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Effect of repetitive potassium iodide on thyroid and cardiovascular functions in elderly rats

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ABSTRACT

Background: To date, paediatric thyroid cancer has been the most severe health consequence of the Chernobyl accident, caused by radioactive iodine (^{131}I) aerosol's dispersion. WHO recommends a single dose of potassium iodide (KI) to reduce this risk. Following the Fukushima accident, it became obvious that repetitive doses of KI may be necessary due to multiple exposures to ^{131}I. Knowledge about the effects of repeated ITB (Iodine Thyroid Blocking) is scarce and controversial. KI may affect the thyroid hormones synthesis; which is crucial for the cardiovascular function. Furthermore, myocardial and vascular endothelial tissues are sensitizes to subtle changes at the concentration of circulating pituitary and/or thyroid hormones.
Objective: In this preclinical study, we aimed to assess the effects of repeated ITB in elderly male rats.
Methods: Twelve months old male *Wistar* rats were subjected to either KI or saline solution for eight days. Analyses were performed 24 h and 30 days after the treatment discontinuation.
Findings: We reported a significant increase (18%) in some urinary parameters related to renal function, a subtle decrease of plasma TSH level, a significant increase (379%) in renin and a significant decrease (50%) in aldosterone upon KI administration. At the molecular level, the expression of thyroid and cardiovascular genes was significantly affected by the treatment. However, in our experimental settlement, animal heart rate was not significantly affected thirty days after KI discontinuation. ECG patterns did not change after administration of KI, and arrhythmia was not observed in these conditions despite the PR-intervals decreased significantly. Cardiovascular physiology was preserved.
Conclusion: Our results indicate that repeated ITB in elderly rats is characterized by molecular modifications of cardiovascular key actors, particularly the Renin-angiotensin-aldosterone axis with a preserved physiological homeostasis. This new scientific evidence may be useful for the maturation of ITB guidelines especially for elderly sub-population.

1. Introduction

Ionizing radiation is still the only established etiologic factor for thyroid cancer in humans, and the thyroid gland is one of the organs most susceptible to the carcinogenic effect of radiation [1]. A large number of epidemiological studies have contributed in improving knowledge about the occurrence of thyroid cancer after accidental radiation exposure [2–4]. Within few years after the Chernobyl nuclear power plant (NPP) accident, an increase in the occurrence of thyroid cancer was reported in the surrounding regions [5]. This is probably associated with the estimated release of 1.760 PBq of ^{131}I into the environment. In 2011, the Fukushima NPP accident raised public health concerns on the potential incidence of thyroid cancer [6]. In Japan, even if the estimated release of ^{131}I into the environment was lesser, a health surveillance program was implemented in 2011, the Fukushima Health Management Survey (FHMS) program.

During a radiological or nuclear emergency, provision of ITB to individuals who are at risk of being exposed to radioiodine should be

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implemented as an urgent protective action, within the frame of a justified and optimized protection strategy. This specific countermeasure complete an action plan including a series of physical countermeasures, *i. e* sheltering – food restriction and evacuation. ITB is based on the consumption of a single dose of KI (130 mg for individuals older than 12 years; 65 mg for children between three and twelve years old; 32 mg for infants between one month and three years old and 16 mg for neonates from birth up to one month) [7]. The optimal period for the intake of stable iodine is less than 24 h prior to, and up to 2 h after, the expected onset of the radiation exposure [8]. It would still be reasonable to administrate KI up to 8 h after the estimated onset of exposure [9]. Though, starting KI intake later than 24 h following radioactive iodine exposure may do more harm than benefit.

A single administration of stable iodine is usually sufficient to protect the thyroid. However, in the case of prolonged, *i.e.* beyond 24 h or repeated exposure, unavoidable ingestion of contaminated food and drinking water, and where evacuation is not easily feasible, repeated administration of stable iodine may be necessary [7,10]. Elderly, over 40 years, should not receive repeated KI intake [7]. As they have a higher risk to develop atrial fibrillation in association with a subclinical hyperthyroidism [11].

Thyroid hormone (TH) signaling is critical for proper heart function by regulating the expression of the genes coding for myosin heavy chain α and β. Moreover, triiodothyronine (T3) stimulates nearly all the enzyme systems involved in Ca^{2+} and ions fluxes. TH also exerts important effects on the electrophysiological properties of cardiac myocytes and affects the peripheral vascular tone [12]. Both diastolic and systolic functions are strictly dependant on the thyroid hormone action. Furthermore, the ventricular contractile function is significantly influenced by the changes of hemodynamic condition secondary to the TH-induced reduction in peripheral vascular tone. The observed rapid changes in systolic pump efficiency after TH administration strongly depends on central and peripheral hormonal effects that act synergistically to improve systolic performance. In addition, the TH-induced reduction in diastolic blood pressure activates the renin-angiotensin-aldosterone axis with a consequent increase in renal sodium resorption and in circulating blood volume. TH homeostasis preserves a positive ventricle-arterial coupling, thus tipping the balance favourably towards heart functioning [12].

In our previous work, we have demonstrated the safety of repeated administration of KI in adult *Wistar* rats [13]. Studies regarding the effect of repeated iodine intake in elderly are scarce; and limited to the thyroid disorder consecutive to the administration of the antiarrhythmic amiodarone [14,15]. These deleterious effects were associated to the excess of iodine used in this therapy. This work aims, for the first time, to evaluate the effects of repeated ITB on the thyroid function and on the cardiovascular homeostasis of the elderly using aged *Wistar* rats as a preclinical model.

2. Materials and methods

2.1. Experimental procedure

2.1.1. Materials

NaCl (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

Male *Wistar* rats, aged 12 months, weighing 547.24 ± 5.69 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 (12hrs/12hrs). Normal-iodized pellet diet 0.3 mg I//Kg pellet (A04-10 SAFE, 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 the 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 KI, rats were divided into two groups, and each group has its own control group as follows:

Group 1 (d9):7 rats/condition, receiving eight consecutive administrations of KI (1 mg/kg/day) or saline solution, and euthanized 24 h later.

Group 2 (d38): 11 rats/condition, receiving eight consecutive administrations of KI (1 mg/kg/day) or saline solution, and euthanized 30 days post-prophylaxis.

The treatment was carried out by gastric gavage; treated rats received 1 mL of KI solution whereas their matched control groups were given 1 mL of saline solution (Fig. 1).

2.1.4. Organs and biofluids collection

Before euthanasia, rats were placed in individual metabolic cages for 24hrs, with free access to food and water. Urine was collected twice a day, and stored at + 4 °C to limit bacterial growth, fractions were pooled, mixed, and centrifuged (3000 rpm/10 min); supernatants were frozen at - 80 °C. Subsequently, rats were euthanized by intracardiac puncture under isoflurane 5% (Abbot France, Rungis, France), blood was collected in heparinized tubes and centrifuged (3000 rpm/10min) subsequently, and the supernatant was immediately frozen at - 80 °C. Thyroid, heart, carotid and aorta were dissected on ice, deep-frozen in liquid nitrogen, and then stored at −80 °C.

2.2. Technical procedure

2.2.1. Biochemical assays

Biochemical indicators were measured in plasma and urine samples with an automated spectrophotometric system Konelab 20i (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, chloride, potassium and sodium), cardiac markers (creatine kinase (CK) and its isoform (CK-MB) and lactate dehydrogenase (LDH)), liver markers (alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT)), and kidney markers (creatinine and urea). In the urine sample, the kidney markers (creatinine, urea and uric acid), substrates (urinary proteins) and electrolytes (calcium, phosphorus, chloride, potassium and sodium) were measured. Phospholipid B (Diagnostic partners, Bormes Les Mimosas, France) and LDH (Diagram, Lille, France) indicators were adapted on the spectrophotometric system.

2.2.2. Hormonal parameters assay

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).

2.2.3. Renin – angiotensin – aldosterone system (RAA)

The hormonal assays for renin and angiotensin II were performed using the "Enzyme-Linked Immunosorbent Assay Kit for Renin" and "Enzyme-Linked Immunosorbent Assay kit for Angiotensin II" kits (Cloud-Clone Corp. USA) which include the test protocols. Absorbance reading was performed at 450 nm. The dosage of aldosterone was prepared with the "ALDOSTERONE Chemiluminescent Immunoassay Kit" (Arbor assays, USA) whose detection limit was determined at 2.35 pg/mL. The apparatus used for reading the absorbance is Tecan (France). For aldosterone, the use of the Berthold automaton (Technologies,

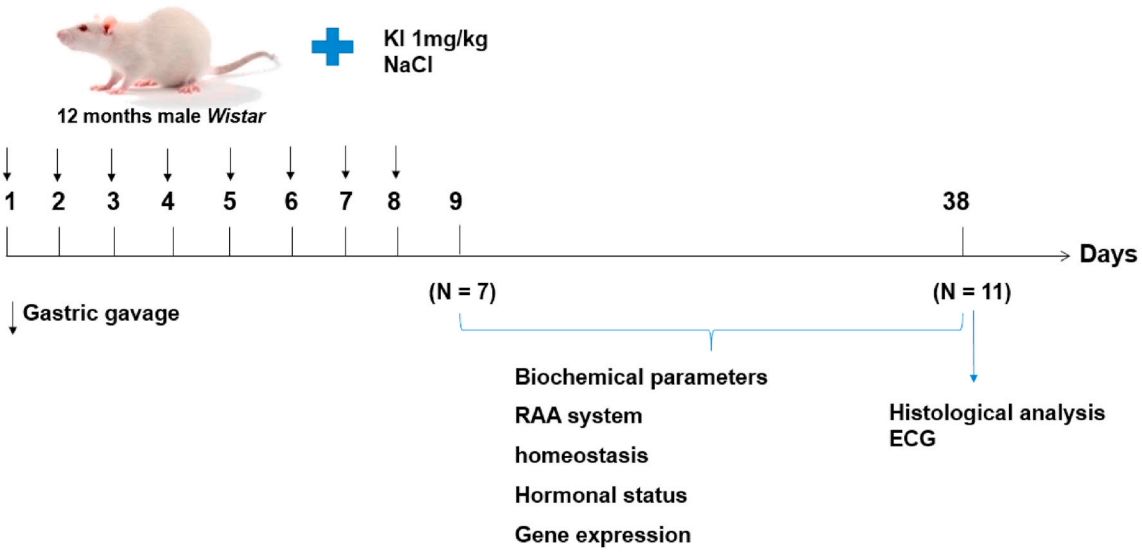

Fig. 1. Prophylactic design of repeated intake of KI in elderly rat.

Germany) allowed the reading of the absorbance at a rate of 0.1 s per well.

2.2.4. Real-time PCR

Total RNA was extracted from both thyroid lobes, heart and aorta (≈25 mg), using mirVana™ miRNA Isolation Kit (Ambion, cat. no.1560). One 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, heart and aorta (Table 1). Relative changes of genes mRNA expression in KI exposed samples were calculated using $2^{-\Delta\Delta Ct}$ method; GAPDH and ACTB were used as internal controls. Results are expressed as mean ± SEM.

2.2.5. Histology and immunohistochemistry

Thyroid, heart, carotid and aorta collected at necropsy were placed in a neutral 10% formalin solution to allow immersion fixation. Each individually fixed tissue was then trimmed, placed in a histological cassette, and impregnated with paraffin according to the SCIEMPATH LABO (Larcay-France) technical procedures. For each thyroid, 3 μm thick sections spaced by more than 100 μm apart from each other were then stained with hematoxylin and eosin. Immunohistochemistry (IHC) for Ki67 was performedaccording toSCIEMPATH LABO protocol. For each carotid or aorta, 4 μm thick sections spaced by more than 100 μm apart from each other were then stained with Verhoeff-Van Gieson, IHC for ICAM and VCAM was performedon carotid and aorta simples. Heart samples were trimmed in order to obtain a complete transverse section and an apical longitudinal section. IHC stains for detection of laminin, myosin heavy chain II, 3-nitrotyrosine and smooth muscle actin α (α-SMA) were performed on heart samples. All stains and immunohistochemistry were performed according to SCIEMPATH LABO protocol. The complete digitization of the tissue sections was performed by the Hamamatsu Nanozoomer at a 20x magnification at the SCIEMPATH LABO.

Table 1
Taqman primers.

Genes	Assay ID number	Gènes	Référence des amorces
Oxidative stress		Cardiac function	
Cat	Rn00560930_m1	Atp2a2-SERCA2	Rn00568762_m1
Sod1	Rn00566938_m1	Pln	Rn01434045_m1
Sod2	Rn00690588_g1	MHCβ	Rn01488777_g1
Inflammation		MHCα	Rn00691721_g1
IL6	Rn01410330	Kv4.2	Rn00581941_m1
Tnf	Rn99999017_m1	Kv4.3	Rn04339183_m1
Tgfb1	Rn00572010_m1	KChIP2	Rn01411450_g1
IL18	Rn01422083	**Thyroid function**	
		TSHR	Rn00563612_m1
IL1b	Rn00580432_m1	NIS	Rn00583900_m1
Endothelial function		AIT	Rn01503812_m1
VEGFa	Rn01511602_m1	PDS	Rn00570082_m1
		Tg	Rn00667257_g1
		TPO	Rn00571159_m1
Vcam1	Rn00563627_m1	MCT8	Rn00596041_m1
Icam1	Rn00564227_m1	DUOX2	Rn00666512_m1
eNOS	Rn02132634_s1	DUOXa2	Rn01512829_g1

2.2.6. ECG measurement

Radio telemetry probes recording electrocardiography (ECG; TL-11M2-C50-PXT; Data Sciences International, St. Paul, MN, USA) were inserted in each animal (n = 6 for each group). Anesthesia was induced with isoflurane inhalation 5% (Forene, Abbott, France) and maintained with isoflurane 2%. A midline incision in the lower abdomen permitted implantation of the radio telemetry probe. The ECG electrodes were tunneled subcutaneously and placed in a lead II configuration. After a 14-days recovery period, ECG was measured. Data were collected telemetrically continuously during 4hrs/2days (we choose the window from 1 to 2 a.m. and 1–2 p.m.) over the last 2 days of the experiment.

The ECG waveforms were sampled at 500 Hz and saved to a PC harddisk with Dataquest Advanced Research Technology (ART) software (Data Sciences International, St. Paul, MN, USA). They were analyzed with Ponemah software (Data Sciences International, St. Paul, MN, USA).

2.3. Statistical analysis

Results are expressed as mean ± SEM, Student's t-test was routinely performed for statistical analysis of data assuming that the data are normally distributed with equal variance. Otherwise, the non-parametric Mann-Whitney Rank Sum Test was performed. Differences were considered statistically significant when $p \leq 0.05$.

3. Results

3.1. Repeated potassium iodide intake impairs biochemical status

At short-term 24hrs post-prophylaxis, repeated KI intake induces a decrease in plasma proteins level. On the other hand, at long-term repeated KI intake induces a significant increase of plasma creatinine level and urinary (chlorine, potassium, sodium, uric acid, urea, creatinine and glucose) levels (Table 2).

3.2. Repeated potassium iodide intake does not impair hormonal status

Repeated KI tended ($p = 0.057$) to downregulate plasma TSH level 30 days post-prophylaxis, free T4 and T3 levels were similar to those of the controls (Fig. 2).

3.3. Repeated potassium iodide intake impairs renin – angiotensin – aldosterone system

For the short-term treatment, plasma (renin, angiotensin and aldosterone) levels were similar between treated and control groups. After 30 days, a significant increase in renin +379.57%, $p = 0.01$) and a significant decrease in aldosterone (−50.28%, $p = 0.039$) were observed. Angiotensin remains unchanged (Fig. 3).

3.4. Repeated potassium iodide intake impairs thyroid genes expression

Repeated KI intake (24hrs post-prophylaxis) induced a significant decrease in NIS – TPO – DUOXa2 and MCT8 mRNA expression (respectively −62.21%, −40.8%, 24.7% and 35.9%, p-values 0.013, 0.037, 0.019 and 0.025). After thirty days, a significant increase in AIT and Tg mRNA expression (+298.98%, $p = 0.00057$ and + 39.37%, $p = 0.03$ respectively) and a significant decrease in TSHR mRNA expression (−51.28%, $p = 0.02$) was observed (Fig. 4).

3.5. Repeated potassium iodide intake impairs cardiovascular genes expression

No difference was observed in cardiac genes expression at 24 h post-prophylaxis (Fig. 5A). A significant decrease in MHCα – SERCA2 and Kv4.2 mRNA expression (−27.4% $p = 0.004$, −31.1% $p = 0.01$, 36.7% $p = 0.0069$) was observed at 30 days post-prophylaxis treatment (Fig. 5).

At the endothelial level, for the 24hrs post-prophylaxis treatment, a significant increase in ICAM1 and VCAM1 mRNA expression (+184% $p = 0.001$ and + 98% $p = 0.001$ respectively) and a significant decrease of TGFB mRNA expression (−77.33% $p = 0.001$) was observed. Thirty days post-prophylaxis, a significant decrease in ICAM1, VCAM1 and IL1BmRNA expression (−62.4% $p = 0.01$,-71.71% $p = 0.006$ and −66.6% $p = 0.03$ respectively) was observed (Fig. 6).

3.6. Repeated potassium iodide intake does not impair thyroid, heart, carotid and aorta histology

The thirty days post-prophylaxis treatment of KI to aged rats did not induce any morphological changes in neither thyroid follicles, nor in nuclear proliferation of the follicular epithelium. No histological changes in the cardiovascular system (vascular and cardiac), evaluated by quantitative histomorphometric approaches, was observed (data not shown).

3.7. Repeated intake of potassium iodide impairs some ECG parameters

The thirty days post-prophylaxis treatment of KI to elderly rats did not induce any changes in heart rate (HR) significantly: (335 average BPM ± 9.8 SEM and 313 BPM ± 7.8 SEM for KI and control, respectively). ECG patterns did not change after one month of KI administration, and arrhythmia was not observed in these conditions (data not shown). The PR decreased significantly (−8.4%, $p = 0.002$) in KI-treated rats, but no alteration was observed for the RR, QT and ST-intervals (Table 3).

Table 2
General status and biochemical parameters.

parameters	d9		d38	
	Control	KI (1 mg/kg/24 h)	Control	KI (1 mg/kg/24 h)
General indicators				
Final body weight (g)	532.00 ± 26.50	561.00 ± 82.73	556.88 ± 21.82	555.92 ± 21.22
TW/BW Ratio	5.23^{E-05} ± 2.05^{E-06}	5.54^{E-05} ± 8.70^{E-06}	6.18^{E-05}± 5.88^{E-06}	6.39^{E-05} ± 3.42^{E-06}
HW/BW Ratio	2.51^{E-03} ± 1.16^{E-04}	2.41^{E-03} ± 3.71^{-04}	2.58^{E-03} ± 5.09^{E-05}	2.50^{E-03} ± 6.02^{-05}
Plasma biomarkers				
Cholesterol (mmol/L)	1.92 ± 0.14	1.73 ± 0.13	2.61 ± 0.09	2.69 ± 0.23
HDL cholesterol (mmol/L)	1.69 ± 0.22	1.91 ± 0.15	1.47 ± 0.12	1.42 ± 010
LDL cholesterol	043 ± 0.08	0.35 ± 0.06	0.28 ± 0.05	0.34 ± 0.05
Phospholipids B (g/L)	1.35 ± 0.07	1.26 ± 0.12	2.15 ± 0.21	2.03 ± 0.15
Triglycerides (mmol/L)	1.15 ± 0.15	0.94 ± 0.30	2.13 ± 0.52	1.88 ± 0.45
Total proteins (g/L)	65.82 ± 1.81	59.90 ± 2.94*	78.81 ± 2.88	86.45 ± 4.62
Calcium (mmol/L)	3.13 ± 0.08	3.11 ± 0.09	3.40 ± 0.05	3.52 ± 0.16
Phosphorus (mmol/L)	1.74 ± 0.08	1.51 ± 0.05	1.58 ± 0.07	1.81 ± 0.10
Iron (μmol/L)	9.33 ± 0.29	8.37 ± 0.67	10.62 ± 0.87	12.47 ± 0.92
Chlorine (mmol/L)	108.75 ± 3.68	113.27 ± 2.86	106.08 ± 1.36	97.61 ± 2.40 *
Potassium (mmol/L)	5.07 ± 0.34	4.59 ± 0.23	4.68 ± 0.09	4.18 ± 0.35
Sodium (mmol/L)	161.99 ± 3.80	158.61 ± 3.90	142.02 ± 1.26	148.36 ± 5.74
ALAT (U/L)	24.03 ± 1.31	26.70 ± 2.85	30.45 ± 4.32	34.57 ± 3.00
ASAT (U/L)	68.74 ± 5.44	84.66 ± 5.74	100.06 ± 5.93	112.61 ± 6.24
Creatinine (μM)	53.74 ± 2.00	56.45 ± 1.63	65.92 ± 3.35	77.83 ± 3.35 *
Urea (mM)	5.77 ± 0.33	6.27 ± 0.43	6.89 ± 0.35	7.52 ± 0.40
CK (U/L)	133.21 ± 21.51	148.64 ± 23.45	154.47 ± 20.10	142.36 ± 14.11
CK-MB (U/L)	336.18 ± 38.04	321.53 ± 54.26	264.17 ± 35.72	284.99 ± 23.87
Glucose (μmol/L)	10.19 ± 0.43	10.75 ± 0.67	13.21 ± 0.94	15.74 ± 0.87
Urine biomarkers				
Chlorine (mmol/24 h)	1.50 ± 0.32	2.04 ± 0.25	1.91 ± 0.13	2.45 ± 0.19 *
Potassium (mmol/24 h)	1.90 ± 0.24	2.16 ± 0.33	2.23 ± 0.09	2.71 ± 0.17 *
Sodium (mmol/24 h)	1.06 ± 0.12	1.02 ± 0.15	1.32 ± 0.08	1.71 ± 0.11 *
Phosphorus (mmol/24 h)	0.18 ± 0.05	0.26 ± 0.07	0.39 ± 0.05	0.47 ± 0.09
Calcium (μmol/24 h)	56.27 ± 19.30	62.90 ± 12.25	90.37 ± 8.93	101.30 ± 13.11
Urinary proteins (mg/24 h)	5.81 ± 1.97	4.58 ± 1.56	29.36 ± 16.55	13.06 ± 2.67
Uric acid (μM/24 h)	14.97 ± 1.93	13.78 ± 1.00	15.21 ± 1.00	20.08 ± 1.56 *
Urea (Mm/24 h)	11.78 ± 1.32	13.51 ± 1.25	14.83 ± 0.87	18.67 ± 1.02 **
Creatinine (μM/24 h)	133.82 ± 13.80	155.74 ± 13.08	147.04 ± .48	199.63 ± 11.44 **
Glucose (μmol/24 h)	10.58 ± 1.7	9.69 ± 0.78	14.92 ± 1.80	21.15 ± 1.78 *

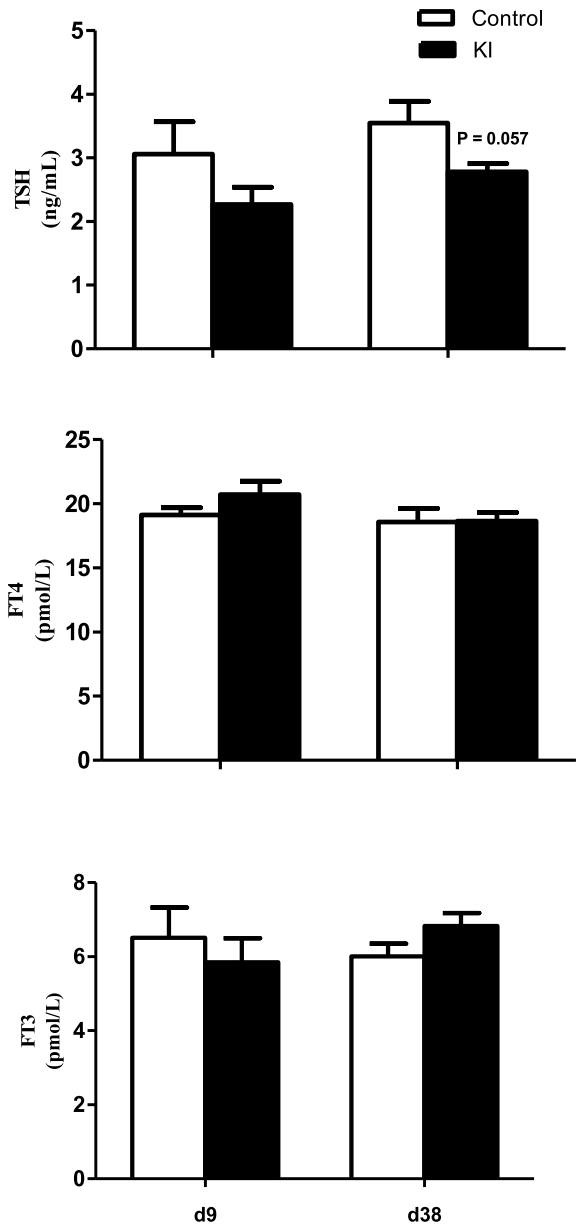

Fig. 2. Plasma levels of thyrotropine (TSH) and, thyroid hormones free thyroxine (FT4) and free triiodothyronine (FT3). Data are expressed as Mean ± SEM. Triiodothyronine (FT3). Data are expressed as Mean ± SEM.

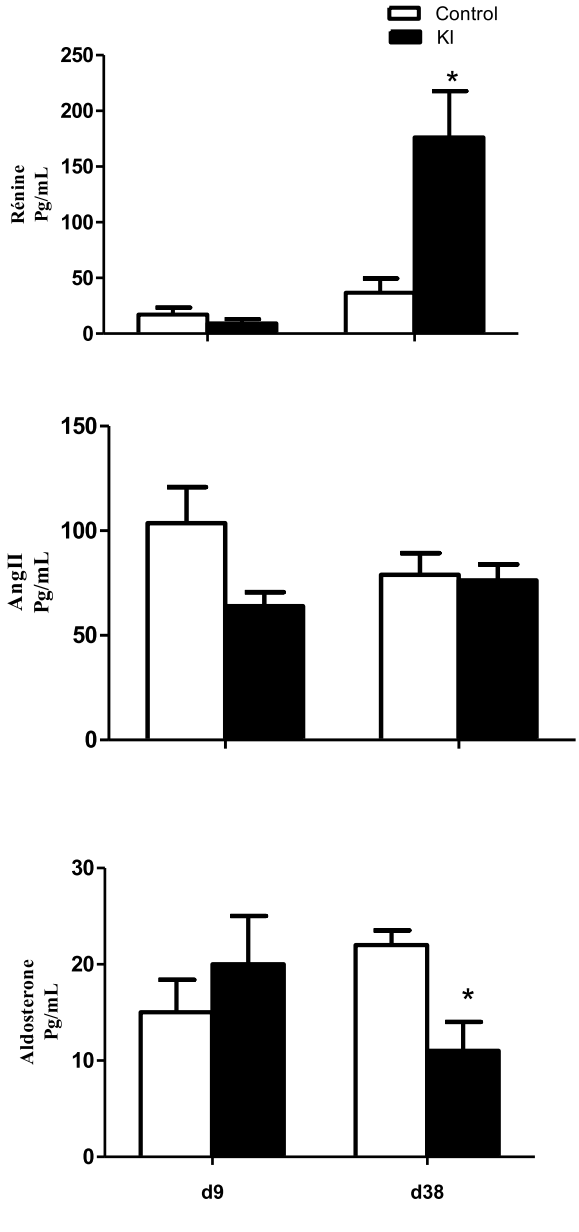

Fig. 3. Plasma level of renin, angiotensin II and aldosterone. Data are expressed as mean ± SEM. *p < 0.05 vs control.

4. Discussion

Repetitive ^{131}I accidently discharged from the Fukushima Daiichi Nuclear Power Plant into the atmosphere combined with the complexity of the evacuation plan consecutive to the earthquake and tsunami raise the question of the risk to the population of possible repetitive exposure to radioactive iodine in the context of radiological and nuclear emergency. Repeated ITB could be justified in case of repeated or prolonged exposure to radioactive iodine. The study of Sternthal et al. [16], in adult humans showed efficient thyroid protection with no toxicological effect linked to the repeated intake of KI for 12 days. However, no scientific evidence is available regarding the effects of repetitive ITB on elderly. In this sub-population, atrial fibrillations secondary to thyroid dysfunction have been described [17]. In addition, different countries, such as Germany, consider that the risk of developing cardiovascular diseases following repeated ITB or even a single dose may be higher than that of developing thyroid cancer. Indeed, in the elderly population, we may wonder if the benefit/risk balance is favorable over the risks of developing thyroid cancer *vs* cardiovascular diseases.

Proper management of chronic diseases is a priority for the various healthcare systems around the world, due to their significant economic burden and because these diseases are the main cause of death and overall disability. Heart failure (HF) is one of these conditions; the lifetime risk of HF at 55 years of age is 33% in males and 28% in females. Also, the proper function and maintenance of thyroid hormones (THs) is of biological and medical significance [18]. THs are essential for optimal function of the cardiovascular system [19,20]. THs synthesis depends on iodine availability as a major substrate [21]. Any variation in iodine level could lead to thyroid dysfunction and could result in the impairment of vital function such as the cardiovascular function [18].

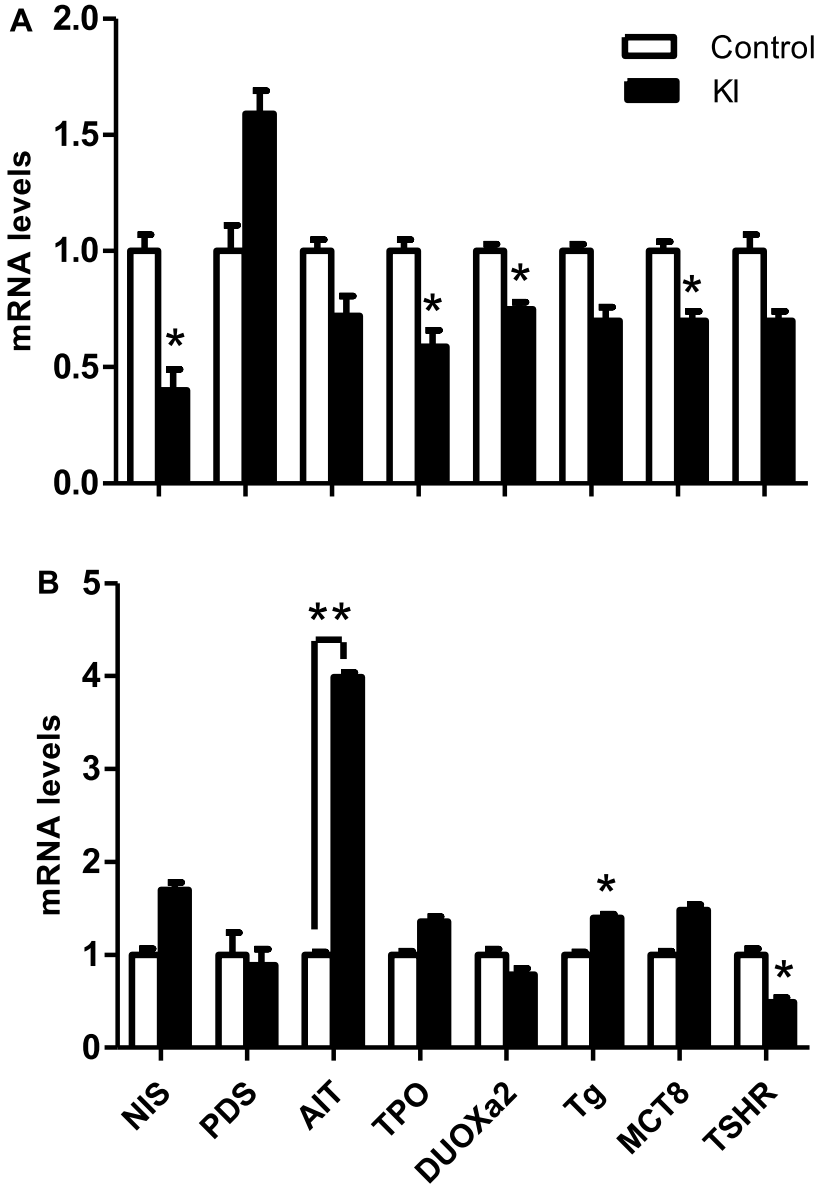

Fig. 4. MRNA expression level of thyroid genes involved in iodine transport (NIS – PDS and AIT), iodine organification (TPO – DUOXA2 and Tg), thyroid hormone transport (MCT8) and thyroid control (TSHR). (A): d9, 24 h post-prophylaxis, (B): d38, 30 days post-prophylaxis. The results are expressed as a ratio to GADPH and ACTB mRNA level. Data are expressed as Mean ± SEM.

In the situation of potential repeated radioactive iodine release upon a nuclear emergency, it is suggested that repeated ITB would be an adapted countermeasure to protect against the incidence of thyroid cancer. It has been clearly demonstrated that the excess of iodine inhibits transiently thyroid function and thyroid hormones synthesis [22]. In the adult population, the safety of repeated KI intake was demonstrated in different species [13,16,23]. However, scientific evidence of repetitive use of stable iodine in elderly are scarce and the use of anti-arrhythmic amiodarone to cope with cardiovascular dysfunction can be highlighted. Repeated use of amiodarone (200 mg contains 75 mg of iodine) in elderly leads to multiple thyroid dysfunctions [14,15]. As far as we know, this study is the first one to evaluate repeated stable iodine prophylaxis in 12-months-old male *Wistar* rats, a model mimicking the human elderly sub-population.

In our experimental conditions, elderly rats were in good general status. Their final body and organs weight did not differ from those of the untreated group. This result is in accordance with our previous study on adult rats and other studies that do not report an impact of repeated iodine intake on the body and/or organs weight [13,24,25]. Routine biochemical evaluation study reveals a significant long-term impact of repeated treatment on standard plasmatic and urinary biochemical status. Thirty days after the treatment discontinuation, we observe slight but significant increase of plasma creatinine level and Cl-, Na+, K+, uric acid, creatinine, urea and glucose levels in the urine. In the literature, elevated creatinine plasma levels is largely used as a biomarker of kidney dysfunction, which is associated with increased risk of adverse outcomes, including cardiovascular events [26]. In addition, Na + level variations suggest a disturbed renin – angiotensin – aldosterone system.

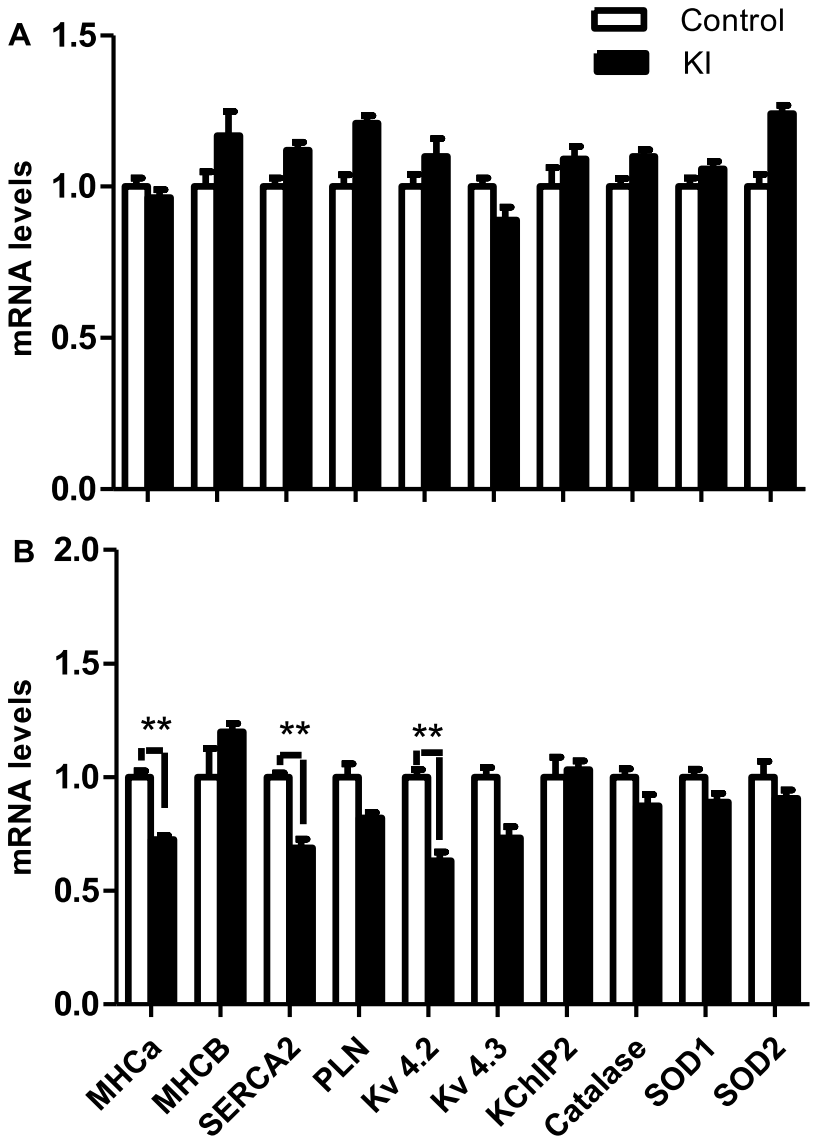

Fig. 5. MRNA expression level of heart genes involved in cardiac function (MHCa – MHCB – SERCA2 – PLN – Kv 4.2 – Kv 4.3 and KChIP2) and oxidative stress (Catalase – SOD1 and SOD2). (A): d9, 24 h post-prophylaxis, (B): d38, 30 days post-prophylaxis. The results are expressed as a ratio to GADPH and ACTB mRNA level. Data are expressed as Mean ± SEM.

In fact, the evaluation of plasma concentration of these key actors reveals a significant increase of renin concentration and significant decrease of aldosterone, whereas, angiotensin II level was not modified. Due to this atypical profile, a regulation through angiotensin conversion enzyme or the contribution of an independent renin pathway is hypothesized.

It has been well described, that an excess of iodine may transiently inhibit the thyroid function: the well-known Wolff-Chaikoff effect [22]. In our study the evaluation of thyroid hormones level shows that free T4 and T3 levels were similar to those of controls. On the other hand, a trend to the downregulation of TSH level at thirty days after treatment discontinuation can be observed and this trend may be associated to transitory subclinical signs of hyperthyroidism. Contrariwise, in the literature it has been reported that aging causes a decrease in serum or plasma levels of T3 and T4 [27,28].

To go in more details, we have investigated the effect of repeated KI intake at the thyroid, heart and aorta molecular level. Usually protein synthesis follows gene expression level [29], so we focus on genetic level of the major actors in these tissues. The function of modified genes is mentioned in Table 4.

A reversible Wolff-Chaikoff effect, 24 h post-prophylaxis was observed, objective by a significant decrease in mRNA expression of key actors of the thyroid hormone synthesis (NIS, TPO, DUOXa2 and MCT8). These observations are in accordance with our previous study [13]. Thirty days post-prophylaxis, expression of these genes were find to resume again and a decrease of TSH receptor mRNA expression was noted. This result complies with the hormonal trend to down-regulate TSH.

Regarding the cardiovascular system, no short term effect of repetitive KI administration was observed. However, a significant

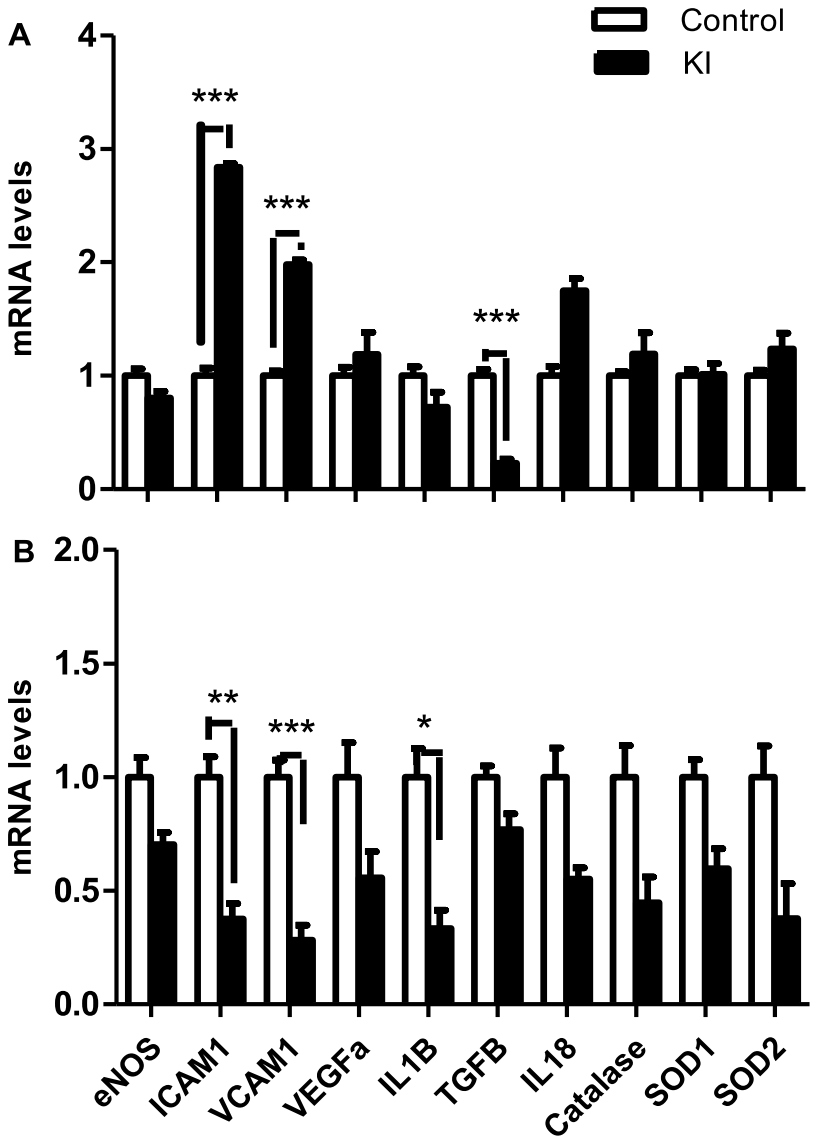

Fig. 6. MRNA expression level of heart genes involved in endothelial function (eNOS – ICAM1 – VCAM1 and VEGFa), inflammation (IL1B – TGFB and IL18) and oxidative stress (Catalase – SOD1 and SOD2). (A): d9, 24 h post-prophylaxis, (B): d38, 30 days post-prophylaxis. The results are expressed as a ratio to GADPH and ACTB mRNA level. Data are expressed as Mean ± SEM.

Table 3
ECG parameters in rats after 30 days post-prophylaxis Heart rate in beats per minute (bpm), other parameters are in milliseconds (ms). Data are expressed as mean ± SEM; n = 4 for each group of rats; * significantly different from control (p = 0 0.002).

Group	HR (bpm)	RR (ms)	PR (ms)	QRS (ms)	QT (ms)	ST (ms)
Control	313 ± 7.8	193.5 ± 4.9	42.9 ± 0.6	19.7 ± 0.5	61 ± 0.9	45 ± 1.4
KI	335.5 ± 9.8	181.3 ± 5.6	39.6 ± 0.9*	18.7 ± 0.2	64.7 ± 1.9	51.1 ± 2.9

downregulation of the main genes involved in cardiac contractility and calcium homeostasis (MHCα – SERCA2 and Kv4.2) was observed thirty days post-prophylaxis. This down-regulation may be a consequence of the repeated KI intake or might be an aging factor [30]. Finally, endothelia ICAM1 and VCAM1 mRNA expression were founded to be increase 24 h post-prophylaxis, while at thirty days post-prophylaxis, a significant decrease in the mRNA expression of these genes was observed. However, in our experimental settlement, the different molecular modifications observed do not have an impact on the cardiac function and in particular on the heart rate. Further preclinical studies could be proposed on predisposed animal model with asymptomatic thyroid or pre-existing cardiovascular pathologies.

In conclusion, the first scientific evidence was produced on the absence of toxic effect of repetitive ITB in a 12-month-old male Wistar rat model. Indeed, we have observed that ITB is responsible for the modifications of the biochemical parameters through affecting particular markers of the renal function. These effects are associated with a change in the regulation of the renin-angiotensin aldosterone axis. In

Table 4
The function of genes modified by the treatment.

Genes		Function	References
Thyroid	NIS	Iodide uptake	[13]
	AIT	Iodide efflux	[13]
	TPO	Key enzyme in thyroid hormone biosynthesis. It catalyzes both iodination and coupling of iodotyrosine residues in TG.	[13]
	DUOXa2	generate H2O2 utilized by thyroid peroxidase (TPO) for the biosynthesis of thyroid hormones	[13]
	Tg	Substrate for the synthesis of the thyroid hormones. Storage of the inactive forms of thyroid hormone and iodine within the follicular lumen of a thyroid follicle enhances gene expression necessary for thyroid hormone secretion	[13]
	MCT8	Thyroid hormones transport and release	[13]
	TSHR	Activates all functional aspects of the thyroid cell	[31]
Heart	MHCa	contractile velocity	[32]
	SERCA2	Calcium homeostasis (transport calcium from the cytosol into the sarcoplasmic reticulum).	[33]
	Kv 4.2	Regulating neurotransmitter release, heart rate, neuronal excitability, epithelial electrolyte transport, smooth muscle contraction, and cell volume	[34]
Aorta	ICAM1	Cell signaling (stabilizing cell-cell interactions and facilitating leukocyte endothelial transmigration)	[35]
	VCAM1	Mediates the adhesion of lymphocytes, monocytes, eosinophils, and basophils to vascular endothelium. It also functions in leukocyte-endothelial cell signal transduction, and it may play a role in the development of atherosclerosis and rheumatoid arthritis.	[36]
	IL1B	Important mediator of the inflammatory response, and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis.	[37]
	TGFB	Performs many cellular functions, including the control of cell growth, cell proliferation, cell differentiation, and apoptosis.	[38]

addition, this prophylaxis causes differential thyroid gene expression of key actor of the Wolff-Chaikoff effect. However, these subtle molecular effects do not alter cardiovascular function.

This new scientific evidence may be useful for the maturation of ITB guidelines especially for elderly sub-population. Our results suggest that the benefit/risk balance of repeated ITB in elderly "normal" rat model is favorable. Before issuing a final judgement it will be of interest to assess repeated ITB toxicity in (i) a preclinical aged animal model predisposed to cardiovascular disease and (ii) in GLP pre-clinical studies using a rodent and a none-rodent animal species. This strategy is necessary to conclude on the merits of repeated ITB in the elderly.

Author statement

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed.

Declaration of competing interest

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

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