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ABSTRACT

Background: A single dose of potassium iodide (KI) is recommended to reduce the risk of thyroid cancer during nuclear accidents. However in case of prolonged radioiodine exposure, more than one dose of KI may be necessary. This work aims to evaluate the potential toxic effect of repeated administration of KI. **Methods:** Adult *Wistar* rats received an optimal dose of KI 1 mg/kg over a period of 1, 4 or 8 days. **Results:** hormonal status (TSH, FT4) of treated rats was unaffected. Contrariwise, a sequential Wolff-Chaikoff effect was observed, resulting in a prompt decrease of NIS and MCT8 mRNA expression (-58% and -26% respectively), followed by a delayed decrease of TPO mRNA expression (-33%) in conjunction with a stimulation of PDS mRNA expression (+62%). **Conclusion**: we show for the first time that repeated administration of KI at 1mg/kg/24h doesn't cause modification of thyroid hormones level, but leads to a reversible modification of the expression of genes involved in the synthesis and secretion of thyroid hormones.

KEY WORLD: Potassium iodide, repeated prophylaxis, thyroid gland, Wolff-Chaikoff effect, thyroid hormones.

HIGHLIGHT:

- KI prophylaxis over many days didn't induce any modification on thyroid hormonal status (TSH –FT3 and FT4 level)
- KI prophylaxis over many days had a reversible impact on the expression of genes involved in iodide transport (NIS and PDS), and those involved in thyroid hormones synthesis (TPO and MCT8)
- The transcriptional effect was time-dependent

1. INTRODUCTION

The use of nuclear technology has always been accompanied by the risk of radioactivity releases into the environment, since 1990 a dramatic increase in the incidence of pediatric thyroid cancer, ensued in Belarus and Ukraine, following the Chernobyl nuclear reactor accident **(Cardis et al. 2005; Jargin 2011; Reiners et al. 2013)**, also four years after Fukushima accident an excess of thyroid cancer has been detected among children and adolescents in the Fukushima prefecture **(Tsuda et al. 2016)**, these incidences are presumably due to ingestion and inhalation of radioiodine in particular ^{131}I a gamma and beta emitter with a physical half-life of 8 days **(Reiners and Schneider 2013)**. To prevent radioactive contamination, the responsible authorities implement series of physical measures like sheltering, evacuation and food restriction, in addition to pharmacologic thyroid blockade by oral potassium iodide (KI) at a dose of 130 mg (2 tablets of KI at 65 mg), fractions of this quantity are used in specific population groups (130 mg in adults, pregnant and lactating women, if necessary; 65 mg in children; 32.5 mg in infant and 16.25 mg in newborn) **(Zanzonico and Becker 2000; Dreger et al. 2015)**. The Polish government successfully applied iodine thyroid blocking (ITB) and as result about 90% of children under the age of 16 years showed thyroid dose commitment below 50 mSv **(Nauman and Wolff 1993)**. In case of a single release of radioiodine, a single administration of KI is regarded as sufficient **(WHO 1999)** it approximately blocks the thyroid 24 h to 36 h, the blocking capacity decreases when time after administration increases **(Dreger, Pfinder et al. 2015)**. Currently, in extreme situations, the responsible authorities order a second administration of KI only for adults **(WHO 1999).** Based on experiences from the Chernobyl accident with the prolonged release of radioiodine over 10 days and Fukushima accident where several releases of radioactivity have occurred over many days **(Reiners and Schneider 2013)**, it was clear that a single dose of KI is not adequate for thyroid blocking, in similar situations repeated administration of KI may be required to ensure adequate protection, unfortunately studies regarding the effects of repeated administration of KI are scarce and consequently the scientific evidence to apply repeated prophylaxis is weak. In 1980, a clinical trial was performed using different doses of KI over 12 days, the study showed a significant level of protection without major effect on the hormonal status of participants **(Sternthal et al. 1980)**. Since 1948, it was demonstrated that an excess of iodide may transiently disrupt thyroid function, the well-known Wolff-Chaikoff effect characterized by decreased and delayed iodide organification **(Wolff and Chaikoff 1948)**, this effect is complex and encompasses

multiple steps including iodide entrance and organification **(Eng et al. 1999)**, iodide efflux through the colloid **(Calil-Silveira et al. 2016)**, intrathyroidal iodide pool regeneration **(Solis et al. 2011)**, and thyroid hormones transport **(de Souza et al. 2015)**. This effect could lead to a temporary variation of thyroid hormones level **(Eng, Cardona et al. 1999; de Souza, Dias et al. 2015)**, which exert a major impact on development, growth, and metabolism **(Calil-Silveira et al. 2016)**. Both the Wolff- Chaikoff effect and its escape are necessary to maintain thyroid hormone synthesis under tight control **(Eng, Cardona et al. 1999)**. Recently, a new prophylactic design, implying the administration of KI at 1 mg/kg/24h over a period of 8 days, was developed **(Phan et al. 2017)**. To go further, the present work aims to apply this design and to assess the toxic effects on the hormonal and biochemical homeostasis in adult *Wistar* rat, also the impact on the main steps of thyroid hormones synthesis (iodide transport, iodide organification and thyroid hormones transport) was investigated.

2. MATERIALS AND METHODS

2.1 Experimental procedure

2.1.1 Materials

Saline solution (pH 7.4) and potassium iodide solution (0.35 g/L), were kindly provided by central pharmacy of armies (Orleans, France)

2.1.2 Animals

Adult male *Wistar* rats, aged 3 months, weighing 335 ± 17 g, were purchased from Charles River Laboratories (L'arbresle, France); and housed 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 (SAFE A04-10, Augy, France) and tap water were available *ad-libitum*. All experimental procedures were approved by the Animal Care Committee of the Institute of Radioprotection and Nuclear Safety, and complied with French regulation for animal experimentation (Ministry of agriculture Act No.87-848, October 19th 1987, modified May 20th 2001).

2.1.3 Experimental groups

In order to evaluate the effect of repeated administration of the optimal dose of potassium iodide, rats were divided into five groups as follows:

Group 1: (d1): 4 rats / group receiving single administration of KI or saline solution, and sacrificed 24 h later, Group 2: (d4): 8 rats / group receiving repeated administration of KI or saline solution over four consecutive days, and sacrificed 24 h later, Group 3: (d8): 8 rats / group receiving repeated administration of KI or saline solution over eight consecutive days, and sacrificed 24h later, Group 4: (d10): 4 rats / group receiving repeated administration of KI or saline solution over eight consecutive days, and sacrificed 48h later and Group 5: (d30): 13 rats / group receiving repeated administration of KI or saline solution over eight consecutive days, and sacrificed 30 days post-prophylaxis.

As shown in figure 1, the treatment was carried out by gastric gavage; treated rats received 1mL of KI (0.35 g/L) whereas their matched control groups were given 1 mL of saline solution (pH7.4).

2.1.4 Organs and biofluids collection

Before sacrifice, rats were placed in metabolic cages for 24h, with free access to diet and water. Urine was collected twice a day, and refrigerated at + 4°C to limit bacterial growth, fractions were pooled, mixed, and centrifuged (3000 rpm / 10 min); supernatants were frozen at - 80°C. After that, rats were anesthetized by inhalation of 5% isoflurane (Abbott France, Rungis, France) and euthanized by intracardiac puncture with blood collected in heparinized tubes. Whole blood was centrifuged (3,000 rpm / 10min) and plasma supernatants were immediately frozen at - 80°C. Thyroid was dissected on ice, weighed, deep-frozen in liquid nitrogen, and stored at -80°C.

2.2 Technical procedure

2.2.1 Urinary Iodine Concentration (UIC) measurement

After slow thawing of samples at (+ 21 °C), the urines were diluted 1/10 000th with 2% ammonia solution (solution made from the Suprapur® 25% ammonia solution, Merck) and then stabilized with a sodium thiosulfate solution at 184 mg/l in 2% ammonia. The total iodine content was determined by direct measurement of three aliquots of each sample by inductively coupled plasma mass spectrometry (ICP-MS, X Series II, Thermo Electron, Courtaboeuf, France) using the standard addition method. The internal calibration range consists of a standard solution of stable iodide at 1000 mg/L (Iodides AVS Titrinorm IC Standard, Prolabo). Tellurium Te-125, used as the second internal standard, was added to the samples at a concentration of 1.25 mg/L from a standard 1000 mg/L solution (Tellurium ICP standard Certipur, Merck). The internal calibration range was constituted in each sample by the following concentrations: 0; 2; 4; 6; 8; 10 and 12 µg/L of stable iodide (Phan, Rebiere et al. 2017).

2.2.2 Biochemical assays

Plasma concentrations of total proteins, urea, creatinine, alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), and urine concentrations of uric acid, urea, creatinine, proteins, were measured by an automated spectrophotometric system Konelab 20i (from thermo electron corporation, Cergy-Pontoise, France) using the manufacturer's biological chemistry reagents (Manens et al. 2016).

2.2.3 Hormonal parameters assay

2.2.3.1 TSH

The TSH rat ELISA test kit (MP Biomedicals Germany GmbH) is a solid phase enzyme immunometric assay in the microplate format, designed for the quantitative measurement of TSH in rat plasma; the microplate is coated with a monoclonal antibody specific for TSH. Calibrators and samples are pipetted into the antibody coated microplate. Afterwards, a polyclonal horseradish peroxidase-labelled antibody is added. The optical density of the

colored solution is measured with a microplate reader at 450 nm. Bi-chromatic measurement with 600-690 nm reference filters is recommended. The analytical sensitivity of TSH was 0.1 ng / mL.

2.2.3.2 FT3 and FT4

Assay was performed at Vebio laboratory (Arcueil, France), using the IMMULIT$^{®}$2000 Systems Analyzers (Siemens healthcare diagnostics, Saint-Denis, France), a solid phase enzyme-labelled chemiluminescent competitive immunoassay, for the quantitative measurement of free thyroxine and free triiodotyronine in heparinized plasma. The CV% was lower than 12% for FT3 and lower than 6% for FT4, the analytical sensitivity of FT4 and FT3 are 2.83 pmol/ L and 1.5 pmol / L respectively.

2.2.4 Real-time PCR

Total RNA was extracted from the entire thyroid sample, using mirVana™ miRNA Isolation Kit (Ambion, cat.no.1560). The NanoDrop apparatus (ThermoFisher Scientific, Cergy Pontoise, France) was used to determine the concentration of RNA ng/µL. 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 12K Flex Real-Time PCR System (ThermoFisher Scientific, Cergy Pontoise, France) using Taqman (applied biosystems, Courtaboeuf, France) , to analyse the mRNA levels of thyroid genes, involved in iodine metabolism and thyroid hormones synthesis : Na^{+}/I^{-} symporter NIS (4331182,Rn00583900_m1), Apical iodide transporter AIT (4331182, Rn01503812_m1), Pendrin PDS (4331182,Rn00570082_m1), Thyroid peroxidase TPO (4331182, Rn00571159_m1), Thyroglobulin Tg (4351372, Rn00667257_g1) , Dual oxidase 2 DUOX2 (4331182, Rn00666512_m1), Dual oxidase maturation factor 2 DUOX a2 (4351372, Rn01512829_g1), monocarboxylate transporter 8 MCT8 (4331182, Rn00596041_m1). Relative changes in genes mRNA expression in iodide treated thyroids were calculated using $2^{-\Delta\Delta Ct}$ method; GAPDH (4331182, Rn01775763_g1) and ACTB (4331182, Rn00667869_m1) were used as internal controls. All RT-qPCR results are expressed as mean ± SEM, and compared to expression levels of non-exposed group.

2.3 Statistical analysis

Results are expressed as mean ± SEM, Student's t-test was routinely performed for statistical analysis of data, and was replaced by Mann-Whitney Rank Sum Test when the equal variance test failed (determined by GraphPad Prism software). Differences were considered statistically significant when $p \leq 0.05$.

3. RESULTS

3.1 Repeated administration of KI increases UIC

Urinary iodine concentration was dramatically increased in group of rats that received KI 1 mg/kg/24h for 1 day, 4 days and 8 days by a factor of 13, 14 and 15 respectively in comparison to their controls ($P < 0.001$). In the group d10 and d30 where the treatment was stopped for 48 h and 30 days respectively, we did not notice a significant difference between controls and treated rats (**Figure2**).

3.2 Repeated administration of KI did not affect the general health parameters and the biochemical status

Body weight was not affected by the treatment; thyroid organ coefficient judged by thyroid weight / body weight was similar between treated and control groups. Liver integrity represented by ALAT and ASAT concentrations, and renal function reflected by urea, total proteins and creatinine concentration in plasma, were not affected either. Also concerning renal function, urinary parameters such as urinary proteins, uric acid, creatinine and urea, were unaffected by the repeated administration of KI (**Table 1**).

3.3 Repeated administration of KI did not affect the activity of the pituitary-thyroid axis

Data presented in Figure 3, showed that repeated iodide administration resulted in non-significant modification of TSH, free T4 and free T3 concentration in all groups.

3.4 Repeated administration of KI generates a sequential genic variation associated to Wolff-Chaikoff effect

The effect of repeated administration of KI on the major actors involved in iodide metabolism and thyroid hormones synthesis; has been studied in rats using 1 mg/kg/24h of KI over different periods of time. After a single administration of KI, the expression of NIS and MCT8 mRNA was significantly decreased by 59% and 26% respectively. After 4 days of KI administration, only the NIS mRNA expression was still decreased by 60% whereas MCT8 mRNA expression was resumed. Then after 8 days of KI administration, NIS and TPO mRNA expression was significantly decreased by 75% and 33% respectively, and PDS mRNA expression was significantly increased by 62%. Two days after the interruption of treatment, NIS mRNA expression was always decreased by 48%, while TPO mRNA expression was resumed. Thirty days later, NIS mRNA expression was returned to normal.

The expression of AIT, Tg, DUOX2 and DUOXa2 involved in iodide efflux and organification, was unaffected by repeated iodide administration, no significant difference was observed compared to the control groups (**Figure 4**).
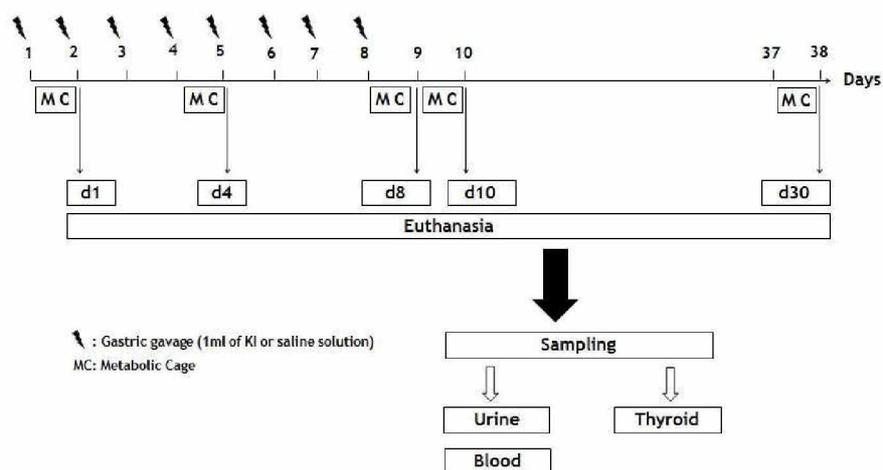
Figure 1: Prophylactic design, three regiments of KI prophylaxis: one administration (group d1), 4 administrations (group d4), and 8 administrations (group d8, d10 and d30).
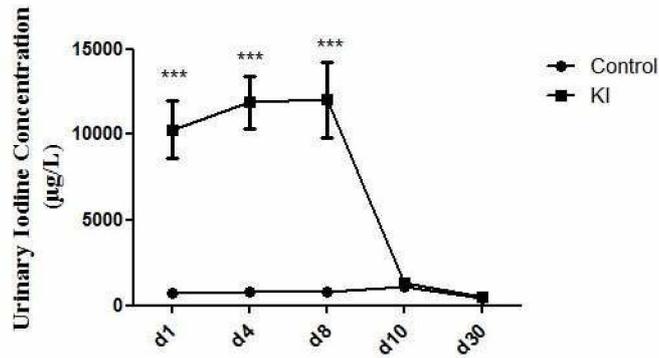
Figure 2: Urinary iodine concentration at different time. Data are expressed as Mean ± SEM.
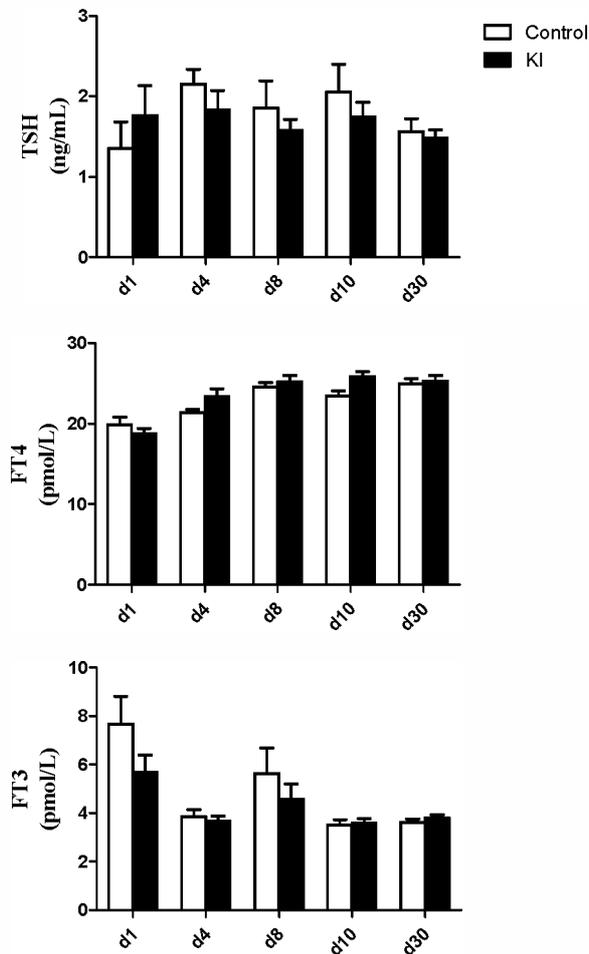
Figure 3: Plasmatic levels of thyrotropine (TSH) and, thyroid hormones free thyroxine (FT4) and free triiodothyronine (FT3). Data are expressed as Mean ± SEM.
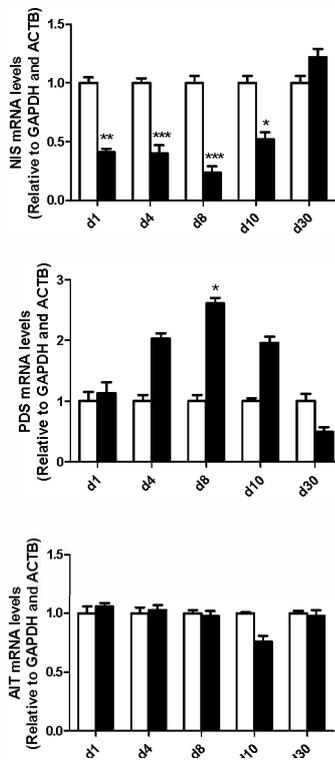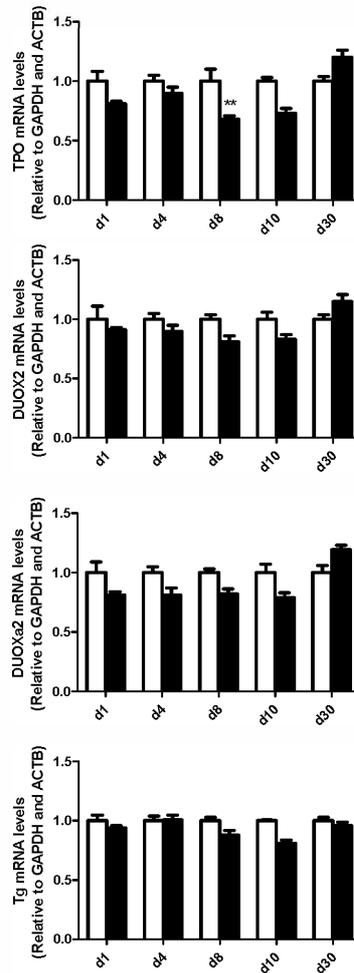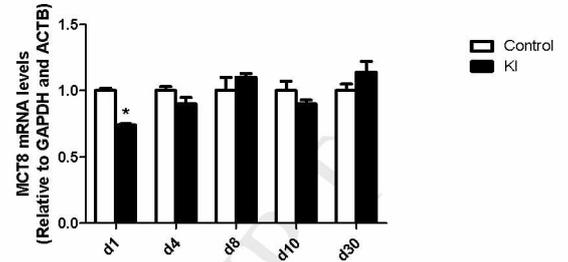
Figure 4: mRNA expression levels of gene involved (A) in iodide transport (NIS: sodium/iodide symporter, PDS: pendrin and AIT: apical iodide transporter), (B) iodide organification (TPO: thyroid peroxidase, Tg: thyroglobulin, DUOX2: dual oxidase 2, DUOXa2: Dual Oxidase Maturation Factor 2), (C) thyroid hormones transport (MCT8: monocarboxylate transporter 8). Measured by real-time PCR in the thyroid of controls (saline solution) and KI-treated rats at 1 mg/kg for 1, 4 or 8 days in the adult model. The results are expressed as a ratio to GADPH and B-actin mRNA level. Data are expressed as Mean ± SEM,*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ significantly different from model-matched control rats.
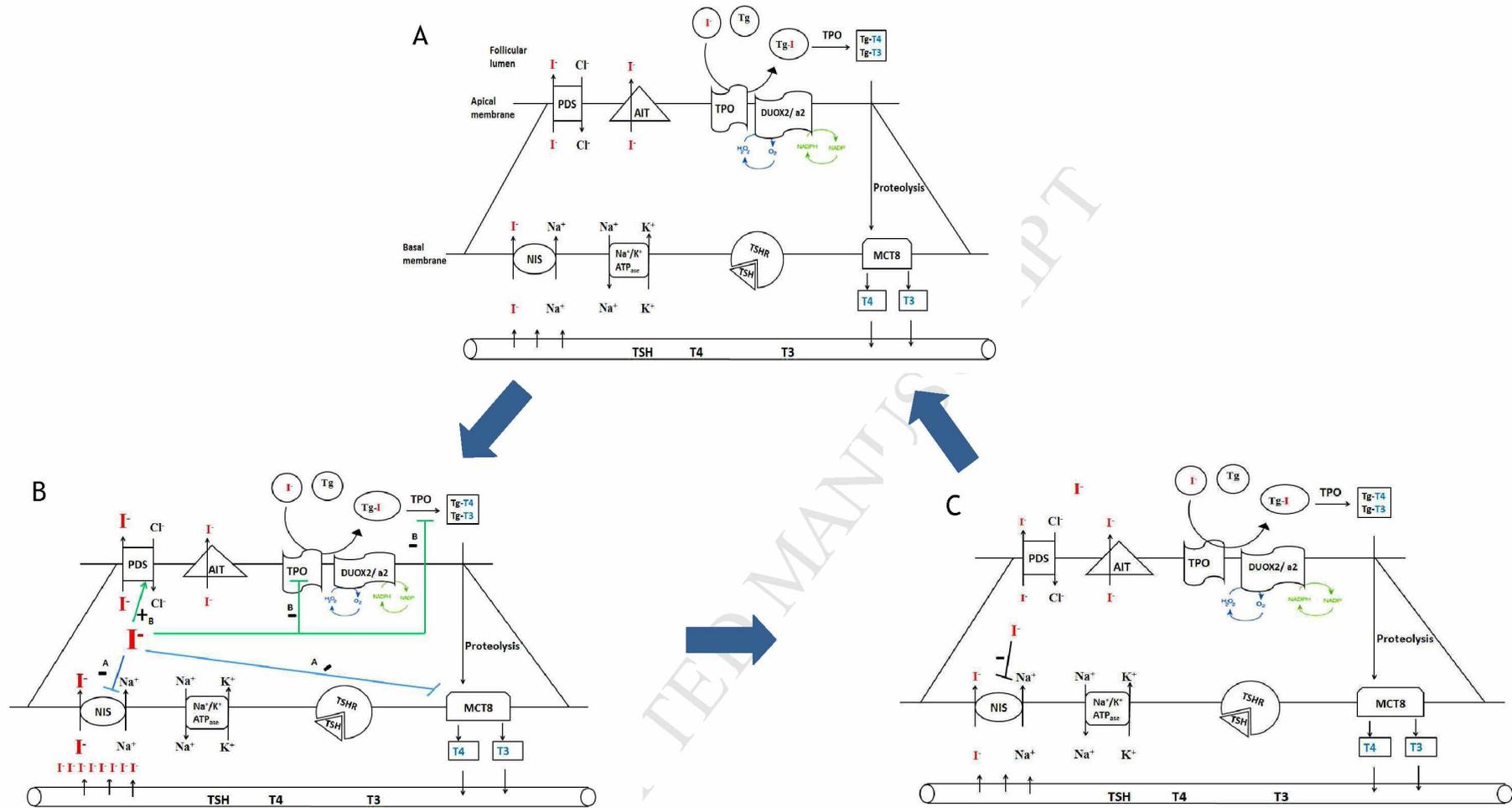
Figure 5: Hypothetical sequence of Wolff-Chaikoff effect in response to repeated potassium iodide administration, A: thyrocyte in basal state, B: a sequential Wolff-Chaikoff effect following 24h to 8 days KI intake, in blue a prompt Wolff-Chaikoff effect and in green a late Wolff-Chaikoff effect, C: escape from Wolff-Chaikoff effect 48 h after the end of the treatment. 30 days after the end of treatment thyrocyte return to basal state.

Table 1: Physiological and biochemical parameters of rats subjected or not to KI administration. Data are expressed as Mean ± SEM. *: p <0.05.

| | | General statuts | | Plasma parameters | | | | | Urine parameters | | | |
| | | Weight | | Renal function | | | Liver integrity | | Renal function | | | |
prophylaxis	KI mg	Body g	TW/BW Ratio mg/g	Creatinine µM	Urea mM	Proteins g/L	ALAT U/L	ASAT U/L	Uric acid µM/24h	Creatinine µM/24h	Urea mM/24h	Proteins mg/24h
d1	0	331.50 ±1.77	0.068±0.005	47.71±2.11	5.59±0.04	55.20±0.71	31.44±1.21	80.90±1.47	17.21±1.56	95.36±10.26	5.5±1.82	11.18±3.6
	1	339.25±10.27	0.073±0.009	49.34±2.53	6.11±0.16	56.33±1.24	32.68±0.28	80.57±1.62	17.10±1.69	92.08±1.02	8.31±0.59	7.08±1.17
d4	0	342.78 ±4.48	0.063±0.002	43.04±0.92	4.73±0.14	52.73±1.25	26.15±1.43	80.49±6.35	12.34±0.88	96.07±3.28	8.28±0.27	6.11±2.22
	1	338.34±3.39	0.071±0.003	42.59±0.56	4.80±0.29	53.17±1.03	25.82±1.56	74.99±5.51	16.39±2.71	115.82±15.13	10.41±1.42	4.36±2.26
d8	0	360.23±6.70	0.060±0.003	43.36±0.69	5.20±0.23	53.56±1.00	29.59±1.60	84.25±6.07	12.17±1.28	97.73±4.52	9.73±0.76	5.00±2.57
	1	350.91±4.59	0.065±0.002	41.92±0.83	4.98±0.26	52.31±0.40	23.70±1.34*	68.48±4.62	12.38±0.90	91.22±3.11	8.98±0.50	6.54±2.95
d10	0	368.09±10.45	0.064±0.003	42.48±0.50	4.89±0.33	54.83±1.71	25.86±3.17	78.59±1.53	14.63±0.82	105.56±11.32	9.14±1.32	13.69±1.38
	1	362.81±7.60	0.073±0.005	42.54±0.74	5.04±0.18	53.36±0.99	25.10±0.70	65.08±3.07*	13.50±0.56	108.89±5.43	11.11±0.39	15.06±6.30
d30	0	437.80±4.27	0.073±0.006	44.54±3.93	5.46±0.72	57.25±1.67	30.63±1.46	124.51±12.69	17.05±0.82	126.27±8.76	11.22±0.55	11.99±3.32
	1	427.73±5.17	0.058±0.005	44.99±1.99	5.62±0.83	56.10±2.09	30.58±1.35	112.18±8.29	17.28±1.08	129.94±6.18	10.40±0.49	10.16±2.26

TW/BW: thyroid organ coefficient, ALAT: alanine aminotransferase, ASAT: aspartate aminotransferase

4. DISCUSSION

Thyroid function can be modulated by many factors, such as TSH, thyroglobulin, and availability of iodine **(de Souza, Dias et al. 2015; Ishido et al. 2015)**. It was demonstrated that an acute excess of iodide could led to a down-regulation of thyroid hormones level, which play an important role in the regulation of body metabolism **(Eng, Cardona et al. 1999; Maia et al. 2011)**. The down-regulation of thyroxine led to the up-regulation of TSH, itself affecting thyroid genes expression **(Zaballos et al. 2008).** These effects could be most pronounced in case of repeated iodide intake.

Urinary iodine concentration was used as a biomarker for iodine status, since it reflects very recent iodine intake in epidemiological studies **(Zhang et al. 2016).** Compared to control groups, KI groups showed a significant increase in terms of urinary iodine level and this was maintained throughout the treatment duration. When evaluated 48 h or 30 days after the end of the treatment, UIC returns to the basal level. This measurement is of major importance to confirm the adequate ingestion of KI by rats.

Despite the prolonged exposure to iodide over 8 days, rats were in good general status: their final body and thyroid weight did not differ from those of untreated rats for all time points. Yoshida et al, 2014 showed that male *Wistar* rats subjected to more than 3 mg/day of KI over 4 weeks didn't show body and thyroid weight variation (Yoshida et al. 2014). Zhang et al, 2016 demonstrated that prolonged KI intake in male *Wistar* rats has no effect on overall weight (Zhang, Jiang et al. 2016). By contrast others studies, demonstrated that females BALB/c and SJL/J mice exposed to chronic iodide administration showed a significant increase of thyroid organ coefficient **(Chen et al. 2015)** and developed goiter and hypothyroidism **(Li and Carayanniotis 2007).** These differences could be explained by the species and the sex difference, also mice were exposed to different iodide doses for a relatively long period of time.

The macroscopic evaluation was confirmed by the levels of routine biochemical parameters: plasma concentration of ALAT and ASAT was similar between treated and control rats; this result is in accordance with the result published by Yoshida et al, 2014. Which indicates that hepatic integrity was not affected by the repeated administration of KI 1 mg/kg/24h, especially that the liver plays an important part in thyroid hormones metabolism **(van der Spek et al. 2017)**. Urea, protein, creatinine and uric acid were unchanged in the treated groups. These parameters are important biomarkers of renal function which account for about two third of iodide clearance from the plasma and more than 90% of iodide excretion from the body **(Cavalieri 1997; Ristic-Medic and Glibetic 2016)**

The negative effect of iodine on serum TH level has been discussed in many studies in human and mice **(Vagenakis et al. 1973; Markou et al. 2001; Miyai et al. 2008)**. In our study, we demonstrated that plasma levels of thyrotropine, free thyroxine and free triiodothyronine were not significantly different between treated and control groups at all-time points. This result is in accordance with other studies that did not report an impact of iodide on the pituitary-thyroid axis activity in rats **(Serrano-Nascimento et al. 2010; Solis, Villalobos et al. 2011; Calil-Silveira et al. 2012; Arriagada et al. 2015)**. By contrast, other experiments showed that an acute or a chronic intake of iodide has an impact on thyroid hormones and TSH level in rat **(Eng, Cardona et al. 1999; de Souza, Dias et al. 2015; Calil Silveira et al. 2016)**. These differences may be due to the differences between studies regarding iodide concentration, the route of administration as well as the duration of treatment.

Since thyroid organ coefficient, biochemical and hormonal status, were unaffected by the repeated administration of KI 1 mg/kg/24h over 8 days, we suggest that this dose is well tolerated by adult *Wistar* rats. Based on these data, we approved the safety of repeated administration of KI at 1 mg/kg/24h in adult rats.

The fact that general health, biochemical and hormonal status remained unchanged did not ensure that repeated iodide administration did not have an impact 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 high level of plasma iodide **(Eng, Cardona et al. 1999)**. Most studies focused on the impact of iodide on NIS expression. Herein a large panel of target genes involved in iodide transport (NIS, PDS, and AIT), iodide organification (TPO, Tg, DUOX2and DUOXa2) and thyroid hormones transport (MCT8) was assessed.

Repeated iodide administration lead to a down-regulation of NIS mRNA expression at all-time points, which is in agreement with other studies; a significant decrease of NIS mRNA expression due to sodium or potassium iodide administration was described **(Eng, Cardona et al. 1999; Anguiano et al. 2007)**. Serrano-Nascimento et al. (2010) showed an acute reduction of NIS mRNA content since 30 min to 24 h after administration; they suggested the involvement of post-transcriptional mechanisms. Another team demonstrated a decrease of NIS mRNA expression after one day and seven days of KI (0.05%) administration **(Solis, Villalobos et al. 2011)**. Also Chen et al. (2015) noted a decrease of NIS mRNA expression after seven months of KIO_3 administration in BALB/c mice. It was also suggested that excess iodide down-regulates NIS expression by transcriptional mechanisms through the activation of PI3K/AKT pathway **(Serrano-Nascimento et al. 2016).**

In conjunction with the inhibition of iodide entrance in thyrocyte, the expression of MCT8 mRNA, the main TH transporter expressed in rats was acutely decreased one day after administration of KI. Our results are in agreement with the study published by de Souza et al. (2015); they reported an impact of NaI (0.05%) on MCT8 mRNA expression and a major effect on MCT8 protein. They suggested that iodide could stimulate MCT8 lysosomal degradation. We propose that this prompt effect of KI on iodide entrance and TH transport after 24 h of administration prevents the occurrence of hyperthyroidism.

Although repeated iodide administration did not affect AIT mRNA level, PDS mRNA expression was increased after 8 days of iodide administration. This probably plays an important role in reduction of intracellular iodine content and prevents deleterious effects on thyroid cells. Calil-Silviera et al. (2012) observed an acute increase of PDS mRNA expression since 30 min that was maintained until 48 h, the same team demonstrates an increase of PDS mRNA expression in male *Wistar* rats after 60 days of NaI (0.005% and 0.05%) administration **(Calil Silveira, Serrano do Nascimento et al. 2016)**. By contrast, Anguiano et al. (2007) demonstrated a decrease of PDS mRNA expression after one day and six days of iodide administration. Chen et al. (2015) also demonstrated a decrease of PDS mRNA expression after seven months of iodide administration in BALB/c mice **(Chen, Lin et al. 2015)**. The mechanisms that underlie the impact of iodide on PDS expression are not well elucidated, it has been demonstrated that PDS activity is increased through cAMP/PKA pathway in kidney **(Azroyan et al. 2012)**, also in PCCL-3 rat thyroid cells TSH increases Pendrin abundance through PKA-dependent pathway **(Pesce et al. 2012)**. Calil Silverira et al.(2016) found that iodide excess led to an increase of level of TSH and pendrine mRNA expression , on the other hand it decreased the TSH receptor mRNA and protein levels, so they suggested that increased pendrine mRNA expression is mainly mediated by iodine per se. Another possible explanation is that NIS inhibition over 8 days could led to a slight deplete of thyroidal iodide store **(McLanahan et al. 2009)**, which could stimulate the expression of PDS that mediates the efflux of iodide to the colloid.

TPO plays a major role in iodide organification, and in the formation of T3 and T4 **(Ruf and Carayon 2006)**. In our experiment TPO mRNA expression was decreased only after 8 days of KI administration, Eng et al, (1999) demonstrated a decrease of TPO mRNA expression after one day of iodide intake, the delayed effect of iodide on TPO mRNA expression in our case can be related to the stimulation of PDS mRNA expression only after 8 days. The well

elucidate mechanism underlying the down-regulation of TPO mRNA expression is the production of an iodo-lipid element 2-IHDA (Ohayon et al. 1994). This down-regulation may also be interpreted as protection against iodide efflux by PDS.

These stimulatory and inhibitory effects on PDS and TPO respectively, were transient, and help to maintain the homeostasis of thyroid function as demonstrated by the level of thyroid hormones, which was unchanged, and perhaps prevents the occurrence of hypothyroidism. The expression of DUOX2, DUOXa2 and Tg was unaffected by repeated iodide administration. Mori et al. (1998) reported a slight decrease of Tg mRNA expression after one month and two months of iodide administration in BB/Wor rats.

Based on these data, a tight Wolff-Chaikoff effect was noticed at the genic level. This effect was produced in two steps: Firstly we observed an early Wolff-Chaikoff effect marked by an acute down-regulation of NIS and MCT8, and then a late Wolff-Chaikoff effect was observed especially marked by a down-regulation of TPO and up-regulation of PDS.

One month after the treatment withdrawal, the expression of genes involved in TH synthesis returned to normal (**Figure 5**).

To conclude, we have shown that potassium iodide administrated to adult rats at 1 mg/kg/24h over a period of 8 days did not modify general health parameters, hormonal status and biochemical homeostasis. We observed a sequential genic variation associated to the Wolff-Chaikoff effect, without any endocrinological perturbations. These prominent data gives the base evidence of the safety of repeated iodide prophylaxis in adult rat model. This is of great interest in the field of radiation protection especially in case of prolonged exposure to radioiodine. These results may contribute as input data for new studies on other species, in order to evolve the current guidelines as well as the marketing authorization of potassium iodide.

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Conflict of Interests

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

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