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Research Article

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Toxicology of Repeated Iodine Thyroid Blocking in Adult Rat

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Abstract

Radioactive iodines emitted following nuclear accidents are responsible for the dramatic increase of the late-onset thyroid cancer. Until the Fukushima disaster, a single dose of potassium iodide (KI) has been considered as an efficient countermeasure. Indeed, recently it has been suggested that repeated administration of KI may be necessary to ensure adequate protection in case of protracted exposure. Whereas, the effect of a single dose of KI has largely been studied ensuring its safety, studies regarding adverse effects of repeated iodine thyroid blocking (ITB) administration are scarce. Our objective was to assess the long term overall impact of KI in adult rats after repeated intake. Adult Wistar rats were subjected to either KI or saline solution over eight days. Biochemical homeostasis, hormones level, autoimmunity status, thyroid morphology and thyroid transcriptome profile were analyzed thirty days after the discontinuation of KI administration. Biochemical parameters, plasma levels of TSH; thyroid hormones; anti-TPO and anti-Tg did not differ between treated and control rats, the thyroid histology was not affected by the treatment and no long term transcriptome signature attributable to the treatment was noticed. Based on these data, we conclude the safety of repeated KI intake in adult rats; these data are prominent and may contribute to the ongoing development of KI guidelines and marketing authorization.

Keywords: Potassium iodide, Nuclear accident, Thyroid gland, Repeated ITB.

Introduction

Exposure to external radiation is associated with harmful effects to the thyroid, such as hypothyroidism, thyroid autoimmunity, thyroid nodules and thyroid cancer [1, 2]. The carcinogenic effect of exposure to ^{131}I was considered to be small compared with that of external photon exposure, until the Chernobyl accident which involved the release of about 1.760 PBq of ^{131}I into the environment [3, 4]. Before the accident, incidence rates of thyroid cancer was less than 1 case per million per year, in the period from 1991 through 1994 this rate increased to more than 90 per million per year in Gomel the most contaminated region of Belarus particularly among young population [5]. Concerns regarding carcinogenic consequences of exposure to ^{131}I arose after the damage to the Fukushima plant, between 2011 and 2014, a survey using ultrasound screening was done among individuals who were under 19 years of age at the time of accident, further long-term follow-up seems necessary to conclude [6-10].

In the event of a nuclear reactor accident and when radioactive material is released into the atmosphere, ^{131}I will be concentrated in the thyroid gland by 10 to 30% through inhalation or ingestion of contaminated food and milk [11-13]. As a part of ^{131}I decay process, β- radiation is emitted and affects the thyroid and its surrounding tissue, and my lead to adverse health outcomes such as thyroid dysfunctions and thyroid cancer [12]. The administration of potassium iodide (KI) is a practical and effective countermeasure to protect the thyroid by preventing the uptake of radioactive isotopes of iodine and thereby reducing the subsequent risk of thyroid cancer [14]. In a clinical trial, KI given prior to exposure reduced the accumulation of ^{131}I in the thyroid gland from an average of 20% to less than 2% [15]. During the Chernobyl reactor accident, the Polish government distributed KI to 95% of Polish children and 23% of the total population. It was estimated that the amount of exposure of the thyroid of individuals who were given KI to ^{131}I was reduced by nearly 40% [12, 14].

There is limited evidence of adverse effects of KI administration from experiences in other contexts. For example, doses between 100 and 200 μg KI/d (rarely up to 500 μg KI/d) are used for treatment of endemic goiter; this treatment is usually continued for years and has occasionally been linked to hyperthyroidism. Nutritional iodine supply is being discussed as playing an important role in changes of thyroid function after administration of additional iodine, e.g. by introducing goiter prophylaxis programs with iodized salt. Here, thyroid autoimmunity and subsequent hypothyroidism are expected as adverse effects. Furthermore, it is suggested that additional iodine in case of iodine deficiency increases the risk for hyperthyroidism, whereas an excess of iodine in case of sufficient iodine supply

increases the risk of autoimmunity and hypothyroidism [16, 17].

Wolff and Chaikoff demonstrated in 1948 that an excess of iodide in rats may transiently disrupt thyroid function, the well-known Wolff-Chaikoff effect is characterized by a decrease in and delayed iodide organification [18]. This affects many steps of thyroid hormone synthesis and secretion; such iodide entrance and organification, iodide efflux through the colloid, intra-thyroidal iodide pool regeneration, and thyroid hormones transport [19-22]. This effect could lead to a temporary variation in thyroid hormones level, which exerts a major impact on development, growth, and metabolism [19, 22]. Normally after a few days, thyroid hormone levels return to normal the so-called escape phenomenon. Both the Wolff- Chaikoff effect and its escape are necessary to keep thyroid hormone synthesis under tight control [19].

The accident of Fukushima, where several releases of radioactivity have occurred over many days, highlighted the limits of the current KI policy which recommends a unique intake of KI tablets; it cannot protect in a satisfactory way the populations repeatedly exposed to radioactive iodine over many days [11, 23]. Repeated administration of KI may be required to ensure adequate protection. Nowadays, the health authorities are unable to face such situations; scientifically sound studies regarding the effects of repeated administration of KI are scarce and consequently the evidence for the application of repeated prophylaxis is weak [15, 24, 25].

The guidelines for the use in planning for and responding to radiological and nuclear emergencies by WHO 2017, identify as one of the four research priorities that need "More data on the dosage, optimal timing and regimens for multiple administrations of stable iodine in case of repeated or protracted releases of radioactive iodine and the adverse health effects of stable iodine administration". In the hope to find an answer to these queries, our work aims to assess the effects of repeated administration of an optimal dose of KI in an experimental model "adult rat", different parameters : general health status, endocrinology parameters, structure and molecular pathways, were assessed in the long term one month after the treatment discontinuation.

Materiels and Methods

Animals and KI administration

Potassium iodide and saline solutions were kindly provided by the central pharmacy of armed forces (Orleans, France).

The study included 3-months old male *Wistar* rats (Charles River Laboratories, L'arbresle, France) divided into KI-exposed and control groups, the treatment was carried out by gastric gavage over 8 days. Each group consisted of 13 animals (Figure 1).

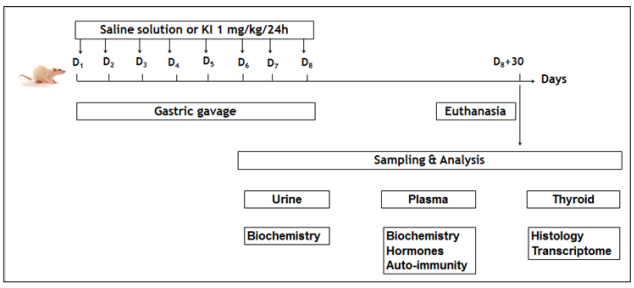


Figure1: prophylactic design, rats (n = 13) were subjected to saline solution or KI over 8 days, then euthanized thirty days later.

The animals were housed and maintained under controlled conditions of temperature (21 ± 2°C), humidity (50 ± 10%) and regular dark/light cycle (12h / 12h). Normal-iodized pellet diet 0.3 mg I / kg of pellet (A04-10 SAFE, Augy, France) and tap water were freely accessible, and their weight and their intakes were monitored on a weekly basis. Thirty days after the treatment discontinuation, rats were placed in metabolic cages for 24h, with free access to diet and water. Urine was collected and centrifuged (3000 rpm / 10 min); supernatant was frozen at - 80°C. Blood was collected by intracardiac puncture under isoflurane anesthesia (Abbot France, Rungis, France) and then centrifuged (3,000 rpm / 10 min); the plasma supernatant was immediately frozen at - 80°C. Thyroid was dissected on ice, instantly deep-frozen in liquid nitrogen and then stored at - 80°C.

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).

Biochemical parameters assessment

Most biochemical indicators were measured in plasma and urine samples with an automated spectrophotometric system (Konelab 20i from Thermo Fisher Scientific, Cergy-Pontoise, France), with the manufacturer's biological chemistry reagent (Brahms, Asnières sur Seine, France). The plasma biomarkers measured were lipids (cholesterol, triglycerides, and phospholipids B), substrates (total protein), electrolytes (calcium, phosphorus, iron, chlorine, potassium and sodium), cardiac markers (creatine kinase (CK) and its isoform CK-MB), liver markers (alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT)), and kidney markers (creatinine and urea). In the urine sample, we measured kidney markers (creatinine, urea and uric acid), substrates (urinary proteins) and electrolytes (calcium, phosphorus, chlorine, potassium and sodium). Phospholipid B (Diagnostic partners, Bormes Les Mimosas, France) indicators was adapted on the spectrophotometric system.

Thyroid markers assessment

Plasma thyroid-stimulating hormone (TSH) was determined with the TSH rat ELISA kit from 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). Plasma anti-thyroid antibodies were determined with anti-thyroid peroxidase antibody (Anti TPO) bioassayTM ELISA kit (Rat) and anti-thyroglobulin antibody (ATGA/ TGAB) bioassayTM ELISA kit (Rat), from USBiological life science (Swampscott, Massachusetts, USA). The analytical sensitivities of TSH, FT4, FT3, Anti-TPO and Anti-Tg are 0.1ng/mL, 2.83 pmol/L, 1.5 pmol/L, 1 ng/mL and 1 ng/mL respectively.

Thyroid histology and immunohistochemistry

Histological and immunohistochemistry analysis were done by an independent laboratory (Biodoxis, Romainville, France). Thyroid glands were excised and incubated in 4 % formaldehyde solution (VWR Chemicals, Fontenay-sous-Bois, France); each individually fixed sample was then intersected and placed in a histological cassette, embedded in paraffin and cut into sections of 3 µm thick, then stained with hematoxylin and eosin.

Ki67, NIS and TPO detection was performed by immunohistochemistry on all the samples. Paraffin sections were first deparaffinized and

antigen retrieval was carried out with the Dako Target Retrieval Solution in a water bath. Then, immunohistochemical staining procedure was performed. After blocking endogenous peroxydase activity (Dako EnVision®+ System-HRP (DAB), Peroxydase Block), the slides were incubated with diluted primary antibody for 1 hour at room temperature. The tissue sections were then washed and incubated with appropriate secondary antibodies for 30 minutes. Immunoreactive signals were detected using DAB substrate solution (Dako EnVision®+ System-HRP (DAB), DAB+ Substrate buffer / Liquid DAB+ chromogen, 5 minutes incubation). Finally, the sections were lightly counterstained with Mayer's hematoxylin. Negative controls were obtained by substitution of the primary antibodies with isotype control immunoglobulin or with antibody diluent alone (negative buffer control) in the immunohistochemical staining procedure.

The approach used for the histomorphometric analysis was adapted from that used by Kot et al. 2013; the first step consisted in selecting stereologically fifty follicles for morphometric analysis. An image of each stereologically selected follicle was then analyzed using image Pro-Premier software (version 9.1) from Media Cybernetics (Rockland, MD, USA). For each follicle, the total area of the follicle ($FOLL_a$), the area of the colloid (Colloid Area), and the respective area of each nucleus were measured. Then, the combined total area of the cytoplasm and nuclei (A_{nc}), the total cytoplasmic area (A_c), the ratio of the nuclei area to the cytoplasmic area (N / C ratio), the mean nuclear area, as well as the ratio of Colloid area to total follicular area (C / F ratio) were calculated [26].

The nuclei of the follicular epithelium were individually segmented and classified into positive or negative nuclei for the Ki67 cell proliferation marker. This classification was performed by a threshold for the positive or negative nuclei combined with a set of filters based on the morphology and size of the segmented objects. Images from the scans of the thyroid section were analyzed with the Image Pro-Premier software.

Histomorphometric analysis of TPO and NIS abundance was performed using the Image Pro Premier software. Images were analyzed for each thyroid. The optical density has also been measured.

Thyroid transcriptome profile

The DNA micro array was performed by an independent laboratory (CRIBIOM, Marseille, France), the samples were divided into two populations according to the experimental treatment applied to the rats, and labeled with either Cyanine 3 or Cyanine 5. Cyanine incorporation was assessed by Nanodrop. The labeled samples were hybridized at 65 ° C, 10 rpm over 17 h, on 3 slides Agilent SurePrint G3 catalog according the supplier's recommendations (Two-Color Microarray protocol-Based Gene Expression Analysis, Low Input Quick Amp Labeling version 6.9.1). The slides were scanned using DNA Microarray Scanner SureSelect (Agilent). The fluorescence data was measured from the images using the software Feature Extraction version 10.7.3.1 (Agilent) and the Quality Control was performed using the GeneSpring software version 12.5 (Agilent). Statistical analysis was performed using R version 3.3.2 in combination with R-studio version 1.0.136. The R-package Limma was used to normalize and to analyze the microarrays [27]. An in-house script was used to run the same normalization method, filtering, and t-tests for all micro arrays after three microarrays have been excluded due to technical problems. Genes were considered differently expressed if the adjusted p-value was below 0.05. A heat map was constructed with all probes obtained from the statistical results and using the R-package gplots.

Statistics

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

Results

General and biochemical parameters

Table 1 shows that the treated rats were in a good general status: their final body weight as well as thyroid weight was not different than the control group. The plasma lipids profile (including levels of total 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), kidney integrity (Creatinine and urea) and heart integrity (CK and CK-MB). After 30 days of KI discontinuation, the levels of the investigated biomarkers of homeostasis in the urine of rats subjected to KI did not differ from those of controls.

Table 1: General and biochemical parameters 30 days post-prophylaxis

Function		Control	KI 1mg/kg
General indicators	Final body weight (g)	391 ± 2.08	392 ± 2.08
	TW/BW Ratio	0.08 ± 0.006	0.06 ± 0.005
Plasma biomarkers			
1. Miscellaneous	Cholesterol (mmol/L)	1.73 ± 0.07	1.65 ± 0.09
	Phospholipids B (g/L)	1.21 ± 0.04	1.19 ± 0.04
	Triglycerides (mmol/L)	1.39 ± 0.18	1.17 ± 0.16
	Total proteins (g/L)	57.25 ± 1.67	56.10 ± 2.09
2. Electrolytes	Calcium (mmol/L)	2.80 ± 0.01	2.79 ± 0.01
	Phosphorus (mmol/L)	2.09 ± 0.05	2.07 ± 0.04
	Iron (µmol/L)	43.01 ± 1.25	40.56 ± 1.17
	Chlorine (mmol/L)	101.48 ± 0.24	101.31 ± 0.28
	Potassium (mmol/L)	4.36 ± 0.08	4.34 ± 0.05
	Sodium(mmol/L)	136.70 ± 0.36	136.62 ± 0.41

3. Thyroid markers	TSH (ng/mL)	1.56 ± 0.17	1.48 ± 0.10
	Free T4 (pmol/L)	24.98 ± 0.61	25.29 ± 0.68
	Free T3 (pmol/L)	3.63 ± 0.12	3.80 ± 0.13
	Anti-TPO (ng/mL)	34.29 ± 4.30	32.65 ± 5.00
	Anti-Tg (ng/mL)	369.31 ± 22.00	328.47 ± 18.00
4. Liver markers	ALAT (U/L)	30.63 ± 1.46	30.58 ± 1.35
	ASAT (U/L)	124.51± 12.69	112.18± 8.29
	ASAT/ALAT	4.02 ± 0.28	3.71 ± 0.25
5. Kidney markers	Creatinine (µM)	44.54 ± 3.93	44.99 ± 1.99
	Urea (mM)	5.46 ± 0.72	5.62 ± 0.83
6. Heart markers	CK (U/L)	579.46 ± 90.62	533.60 ± 57.54
	CK-MB (U/L)	741.88 ± 225.22	843.36 ± 198.07
Urine biomarkers			
1. Electrolytes	Chlorine (mmol/24h)	4.80 ± 0.5	4.80 ± 0.3
	Potassium (mmol/24h)	1.80 ± 0.08	1.70 ± 0.09
	Sodium (mmol/24h)	0.90 ± 0.08	0.90 ± 0.05
	Phosphorus (mmol/24h)	0.10 ± 0.02	0.10 ± 0.02
	Calcium (µmol/24h)	23.10 ± 3.50	19.70 ± 3.40
2. Miscellaneous	Urinary proteins (mg/24h)	11.99 ± 3.32	10.16 ± 2.26
	Uric acid (µM/24h)	17.05 ± 0.82	17.28 ± 1.08
	Urea (Mm/24h)	11.22 ± 0.55	10.40 ± 0.49
	Creatinine (µM/24h)	126.27± 8.76	129.94± 6.18

Data are expressed as mean ± SEM (n = 13 /group), ALAT: alanine aminotransferase, ASAT: aspartate aminotransferase, TW: thyroid weight, BW: body weight, TSH: thyroid stimulating hormone, T4: thyroxine, T3: triiodothyronine, Anti-TPO: thyroid peroxidase antibody, Anti-Tg: thyroglobulin antibody, CK: creatine kinase, CK: creatine kinase myocardial band.

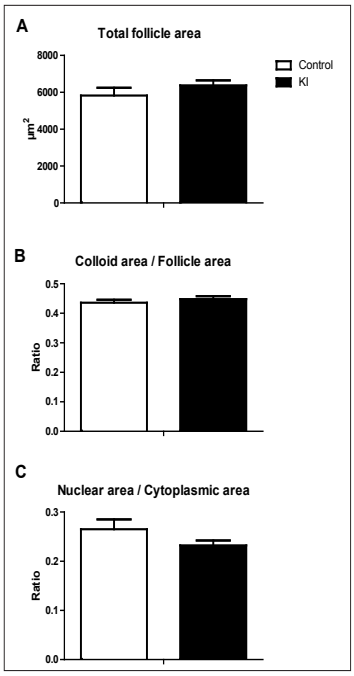
Pituitary- thyroid axis

Table 1 shows that repeated potassium iodide administration resulted in non-long-term significant modification of TSH, free T4 and free T3 concentration compared to control rats. Also the treatment did not show a long-term effect on thyroid autoimmune status; the plasma level of anti-thyroid antibodies (Anti-TPO and Anti-Tg) did not differ between control and treated rats 30 days after the KI treatment discontinuation.

Histological and immunohistochemical characterization of thyroid tissue

Follicular histomorphometry

A total of nine histomorphometric parameters of the thyroid follicles were measured or calculated. First, the mean surface area of the fifty follicles measured individually is 6379.47 ± 852.62 µm^2 for the treated rats *vs* 5826.18 ± 1325.06 µm^2 for the untreated rats (Fig 2A). The mean area of the nuclei / follicle is 597.96 ± 84.98 µm^2 and 600.97 ± 84.70 µm^2 for treated and untreated rats, respectively. On the other hand, the mean cytoplasm / follicle area is 2700.85 ± 486.59 µm^2 and 2438.11 ± 542.71 µm^2 for treated and control groups, respectively. The ratio of nuclear area / cytoplasmic area (N / C ratio) was then calculated for each follicle analyzed, the average N / C ratio is also comparable between the two groups 0.232 ± 0.032 for treated rats vs 0.265 ± 0.059 for controls (Fig 2C). The average number of nuclei per follicle is 21.50 ± 2.34 for treated rats vs 21.42 ± 2.45 for controls. Regarding the average size of the follicular nuclei, treated rats have an average size of 28.05 ± 3.18 µm^2 whereas the average size in control group is 28.28 ± 2.90 µm^2 (Fig 2D, 2E). The average colloidal area of the individual follicles was also measured and the ratio of the colloidal area to the total area (C / F ratio) was calculated for each follicle analyzed. The results of these two parameters show that the average colloid / follicle area is 3080.67 ± 522.64 µm^2 in treated animals vs 2787.11 ± 789.48 µm^2 in control animals. The calculated C / F ratios have an average of 0.448 ± 0.045 for treated rats vs 0.436 ± 0.030 for control rats (Fig 2B).



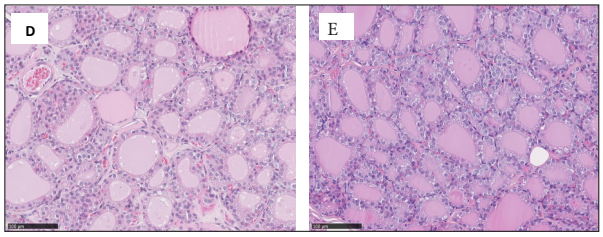


Figure 2 (A): The mean surface area of the fifty follicles, (B): The ratio of colloid /follicle area, (C): The ratio of nuclear/ cytoplasm area, (D) and (E): Histological sections of the thyroid illustrating follicles (H&E) in control and treated rats, respectively (scale bar 100 µ m).

Histomorphometry of Follicular protein abundance of Thyroid peroxidase

The mean positive signal, as determined by the integrated optical density for TPO in immunohistochemistry is 64.12 ± 8.52 lum / mm^2 in treated group *vs* 67.32 ± 16.95 lum / mm^2 in untreated group (Fig 3B).

Histomorphometry of follicular protein abundance of sodium / iodide symporter

The mean integrated immunohistochemistry optical density for NIS is 40.78 ± 16.74 lum / mm^2 in treated group *vs* 40.16 ± 17.57 lum / mm^2 in untreated group (Fig 3B).

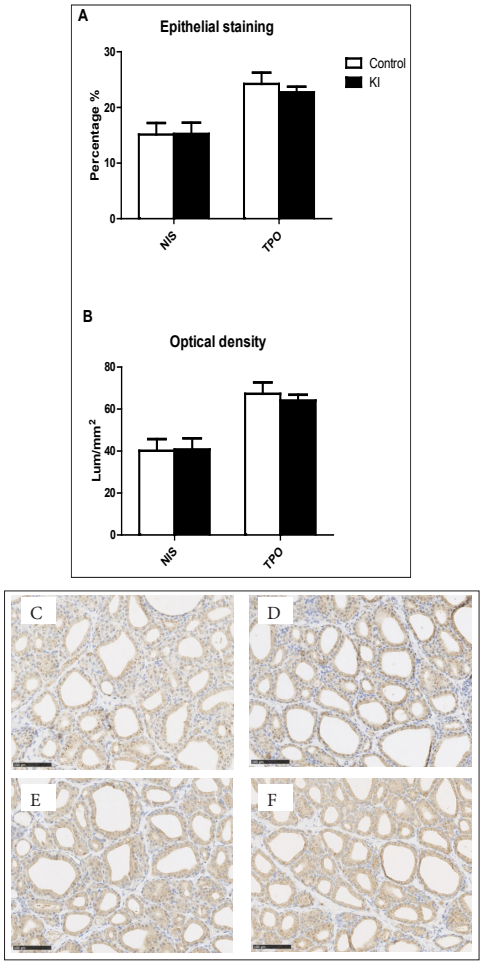


Figure 3:(A) The percentage of sodium iodide symporter (NIS) and thyroid peroxidase (TPO) positive cells, (B) The mean integrated optical density of NIS and TPO in the thyroid of control and treated rats. Immunohistochemical expression of biochemical pathways of thyrocytes in thyroid tissue (scale bar 100 µ m): (C) and (D) Sodium/Iodide symporter (NIS) staining in control and treated rats respectively. (E) and (F) thyroid peroxidase cytoplasmic staining in control and treated rats, respectively.

Epithelial proliferation index

The proliferation rate is significantly not different for both groups: an average of 2.66 ± 1.01% and 2.70 ± 0.81% of Ki67 positive nuclei for treated and untreated rats, respectively (Fig 4).

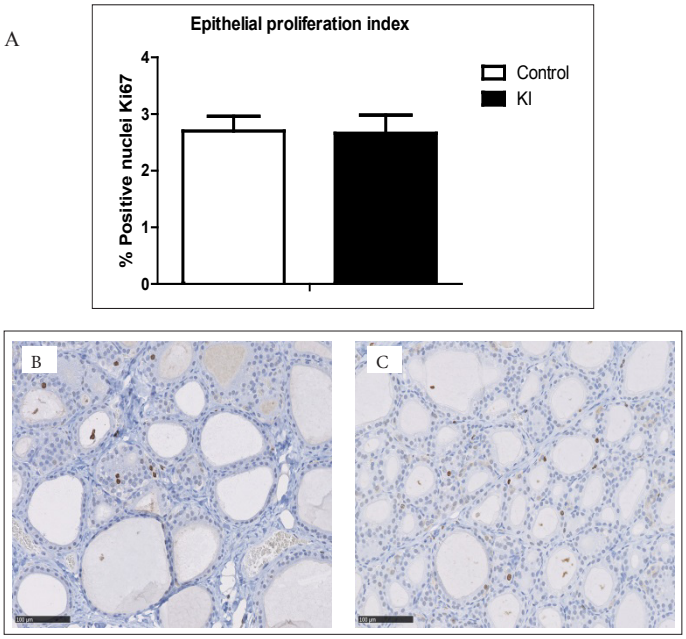


Figure 4: (A) The percentage of positive nuclei to Ki67. (B) and (C): Immunohistochemical expression of Ki67 positive nuclei in control and treated rats respectively (scale bar 100 µ m).

Thyroid Transcriptome signature

The statistical analysis showed no significant difference in gene expression between the control and the KI-administered group among 31951 transcripts assessed (Fig 5A). We choose several genes implicated in the thyroid hormone biosynthesis, oxidative stress, apoptosis and immune system and looked in more detail at their gene expression pattern (Fig 5B). The heat map showed that no clear distinguishable profile in gene expression can be observed between the two groups.

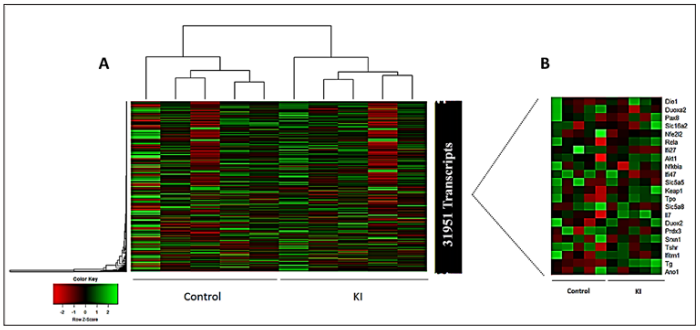


Figure 5: (A) Heat map analysis of the transcriptome (31951 transcripts) for treatment effect, (B) heat map analysis of selected transcripts involved in thyroid hormone metabolism (Dio1:

iodothyronine deiodinase 1, Duoxa2: dual oxidase maturation factor 2, Pax8: Paired-box gene 8, Slc16a2: monocarboxylate transporter 8 MCT8, Tshr: thyrotropin receptor, Tg: thyroglobulin, Ano1: anoctamine1, Slc5a8: apical iodide transporter, Slc5a5: sodium iodide symport (NIS), Tpo: thyroid peroxidase, Rela: p65, Akt1: protein kinase B), oxidative stress (Nfe2l2: nuclear factor (erythroid-derived 2)-like 2, Keap1: Kelch-like ECH-associated protein 1, Srxn1: Sulfiredoxin 1, Prdx3: Peroxiredoxin 3), apoptosis and inflammation (Ifitm1: Interferon Induced Transmembrane Protein 1, Ifi27: Interferon Alpha Inducible Protein 27, Nfkbia: Nuclear factor-kappa-B-inhibitor alpha, Il7: Interleukin-7, Ifi47: interferon gamma inducible protein 47). The genes were clustered using linkage hierarchical cluster with Euclidean distance, and the expression of each of the transcripts are shown with red for low, black for middle, and red for low expression.

Discussion

Thyroid cancer is considered as the most severe health consequence of a nuclear reactor emergency with release of radioiodine into the atmosphere [16]. Thyroid gland is mainly regulated by two systems: iodine and TSH [28]. The ability of the thyroid to concentrate iodine is essential for the synthesis of thyroid hormones (TH), a complex mechanism involving several genes (i.e. NIS, PDS, TPO, DUOX, Tg, MCT8…) [29, 30], circulating TH levels is tightly regulated under physiological circumstances and exert a major impact on the body homeostasis. Acute excess of iodine could lead to a transient down-regulation of thyroid hormones level, the so-called Wolff-Chaikoff effect, this down-regulation leads to the up-regulation of TSH, thereby affecting gene expression in the thyroid [19, 22, 31, 32].

During the Fukushima nuclear power plant emergency due to the earthquake, KI was not administered to the general population [33]. Repeated administration of potassium iodide could be used as an option in case of prolonged exposure to radioiodine, but there is real gap of knowledge concerning the repeated intake of stable iodide and the long-term sanitary consequences related to it. Nowadays, according to the WHO 2017 guidelines, the authorities recommend a single intake of potassium iodide.

In our study to mimic experimentally a such prophylaxis, we have chosen a dose of 1 mg/kg/day as it has been demonstrated to be an optimal dose [34]. The concentration of iodine in urine (UIC) was measured at the end of the treatment and thirty days after the end of the treatment to evaluate iodine status, we observed that after the end of the treatment UIC was significantly higher in the treated group, but thirty days after we didn't notice a significant difference between treated and control groups [35]. Thirty days post-prophylaxis, rats were in good general health status; their final body weight and thyroid weight didn't differ from those of no treated rats. Our results are in accordance with other preclinical studies where excess iodide for relatively long period of time didn't alter the overall body weight of *Wistar* rats [36-38]. Regarding the thyroid weight, our result is in accordance with other studies [36, 38]. In contrast to our results, Chen et al, 2015 has demonstrated that a higher iodine concentration is associated with a greater thyroid organ coefficient in female BALB/c mice [39]. The difference could be due to various parameters (animal species and sex and treatment dose and duration). Moreover, plasma and urine biochemical parameters didn't differ between rats subjected to iodide and controls. Yoshida and colleagues has also demonstrated that after the administration of iodide more than 3 mg/day in rats for 4 weeks, no change in some targeted serum biochemical parameters (alanine aminotransferase, aspartate aminotransferase, total cholesterol and triglyceride) has been observed [36].

To evaluate thyroid functioning, we have assessed the blood TSH and free thyroid hormone levels as relevant biomarkers. There has been no difference in neither TSH concentration nor plasma thyroid hormone levels observed between treated and control groups, respectively; this may suggest that the thyroid function has not been affected by the repeated administration of iodide. These results are in accordance with other clinical and preclinical studies that reported no impact of iodide on the pituitary-thyroid axis activity [15, 21, 33, 37, 39-43].

Since it has been suggested that excess iodine is associated with thyroid autoimmunity, we selected two biomarkers of thyroid autoimmunity status anti-TPO and anti-Tg, our result show that 30 days post-prophylaxis anti-thyroid antibodies level didn't differ between treated and control rats [17].

The histological analysis of thyroid showed no modification attributable to iodide intake, the shape and the size of follicles and cell proliferation index did not show difference between treated and control rats. In a previous study in rats, thyroid histological analysis showed a significant enlargement of thyroid follicles and an increased amount of extracellular matrix around them in response to excess iodine treatment [38]. Mice subjected to an excess of iodine were characterized by an increased follicles area and a small of follicular cavity, their thyroid cells became flat [39]. Thyrocytes are polarized cells, with a basal membrane in contact with blood vessels and an apical membrane in contact with the follicular lumen that contains the colloid. Two proteins play an important role in iodine transport and TH synthesis, *i.e*. NIS and TPO. In our study, the immunohistochemical characterization of thyroid tissues indicates that the protein abundance and localization of these two important proteins have not been affected 30 days after the discontinuation of KI intake, our results are in accordance with Faggioano and colleagues [44]. Under normal physiological conditions, NIS staining is heterogeneous and restricted to the basolateral membrane, whereas TPO staining is homogenous in the cytoplasm of the majority of thyroid cells. The results of histological analysis, proliferation assessment and immunohistochemical characterization show no adverse effect of KI 1mg/kg/ day over 8 days on thyroid cytorachitecture and function.

To elaborate further, we have evaluated the long-term impact of repeated administration of iodide on thyroid function at the molecular level. It is well known that the Wolff-Chaikoff effect and its escape occur during the exposure of the normal thyroid to supraphysiological iodide concentrations [19]. Leoni and colleagues have analyzed the transcriptome profile of rat follicular cell lineage PCCl3 under normal and excess iodine conditions; they have found that 84 transcripts (especially classified in a protein metabolic category) were differentially expressed in response to iodide [45]. In our experimental conditions, we have analyzed the transcriptome profile of rat thyroid under treated and untreated conditions. Among 31951 transcripts we have not been able to observe a distinguishable profile of gene expression between treated and control groups. Furthermore, even the expression of the genes involved in iodide metabolism and thyroid hormone synthesis was similar between treated and control group 30 days post-prophylaxis. This indicates that the KI treatment with

a dose of 1 mg/kg/day does not affect important cellular processes. In summary, the results obtained from the current study shows that repetitive intake of potassium iodide with a dose of 1 mg/kg/day for eight days has no long-term harmful impact on the general health status, thyroid structure and function in rat experimental model. In addition, transcriptomic analysis of gene expression shows no significant signature between treated and control groups. In order to evolve the marketing authorization of KI and to update the current iodine guidelines, our data can be used in the future as input data for additional studies on other animal species. In the near future, our perspective is to assess the effect of this prophylactic design in more sensitive individuals like pregnant and elderly populations, in order to fill the gap of knowledge and improve the KI marketing authorization in case of prolonged exposure to radioactive particle [46].

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