5.49	15.88 ± 4.96	8.68 ± 5.50	10.66 ± 2.82	8.79 ± 3.69	6.85 ± 3.32
	Uric acid (μM/24 h)	7.21 ± 1.46	**10.75 ± 3.21***	10.55 ± 3.65	11.51 ± 3.97	9.79 ± 1.46	9.11 ± 1.05	10.17 ± 2.78	10.08 ± 1.16
	Urea (Mm/24 h)	4.18 ± 1.55	4.87 ± 1.43	5.31 ± 1.93	5.90 ± 2.03	4.32 ± 0.59	3.95 ± 0.52	4.52 ± 1.44	4.39 ± 0.8
	Creatinine (μM/24 h)	54.41 ± 10.09	58.83 ± 22.6	54.21 ± 19.49	59.53 ± 23.64	48.49 ± 6.92	47.18 ± 4.91	52.32 ± 13.51	48.71 ± 3.68
	Glucose (mM)	7.99 ± 2.03	10.57 ± 3.22	12.2 ± 5.32	14.05 ± 5.49	10.23 ± 1.79	10.72 ± 1.59	10.37 ± 3.16	10.14 ± 1.38

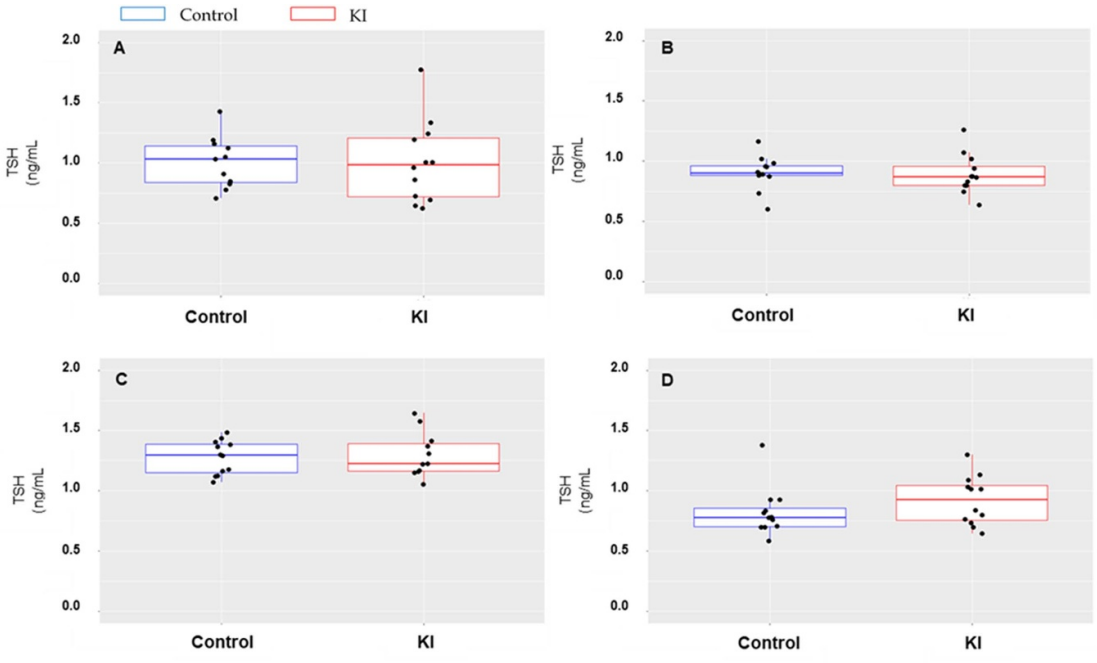

Figure 2. Plasma levels of thyrotropine (TSH) Data are expressed as mean ± SEM. Triiodothyronine (FT3). PW1: early two administration (G1), early four administration (G2) and PW2: late two administration (G3), late four administration (G4). Data are expressed as mean ± SEM. $N = 20$/group (A): G1, (B): G2, (C): G3, (D): G4.

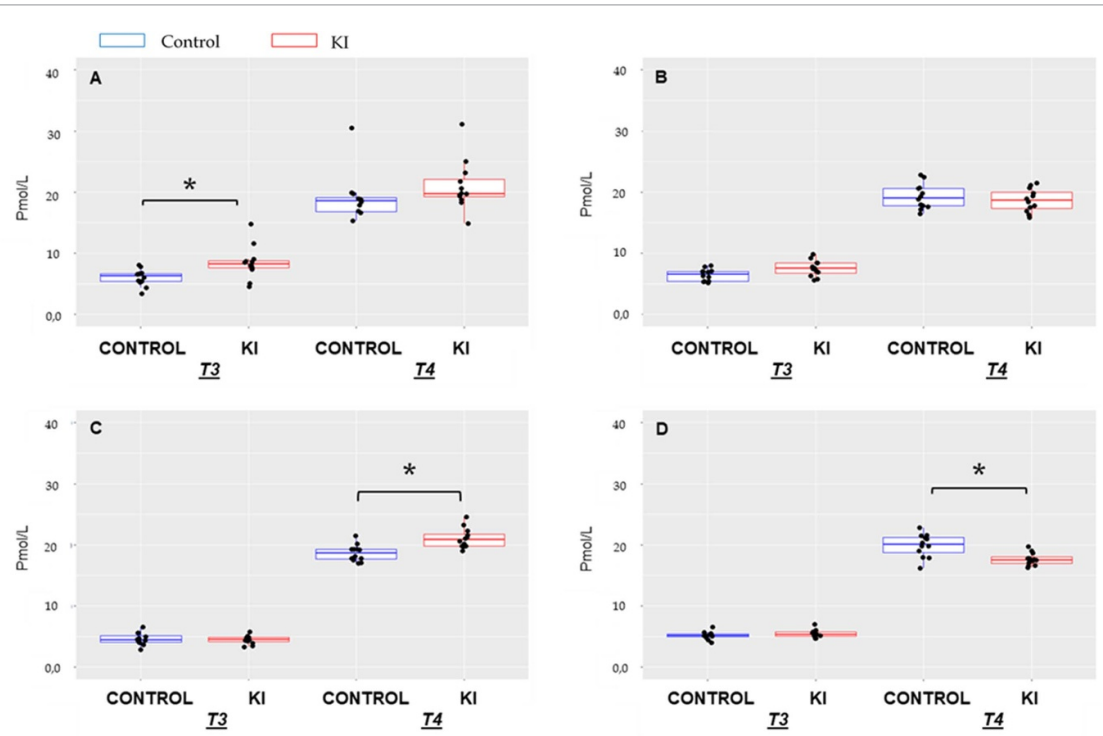

Figure 3. Plasmatic levels of hormones free thyroxine (FT4) and free triiodothyronine (FT3) and the for the progeny exposed *in utero* to KI, PW1: early two administration (G1), early four administration (G2) and PW2: late two administration (G3), late four administration (G4). Data are expressed as mean ± SEM. $^{*}p < 0.05$ vs control. $N = 15$/group (A): G1, (B): G2, (C): G3, (D): G4.

the other hand, we observed a slight modification of thyroid hormones (increase in FT4 and decrease in FT3) in pregnant rats 24–48 h after treatment over the period GD13–GD16 (group 4). However, these changes were transient, and FT3 and FT4 levels returned to normal concentration at weaning. These results are not consistent with previous data that have demonstrated thyroid dysfunction in rats, mice and men exposed to

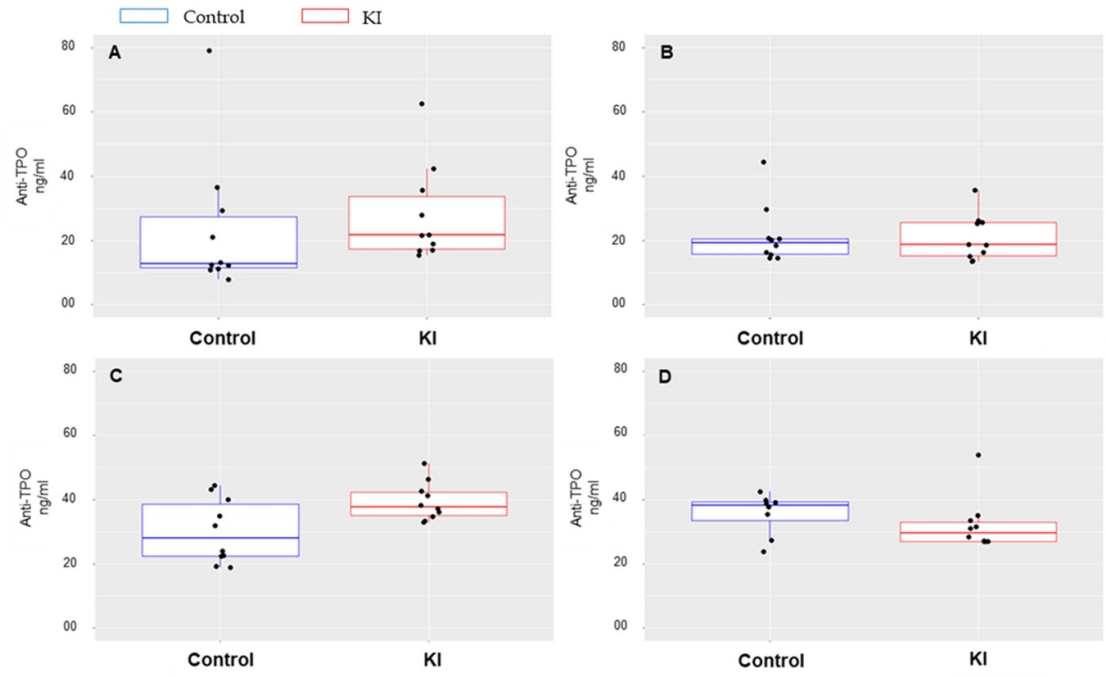

Figure 4. Plasmatic levels of TPO-AB for the progeny exposed *in utero* to KI PW1: early two administration (G1), early four administration (G2) and PW2: late two administration (G3), late four administration (G4). Data are expressed as mean ± SEM. $N = 15$/group (A): G1, (B): G2, (C): G3, (D): G4.

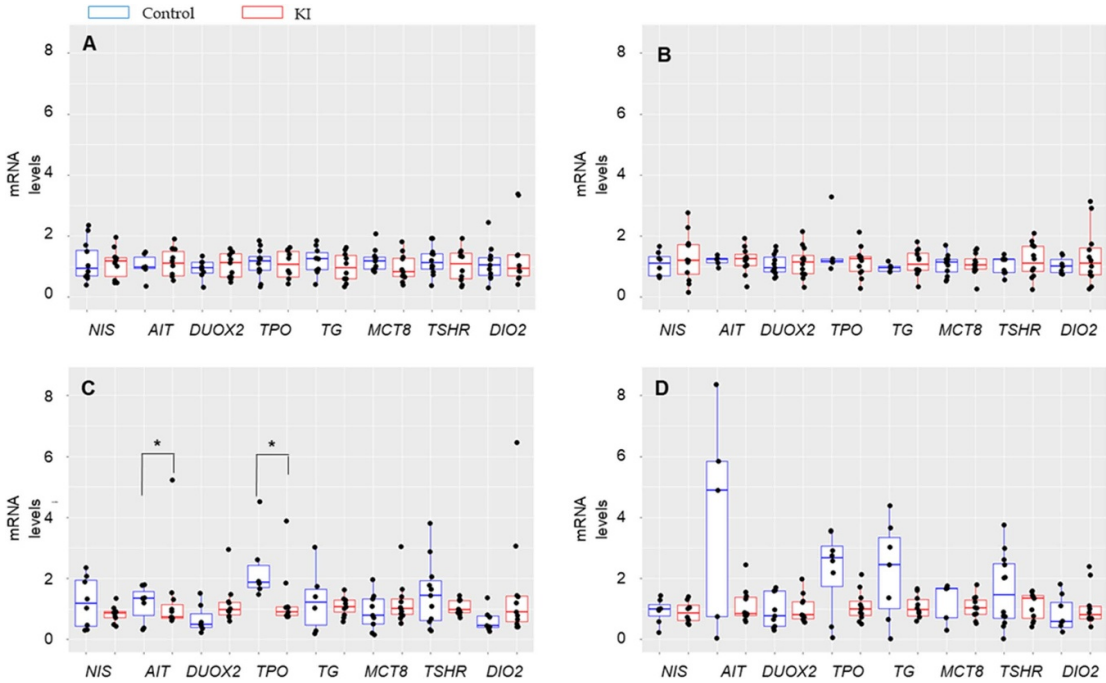

Figure 5. mRNA expression level of thyroid genes involved in iodine transport (NIS—PDS and AIT), iodine organification (TPO—DUOX2 and Tg), thyroid hormone transport (MCT8) and thyroid control (TSHR) PW1: early two administration (G1), early four administration (G2) and PW2: late two administration (G3), late four administration (G4). $N = 15$/group (A): G1, (B): G2, (C): G3, (D): G4. The results are expressed as a ratio to GADPH and ACTB mRNA level. Data are expressed as mean ± SEM.

high doses of iodine [18, 23–25]. In particular, chronic treatment with NaI at a dose of 0.6 or 7.3 mg l^{-1} during gestation and lactation in rats has been shown to induce a significant decrease in FT3 and FT4 serum levels associated with a significant increase in TSH [25]. This could be due to the difference of duration and method of KI administration adopted in the two studies. The study of Serrano *et al* consisted on chronic treatment in drinking water covering the entire period of gestation and lactation. In contrast the present work, proposes a short KI treatment (1 mg kg d^{-1}) of 2–4 d during gestation by gavage. In addition, our

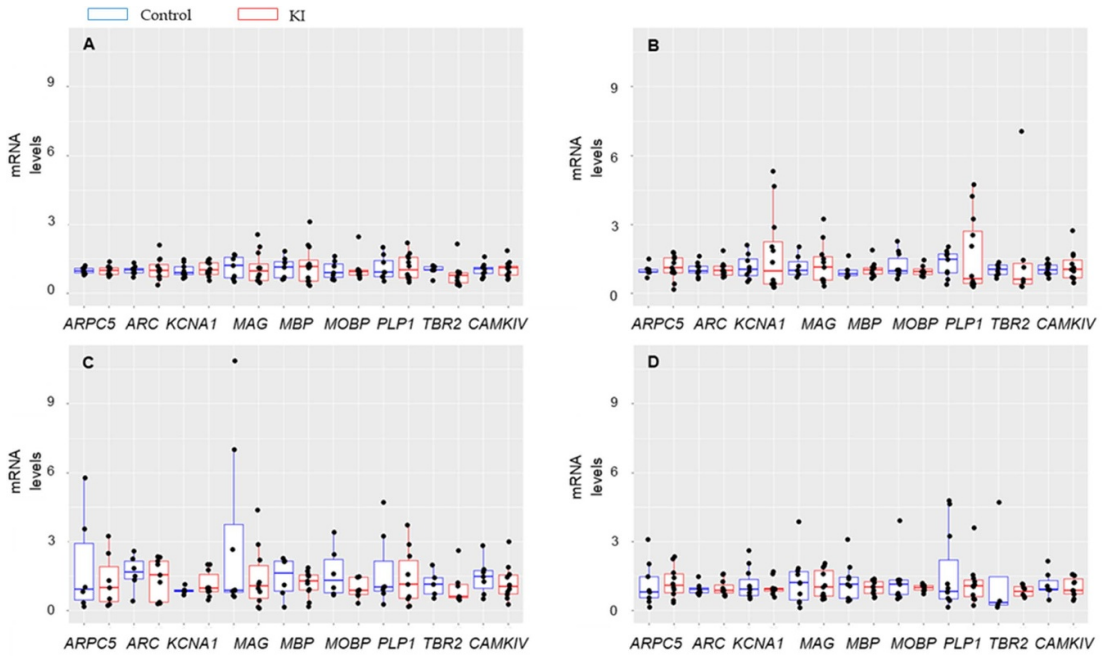

Figure 6. Cortex mRNA expression level of genes involved in transcriptional regulation (Tbr2), myelination (MBP—MOBP—MAG and PLP1), synaptogenesis (ARC—Kcna1 and APRC5) and maturation (CamkIV), at the cortex. Measured by real-time PCR in the cortex of controls (saline solution), and KI-exposed progeny PW1: early two administration (G1), early four administration (G2) and PW2: late two administration (G3), late four administration (G4). $N = 15$/group (A): G1, (B): G2, (C): G3, (D): G4. The results are expressed as a ratio to GADPH and ACTB mRNA level. Data are expressed as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$ vs control.

protocol allows us to control the daily amount of iodine administered to each animal, which was not the case in the Serrano *et al* study. Overall, our results indicate the safety of repeated treatment with KI for 2 or 4 d in pregnant females, with the exception of slight transient changes in TH after treatment over the GD13–GD16 period. They suggest the safety of repeated KI prophylaxis on thyroid function in an adult individual and without impacting the foetal development during the gestational period in our experimental model.

Indeed, the rat foetus is totally dependent on maternal TH until birth [26]. However, endocrine disruption during the intrauterine period is generally associated with increased risk of neuronal, cardiovascular, endocrine, or metabolic diseases in adulthood [27]. In addition, excess iodine significantly increases the risk of developing hypothyroidism in the foetus, as demonstrated by Serrano-Nascimento *et al* who observed a dysfunction of the hypothalamic-pituitary-thyroid axis in adult offspring following chronic exposure of pregnant rats to NaI in drinking water during the whole gestation and lactation period [27]. Furthermore, the study by Lebsir *et al* indicates that repeated exposure of pregnant rats to KI for 8 d significantly decreases TSH and T4 levels in adult offspring, which could reflect congenital hypothyroidism [20]. Contrary to these previous results, our study indicates that 2/4 d KI treatment does not impact TSH levels in adult offspring and suggests the absence of central TSH-related changes in this sensitive population, in agreement with the lack of effect on maternal TH.

On the other hand, we observed a significant increase of FT3 in individuals of group 1, as well as a significant increase in FT4 in offspring exposed for 2 d to KI (group 3) and a significant decrease in those treated for 4 d (group 4). Nevertheless, the variations in TH observed in our experimental conditions remained within the range of values obtained for these hormones in all the control individuals of the study. Moreover, reversibility studies recently conducted in the laboratory, based on the same experimental modalities, have shown a normalisation of FT3 and FT4 levels around PND100 (data not shown). These results suggest that the slight changes in FT3 and FT4 levels observed in response to 2/4 d prophylaxis during gestation in male offspring are transient and do not lead to excess iodine toxicity. In the absence of any changes in TSH, these results may suggest that the changes in FT3 and FT4 could result from peripheral disturbance, for example in the liver and kidney, the main sites of deiodinase 1 (D1)-induced thyroid hormone deiodination [28]. In this regard, it is interesting to note that Serrano-Nascimento *et al* found a decrease in D1 activity in liver and kidney in offspring exposed to excess iodine, associated with a decrease in TH levels, particularly FT3 [25].

In addition, analysis of liver and kidney plasma markers gave comparable results between KI treated animals and control individuals, apart from a slight change in total protein, calcium and glucose levels. Urinary markers showed an increase in uric acid and potassium in treated individuals. These changes did not exceed the range of values obtained for these parameters in all study controls and probably have no physiological impact. These results are comparable to the study by Lebsir *et al* which did not reveal any major impact on these markers following *in utero* exposure to excess iodine for 8 d in the offspring [20]. It has been suggested that excess iodine could be considered as an environmental factor that could trigger autoimmune thyroid disease [29]. Several mechanisms have been suggested that may explain this link, including inflammation, oxidative stress, or modulation of thyroglobulin antigenicity [29]. In particular, some studies have shown that treatment of adult mice with 150–500 mg l^{-1} NaI in drinking water for several weeks could lead to the development of autoimmune thyroiditis, characterised by lymphocyte infiltration of the thyroid [30, 31]. Our work did not reveal an autoimmune response, as measured by anti-TPO antibody assay, following prophylaxis with 1 mg kg d^{-1} of stable iodine for 2–4 d during gestation. Our autoimmune results are similar to those obtained by Lebsir *et al* suggesting that KI is well tolerated in our experimental model after repetitive ingestion [20].

In order to go further, we analysed the expression of a number of thyroid genes involved in the metabolism of iodine and thyroid hormones (Wolff–chaikoff effect), whose expression is known to be sensitive to an excess of iodine [10]. Similar to Lebsir *et al* we did not observe any variation in thyroid metabolism gene expression in KI-exposed offspring, except for a small decrease in TPO and AIT gene expression in group 3 individuals. These results are not in line with those described by Serrano-Nascimento *et al* [27], who showed that an excess of iodine significantly reduced the gene expression of all investigated genes involved in iodine metabolism, synthesis and secretion of TH in the thyroid of male offspring. This observation suggests that 2 or 4 d KI prophylaxis is safer than 8 d prophylaxis or over the period of gestation and lactation.

Thyroid Hormone is essential for the proper development of the CNS during the intrauterine period [16, 17]. Indeed, iodine deficiency or hypothyroidism during foetal life could lead to the development of neuronal pathologies. In order to explore potential effect of daily 1 mg iodine on neuronal development, we have studied the expression, in the cortex of the offspring, of genes involved in myelination, differentiation and neuronal plasticity. Contrary to the study by Lebsir *et al* we did not observe any significant variations in expression of the genes investigated in the different groups, suggesting the absence of neuronal toxicity due to excess iodine in all experimental conditions studied.

As a conclusion, this work proposes, for the first time, a 1 mg daily KI prophylaxis over several days for pregnancy condition (preclinical model). These data open new perspectives for the possibility of, a more suitable strategy in case of prolonged release of I^{131} than the current single KI intake recommended by World Health Organization. The absence of toxicity regarding TH metabolism and neuronal development in the offspring presented in this study suggests that repeated ITB for 2 or 4 d during gestation seems favourable in terms of benefit/risk balance in our experimental model. These results need to be implemented with GLP studies in large animals in order to validate the safety of repeated KI prophylaxis with sufficient acceptable security factor for a human application.

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Conflict of interest

The authors do not report any conflict of interest regarding the publication of this paper.

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