

# **Iodinated Contrast Agents Perturb Iodide Uptake by the Thyroid Independently of Free Iodide**

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## ABSTRACT

The perturbation of thyroid iodide uptake is a well-documented side effect of the utilization of iodinated contrast media (ICM) administered intravenously. This side effect is thought to be mediated by free iodide in ICM formulations but this hypothesis has never been formally demonstrated. The aim of the present paper is to assess the validity of this hypothesis.

## Methods

We used mass spectrometry analysis to quantify free-iodide contamination in ICM. Established cell lines expressing the Na/I symporter (NIS) were used to quantify the effect of ICM on iodide uptake. Single-photon emission computed tomography/computed tomography (SPECT/CT) imaging was used to measure the *in vivo* uptake of  $^{99m}\text{Tc}$ pertechnetate and  $^{123}\text{I}$  in two NIS-expressing mouse tissues, thyroid and salivary glands. Scintigraphies of naïve and ICM-administered patients were compared. Immunohistological and western blot analyses were performed to evaluate NIS protein expression in these organs.

## Results

Although free iodide was present in ICM formulations, *in vitro* uptake of iodide by NIS-expressing cells was not significantly affected by ICM. In mice, intravenous or sublingual administration of ICM led to a reduction in radiotracer uptake by the thyroid, accompanied by a dramatic reduction in NIS protein expression in this tissue. In the salivary glands, neither radiotracer uptake nor NIS protein expression were affected by ICM. The thyroid-selective effect of ICM was also observed in humans. Administration of potassium iodide (KI) as a source of free iodide led to a diminution of  $^{99m}\text{Tc}$ pertechnetate uptake in both mouse thyroid and salivary glands. Altogether, these data rule

out a direct intervention of free iodide in the perturbation of thyroid uptake and suggest a direct and selective effect of ICM on the thyroid.

## **Conclusions**

We demonstrate that ICM reduce thyroid uptake of iodide independently of free iodide. This effect is due to a specific and dramatic decrease in NIS expression in thyrocytes. These data cast serious doubt on the relevance of measuring urinary iodide concentration to evaluate the delay between ICM administration and radioiodine therapy in patients with differentiated thyroid carcinoma. Finally, the property of ICM to perturb iodide uptake in the thyroid may be used in radioprotection.

## INTRODUCTION

ICM are routinely administered to patients as contrast media for X-ray imaging. Considering their widespread utilization, they can be considered as safe. However, a well-documented side effect of intravenous ICM administration observed in most patients is compromise of diagnostic thyroid scintigraphy and radioiodine treatment of thyroid malignancies (1-5). For the latter, guidelines recommend delaying radioactive iodide treatment in patients who have been exposed to ICM (4,6-8). Mechanistically, this reduced iodide uptake by thyroid tissues in response to ICM is thought to be the result of injection of high amounts of free iodide contaminating the ICM formulation (9). Many studies have reported that urinary iodide concentration remains high for days and even weeks after ICM administration (10,11). This high urinary iodide concentration may reflect a high blood iodide content that could be consistent with the long-lasting effect of ICM on thyroid iodide uptake. The source of this free iodide may be free iodide associated (9) with ICM and/or released from (4,12) ICM upon injection. However, although this hypothesis is generally-accepted, it has, to our knowledge, never been formally demonstrated.

The protein responsible for iodide uptake is NIS which is located at the basolateral membrane of some epithelial cells including thyrocytes in the thyroid (13,14) and duct cells in the salivary gland (15). Ingestion of a large amount of KI leads to a high blood iodide content which in turn dramatically reduces the uptake of radio-iodide or its surrogate  $^{99m}\text{TcO}_4^-$  in the thyroid and the salivary glands (16). However, this effect is transient and a return to normal uptake capacities of both tissues is observed within 24 hours after a single administration of a large amount of KI.

In this context, the aim of the present paper was to determine experimentally whether the free iodide that contaminates ICM (9) or which can be released *in vivo* through deiodination of

ICM in tissues (4,12) is responsible for the reduced iodide uptake by thyroid tissues. If either or both these hypotheses are correct, Iomeron (as a prototype of ICM) would be expected to act in the same way as free iodide on iodide uptake in NIS-expressing cells and tissues.

## **MATERIALS AND METHODS**

### **Animals**

Eight-week-old female C57BL/6JRj mice were obtained from Janvier (Le Genest Saint Isle, France). The animals were treated in accordance with the French Agriculture Ministry guidelines and the experiments were approved by the University of Nice Sophia Antipolis animal care user and ethics committee (Ciepal NCE/2014-211).

### **Contrast Agents**

Experiments were performed using iomeprol (Iomeron 350; lot LP4557) and iodixanol (Visipaque 320; lot 125 78 776).

### **Cell Lines**

The human colorectal cancer cell line HT-29 (HTB-38, American Tissue Culture Collection) was stably transfected with the expression plasmid pcDNA3.1-mNIS (murine NIS) (17) and one clone with high functional expression of NIS was selected (18). The rat follicular thyroid cell line PCCL3 was obtained from Dr. A. De La Vieja (Madrid, Spain) and cultured as previously described (19). Measurement of *in vitro* iodide uptake was performed as previously described (20).

### **Mass Spectrometry**

Iomeron, Visipaque and standard solutions of NaI were characterized by high-resolution mass spectroscopy in negative mode electrospray. For the direct-infusion experiments a flow of 5  $\mu\text{L}/\text{min}$  was provided by a syringe pump (11 Plus, Harvard Apparatus, Holliston, MA, USA) using a 500  $\mu\text{L}/\text{min}$  syringe (Hamilton, Reno, NV, USA). The mass spectrometer (Q Exactive Plus, Thermo Fisher Scientific, Bremen, Germany) was operated with an electrospray source for the

direct-infusion method development. The direct-infusion Orbitrap measurements were carried out using the Ion Max source from Thermo Fisher Scientific and applying the following parameters: sheath gas flow, 15 arbitrary units; auxiliary gas flow, 5 arbitrary units; capillary temperature, 275°C. The automatic gain control target was set to  $10^6$  and the maximum injection time was 50 ms. In the high-resolution measurements with setting of 140 000 at  $m/z$  200 one microscan was recorded. The spray voltage in negative mode was selected at -2.5 kV.

### **Administration of ICM in Mice In Vivo**

Intravenous and intraperitoneal administration was performed by injecting 100  $\mu$ L Iomeron diluted 50/50 (vol/vol) with phosphate-buffered saline (corresponding to 18 mg iomeprol). Enteral administration was performed by gavage of 100  $\mu$ L Iomeron diluted 50/50 (vol/vol) with phosphate-buffered saline (corresponding to 18 mg iomeprol). Sublingual administration was performed on anesthetized mice by five sublingual depositions of 5  $\mu$ L Iomeron, with a delay of 10 minutes between two administrations. This procedure led to the administration of 9 mg iomeprol.

### **Small-Animal MicroSPECT/CT Scans**

$^{99m}\text{Tc}$  pertechnetate ( $^{99m}\text{TcO}_4^-$ ) was obtained from a freshly eluted  $^{99}\text{Mo}/^{99m}\text{Tc}$  generator.  $^{123}\text{I-NaI}$  was purchased from IBA (Gif sur Yvette, France). Animals were administered intraperitoneally with activities of 20 MBq  $^{99m}\text{TcO}_4^-$  or 10 MBq  $^{123}\text{I}^-$ . Thyroid and salivary gland tracer uptake were measured at different times using a dedicated microSPECT/CT scanner (eXplore speCZT CT120, GE) as previously described (21). Reconstructed images were analyzed and quantified using AMIDE software (22). Tri-dimensional regions of interest were drawn

manually around the thyroid and salivary glands, as previously detailed (23,24). Uptakes were expressed as percentages of the injected activity after decay correction (21).

### **Kinetics of Inhibition of $^{99m}\text{Tc}$ pertechnetate or $^{123}\text{I}$ -NaI uptake by ICM and KI**

For ICM inhibition of  $^{99m}\text{Tc}$ pertechnetate or  $^{123}\text{I}$ -NaI uptake, SPECT/CT imaging of mice administered 20 MBq  $^{99m}\text{Tc}$ pertechnetate or 10 MBq  $^{123}\text{I}$ -NaI was performed (basal uptake, day 0). At the end of the scan, ICM was administered. At different times after ICM administration, animals were injected with 20 MBq  $^{99m}\text{Tc}$ pertechnetate or 10 MBq  $^{123}\text{I}$ -NaI and new scans were performed.

For KI inhibition of  $^{99m}\text{Tc}$ pertechnetate uptake, SPECT/CT imaging of mice administered 20 MBq  $^{99m}\text{Tc}$ pertechnetate was performed (basal uptake). Twenty-four hours later, animals were injected intraperitoneally with KI (9 mg in 180  $\mu\text{L}$ ). The animals were then injected 30 min later with 20 MBq  $^{99m}\text{Tc}$ pertechnetate and scanned. Twenty-four hours later, animals were injected with 20 MBq  $^{99m}\text{Tc}$ pertechnetate and new scans were performed.

### **Thyroid and Salivary Glands Uptake of $^{99m}\text{Tc}$ pertechnetate in Humans**

A “naïve” patient and a patient who had received an intravenous injection of Iomeron 350 two weeks before scintigraphy were involved. On the day of the scintigraphy, patients were injected intravenously with an activity of 1 MBq/kg and 600 s scans was performed 15 min later. The institutional review board approved this retrospective study and, considering that the images are anonymous, the requirement to obtain informed consent was waived.

### **Immunohistochemistry and Western Blot Analyses**

After culling the animals, thyroids and salivary glands were dissected. Some thyroids and salivary glands were paraffin-embedded and cut into 4- $\mu\text{m}$ -thick sections. The paraffin was then

removed and the sections were rehydrated and subjected to an antigen retrieval treatment with a solution of citrate buffer, pH 6, using an automated station (PT Link, Dako). NIS immunostaining was performed as previously described (21). For western blotting thyroid and salivary gland membrane proteins were subjected to electrophoresis as previously described (21). Western blotting was performed with antibody 25 anti-mouse NIS, an affinity-purified rabbit immunoreactive serum fraction, or with an anti- $\beta$ -actin antibody (Sigma).

### **Statistical Analysis**

Statistical analysis was performed using Prism (GraphPad software). Dual comparisons were made using a Student's t-test and comparisons between multiple conditions were analyzed using analysis of variance. Statistical significance was set at  $P < 0.05$ .

## **RESULTS**

### **Free Iodide Content of ICM**

We first evaluated whether free iodide was present in commercially available ICM. Mass spectrometry analysis revealed that the concentrations of free iodide in Iomeron and Visipaque were in the range of 30  $\mu$ M and 100  $\mu$ M, respectively. The details of this dataset are available in Supplemental Figure 1.

### **Effect of ICM on the Uptake of Iodide in Cell Lines**

We next examined whether iomeprol and its contaminating free iodide could affect iodide uptake in NIS-expressing cell lines. PCCL3 (a rat follicular thyroid cell line) cells were incubated with  $^{125}$ I in the presence of either saline buffer, Iomeron, NaI, or perchlorate ( $\text{NaClO}_4$ ). After one hour, cells were washed and cellular  $^{125}$ I content was determined. As expected, both NaI and perchlorate inhibited iodide uptake in PCCL3 cells, but Iomeron failed to affect this uptake significantly (Fig.1). Similar data were obtained using the HT29-NIS (a human colorectal carcinoma cell line expressing NIS) cell line (Supplemental Fig. 2).

### **Effect of ICM on $^{99\text{m}}$ pertechnetate Uptake in Mice and Humans**

We evaluated whether Iomeron could affect the uptake of the iodide analog,  $^{99\text{m}}$ pertechnetate, by the mouse thyroid and the salivary glands. Basal uptake was measured before (T0) and after intravenous administration of Iomeron. Figure 2A shows that a dramatic reduction in radiotracer uptake by the thyroid was observed 24 hours after Iomeron administration. This uptake capacity remained low for eight days and a recovery was observed at day 12. In the same animals, Iomeron administration failed to affect iodide uptake by another NIS-expressing tissue,

the salivary glands (Fig. 2B). This differential effect was also obtained when  $^{123}\text{I}$  was used as the radiotracer (Supplemental Fig.3). The differential uptake of  $^{99\text{m}}\text{pertechnetate}$  by the thyroid and salivary glands was also observed using Visipaque (Supplemental Fig. 4). This observation suggests that ICM affect the mouse thyroid and the salivary glands differently. To evaluate whether this differential action is observed in humans, we compared  $^{99\text{m}}\text{pertechnetate}$  uptake in the thyroid and salivary glands of a naïve patient (Fig. 3A) or a patient who had received an intravenous injection of Iomeron two weeks before (Fig. 3B). Figure 3A shows the scintigraphy of a naïve patient in which both the thyroid and salivary glands are taking up  $^{99\text{m}}\text{pertechnetate}$ . The scintigraphy of a patient treated with Iomeron shows radiotracer uptake in the salivary glands and a lack of fixation in the thyroid region (Fig. 3B).

### **Effect of KI on $^{99\text{m}}\text{pertechnetate}$ Uptake in Mice**

A similar experiment was carried out to evaluate the effect of KI on radiotracer uptake by the thyroid and salivary glands. Figure 4A shows that the ability of the thyroid to take up  $^{99\text{m}}\text{pertechnetate}$  was reduced 30 minutes after intraperitoneal injection of KI. This capacity was recovered 24 hours after injection. A similar pattern was observed when  $^{99\text{m}}\text{pertechnetate}$  uptake to the salivary glands was measured (Fig. 4B). This dataset demonstrates that potassium iodide affects both thyroidal and salivary gland  $^{99\text{m}}\text{pertechnetate}$  uptake.

### **Effect of ICM on NIS Expression In Vivo**

We next evaluated the effect of Iomeron on NIS expression in the thyroid and salivary glands. Immunohistological analysis revealed that NIS expression was hardly detectable in thyroids four days after Iomeron injection (Fig 5B), as compared with control mice (Fig. 5A). By contrast, NIS expression was detectable in the ductal cells of the salivary glands of both control

and Iomeron-treated mice (Fig. 5C and D). Semi-quantitative analysis by western blot confirmed a dramatic decrease in NIS expression in the thyroid glands of Iomeron-treated mice compared with controls (Fig.5E). No significant difference between NIS expression was detected in the salivary glands in both groups.

### **Modes of Administration of ICM in Mice**

We next compared the efficacy of different modes of administration of Iomeron on <sup>99m</sup>pertechnetate uptake by the thyroid and the salivary glands. Figure 6A shows that intravenous and sublingual administration of the ICM resulted in a marked reduction in radiotracer uptake (Fig. 6A). A similar effect was obtained with Visipaque (Supplemental Fig.4 A), while neither enteral nor intraperitoneal administration affected radiotracer uptake by the thyroid. Uptake to the salivary glands was not affected in any condition (Fig. 6B and Supplemental Fig. 4).

## DISCUSSION

Although rare, side effects of the utilization of ICM, such as thyrotoxicosis, hyperthyroidism, and hypothyroidism have been reported, both in populations at risk and in some cases in individuals without previous thyroid dysfunction (4,25,26). However, the main side effect observed is the perturbation of iodide uptake by the thyroid. This effect does not appear to affect total intrathyroidal iodine concentration (27). This perturbation has been attributed to the free iodide associated with and/or released from ICM on administration. We first determined whether free iodide contamination associated with the ICM could mediate this effect. Mass spectrometric analysis was used to quantify the free iodide in Iomeron (25 to 30  $\mu\text{M}$  free iodide) and Visipaque (100  $\mu\text{M}$  free iodide). In addition, competition experiments using NIS-expressing cells demonstrated that Iomeron did not affect iodide uptake. These *in vitro* results suggest that if free iodide is involved in the perturbation of iodide uptake by the thyroid *in vivo*, it must be released in the body from the deiodination of ICM in tissues that may store ICM. The possibility of such ICM deiodination has been demonstrated (4,12) and, considering the high amount of iodide associated with the organic backbone of the ICM, deiodination may be the source of a delayed release of sufficient iodide to the circulation to perturb thyroid uptake.

Free iodide perturbed uptake of the iodide analog,  $^{99\text{m}}$ pertechnetate, by both the mouse thyroid and salivary glands. Kinetically, for both organs, uptake decreased rapidly (30 minutes after KI injection in our experiment), and the capacity of the thyroid to take up the radiotracer was restored within 24 hours. In comparison, iomeprol affected uptake of  $^{99\text{m}}$ pertechnetate to the mouse thyroid selectively. As expected from the well-known biodistribution of these two radiotracers (28), this effect was also observed with  $^{123}\text{I}$ . The images presented in Figure 3 represents two scintigraphies obtained from human patients. These human data are consistent with the animal

results. However, considering the small number of cases, this dataset can only be taken as an indication rather than a formal demonstration of a differential uptake of  $^{99m}\text{Tc}$  pertechnetate by the thyroid and salivary glands upon ICM administration. Nevertheless, overall, our data rule out a direct intervention of free iodide in the perturbation of uptake to the thyroid and suggest a direct and selective effect of iomeprol on the thyroid. Considering that similar data were obtained with Visipaque, it is tempting to hypothesize that ICM, in general, act directly and selectively on the thyroid.

In patients, urinary iodide content has been shown to be elevated for a number of weeks after ICM injection and a four-to-six-week period is considered necessary for urinary iodide to return to normal levels (10,11,29,30). These observations provide the basis for the advised four-to-six-week delay between ICM injection and radioiodine therapy in thyroid cancer patients (7). This implies that urinary iodide has been used as a surrogate marker of the restoration of normal iodide uptake by thyroid tissues. Our results cast serious doubt on the relevance and usefulness of the measurement of urinary iodide concentration in the context of ICM and radioiodine therapy. In our experimental conditions, in mice, the ICM-mediated thyroid “stunning” lasted for at least eight days and a recovery was observed by day 12. These data are consistent with a long-lasting effect of a single injection of ICM in humans. The recommended delay between ICM injection and radioiodine therapy is currently between four to eight weeks. If the recommended delay between ICM injection and radioiodine therapy were to be reduced or optimized, we advocate that dedicated trials should be performed and based on functional imaging of the thyroid.

The mechanism by which ICM reduces iodide uptake by the thyroid involves a dramatic reduction in the expression of the NIS protein. Four days after ICM injection, the NIS protein was hardly detectable using immunohistochemistry. Western blot analysis confirmed this observation.

As expected from the functional imaging data, NIS protein expression was unaffected in the salivary glands. To exert this effect, it is likely that ICM trigger an intracellular molecular mechanism that results in reduced NIS protein expression. ICM have been shown to trigger various intracellular signaling pathways in kidney cells (31-33), which could be involved in the effect observed on the thyroid. The molecular mechanisms involved in the thyroid-specific effect of ICM are currently under investigation in our laboratory.

Although effective and safe overall, the utilization of KI tablets to protect populations in the event of a nuclear incident is associated with some problematic issues. In cases of prolonged  $^{131}\text{I}$  exposure, the United States Food and Drug Administration recommends KI tablets to be ingested on a daily basis (<http://www.fda.gov/Drugs/EmergencyPreparedness/BioterrorismandDrugPreparedness/ucm072265.htm>), with potential logistic and compliance problems. In this context, "one shot" measures that could replace KI tablets or reduce the requirement for a stringent compliance to the daily intake of KI tablets would be welcome. Formulations based on ICM could provide this benefit. Given that the intravenous mode of administration is hardly relevant for an application in radioprotection, the sublingual delivery of ICM is efficient in mice and could be considered in humans. In addition, ICM could be chemically modified to be absorbed by the gut epithelium, leading the way to a compound administered orally. Furthermore, elucidation of the molecular mechanisms involved in the effects of ICM on the thyroid could provide a basis for the selection of new compounds that could be used in radioprotection.

## CONCLUSIONS

One side effect of the utilization of ICM is alteration of iodide uptake by the thyroid. This effect is thought to be mediated by free iodide associated with and/or released from ICM. In the present report we demonstrate that ICM induces thyroid stunning to a greater and longer-lasting degree than the free iodide found in ICM would explain. The effect is due to a specific and dramatic decrease in NIS expression in thyrocytes. These data cast serious doubt on the relevance and usefulness of the measurement of urinary iodide concentration to evaluate the delay between ICM administration and radioiodine therapy of patients with differentiated thyroid carcinoma. Finally, the ability of ICM to perturb iodide uptake in the thyroid on a relatively long-term basis could be exploited in radioprotection.

## **DISCLOSURE**

A patent on the utilization of ICM in radioprotection is currently being processed.

## **ACKNOWLEDGEMENTS**

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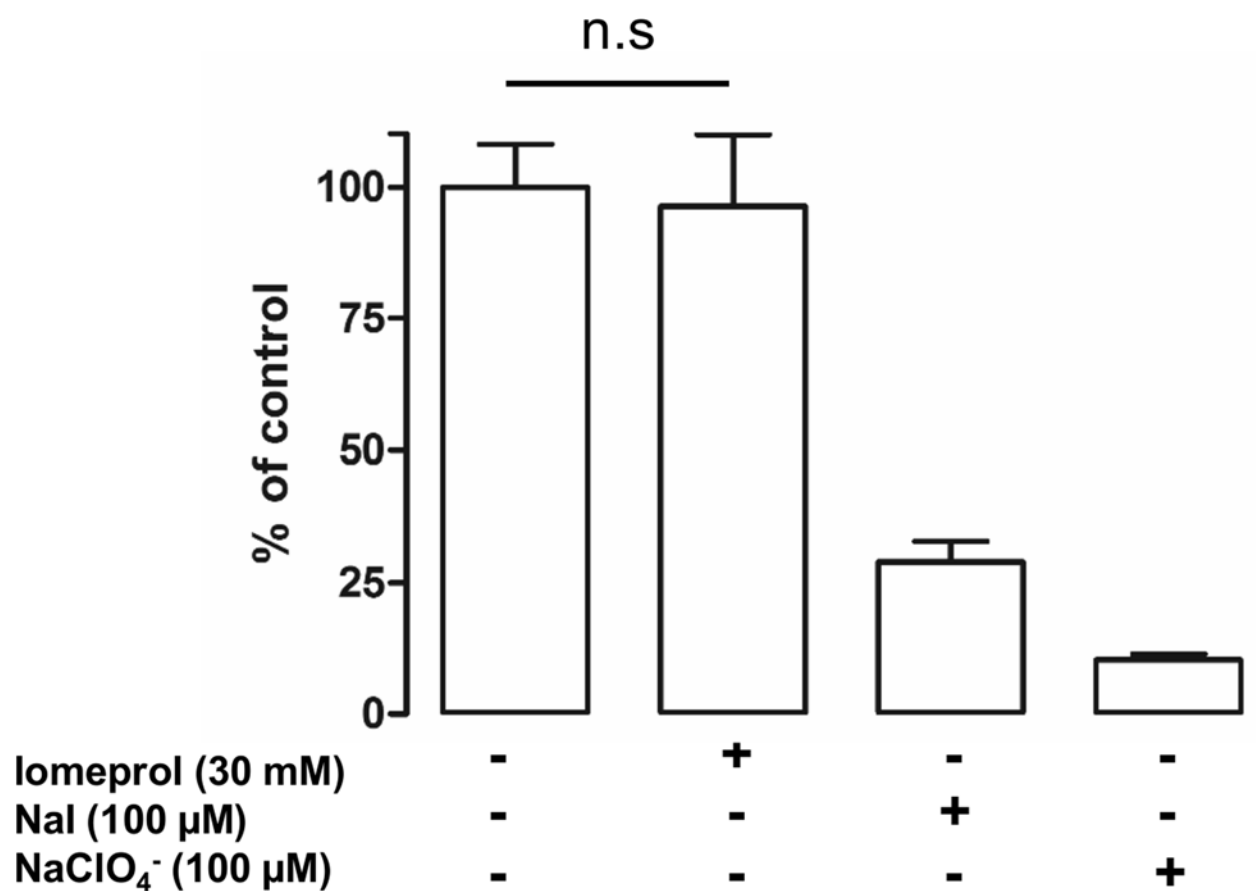
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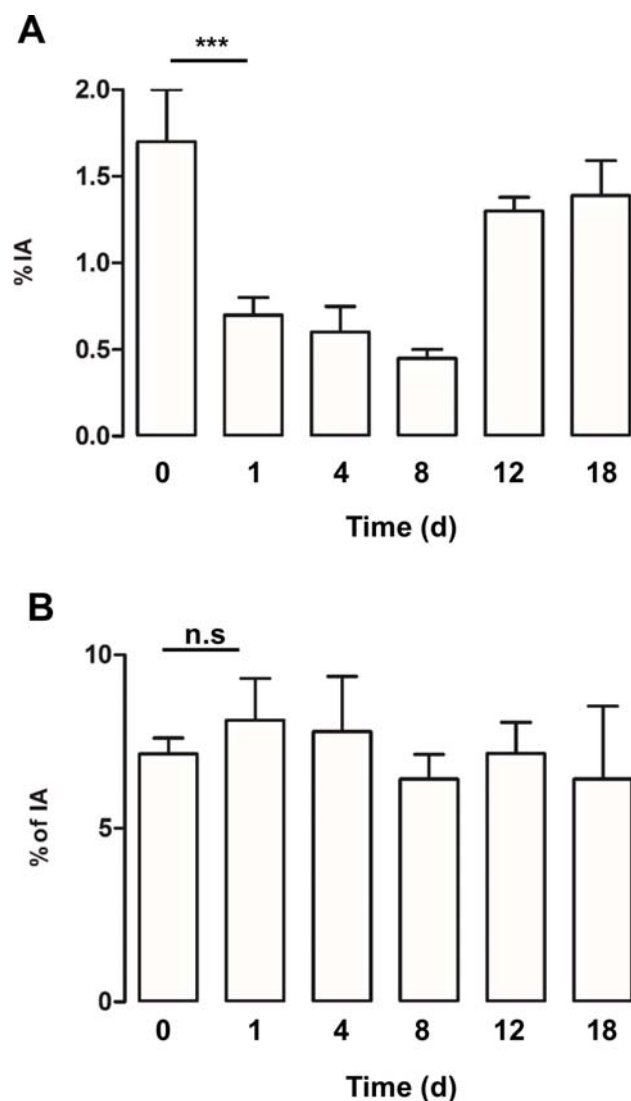
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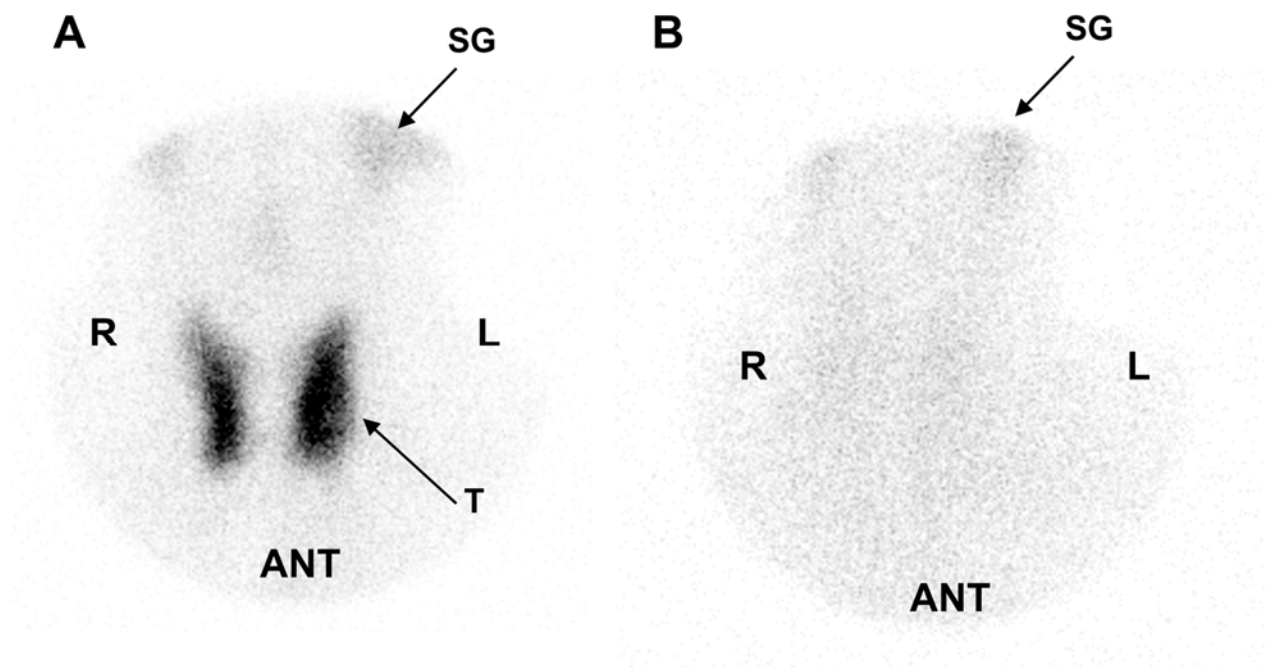
## FIGURE LEGENDS



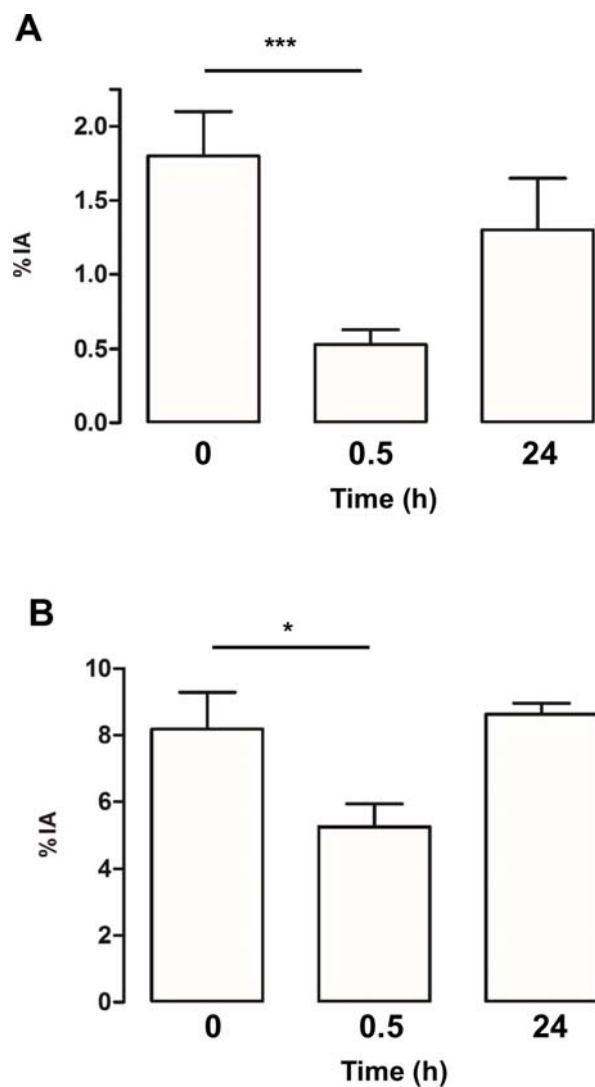
**Figure 1:** Effect of Iomeron on iodide uptake of PCCL3 cells. PCCL3 cells were incubated for one hour with  $^{125}\text{I}$  in the presence of an equal volume of either saline, Iomeron (100  $\mu\text{L}$  corresponding to 71.4 mg of iomeprol in a total of 3 mL of medium), NaI or perchlorate. Cells were then rapidly washed with saline buffer and lysed. Aliquots of lysates were counted in a  $\gamma$  counter. The results are expressed as percentage of uptake in the control condition. The data presented are the mean  $\pm$  SEM of triplicates and are representative of three independent experiments. n.s : non statistically significant.



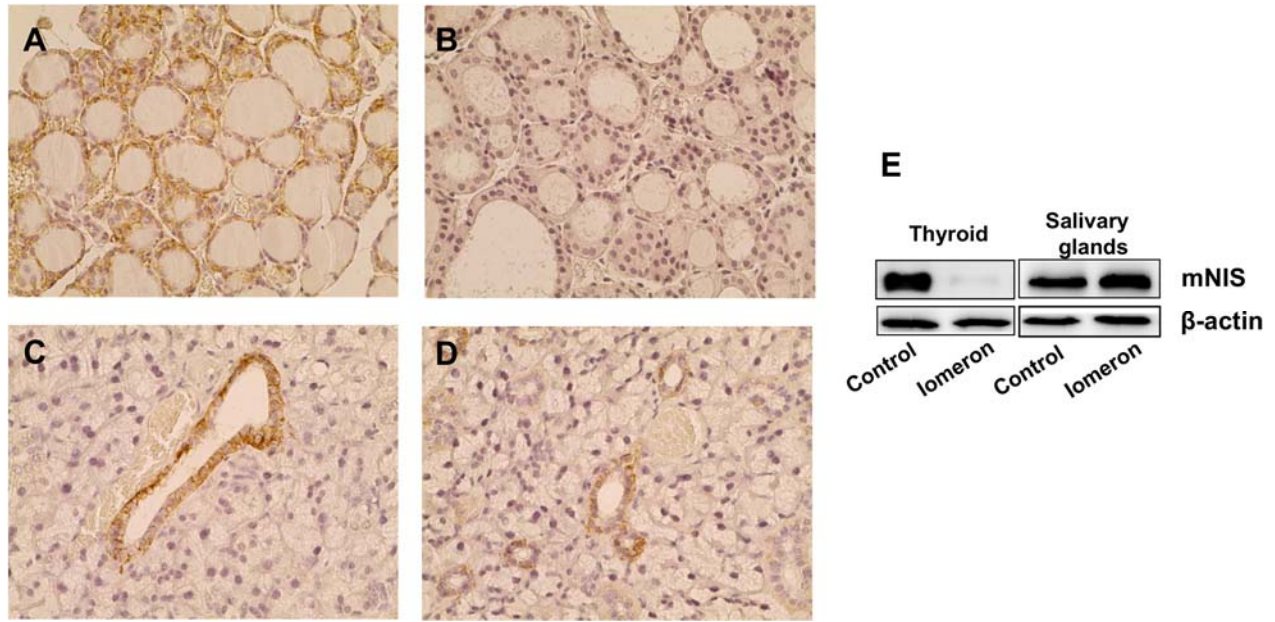
**Figure 2:** Effect of Iomeron on the uptake of  $^{99m}\text{Tc}$  pertechnetate by the mouse thyroid and salivary glands. SPECT/CT imaging of mice administered 20 MBq  $^{99m}\text{Tc}$  pertechnetate was performed (day 0). At the end of the scan, Iomeron was administered intravenously. One, four, eight, twelve and eighteen days later, animals were injected with 20 MBq  $^{99m}\text{Tc}$  pertechnetate and new scans were performed. The data presented represent the percentage of radiotracer injected taken up by the thyroid (A) and salivary glands (B). (n = 3 per condition). \*\*\* p < 0.001; n.s : non statistically significant. % IA: percentage of the injected activity.



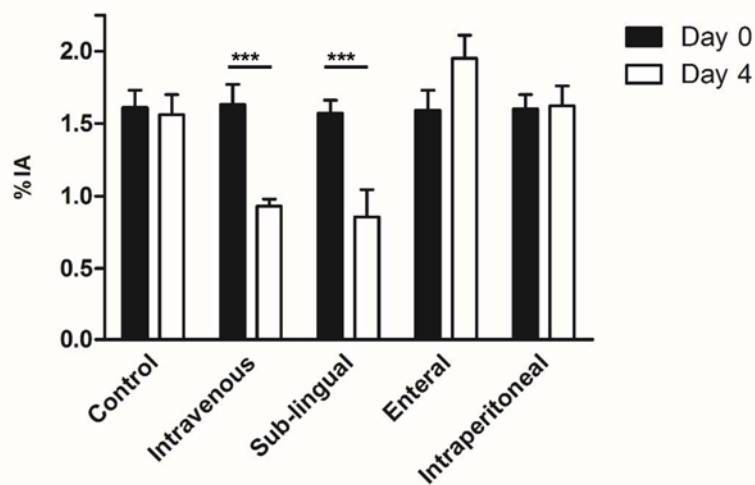
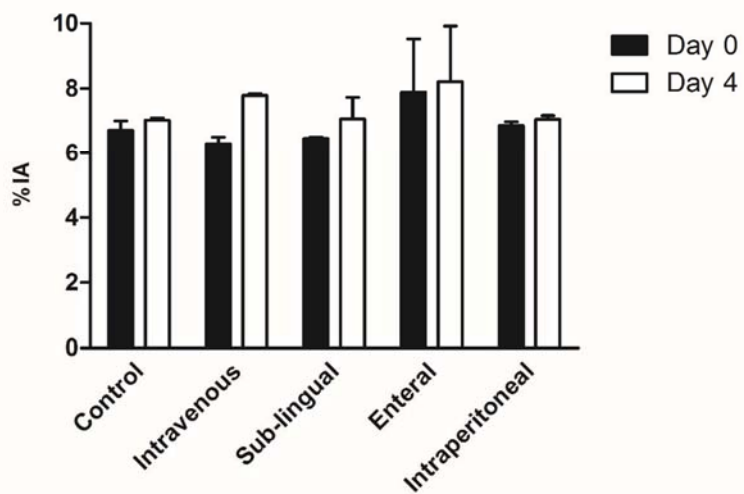
**Figure 3:** Effect of Imeron on the uptake of  $^{99\text{m}}$ perchnetate by the human thyroid and salivary glands. Scintigraphies of a naïve patient (A) or a patient who has been administered with Imeron two weeks before the scintigraphy (B). ANT: anterior; SG: salivary glands; T: thyroid; R: right; L:left.



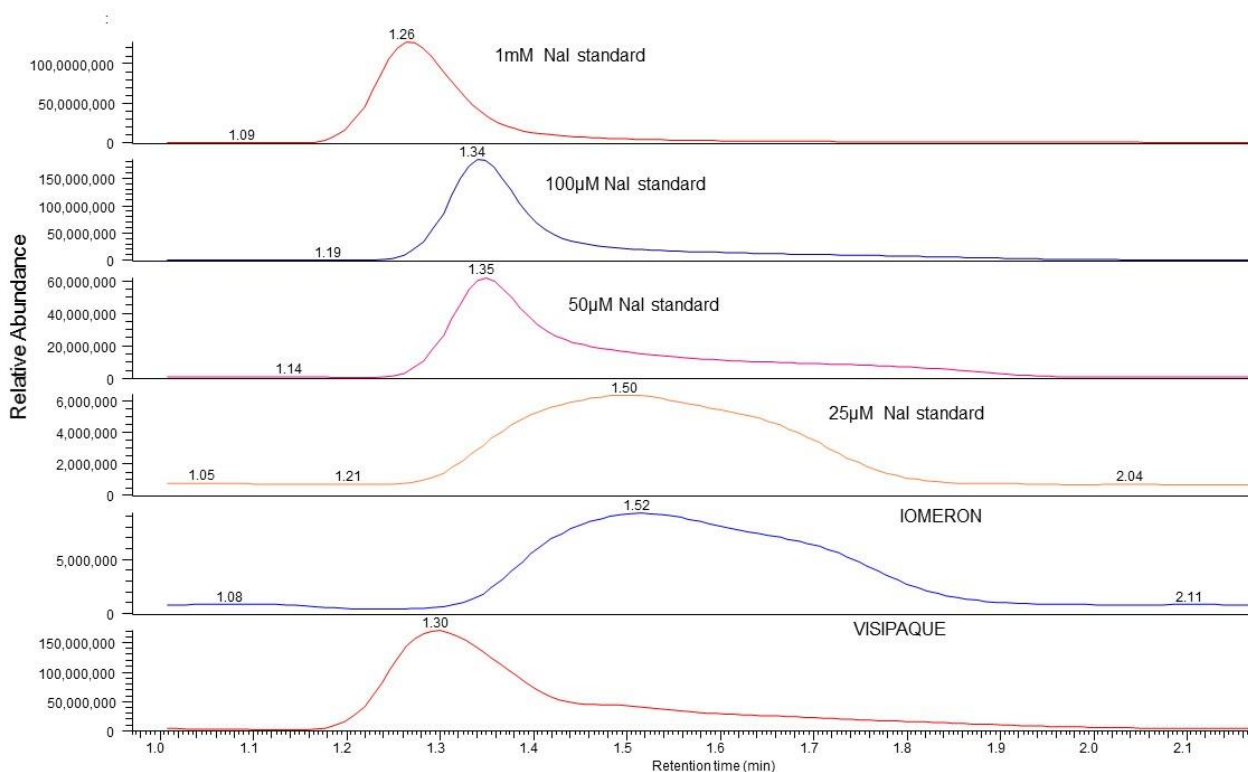
**Figure 4:** Effect of KI on the uptake of  $^{99m}\text{Tc}$  pertechnetate by the mouse thyroid and salivary glands. SPECT/CT imaging of mice administered 20 MBq  $^{99m}\text{Tc}$  pertechnetate was performed (day 0). Animals were injected intraperitoneally with KI (9 mg in 180  $\mu\text{L}$ ) and scanned 30 minutes later. Twenty-four hours later, animals were injected with 20 MBq  $^{99m}\text{Tc}$  pertechnetate and new scans were performed. The data presented represent the percentage of radiotracer injected taken up by the thyroid (A) and salivary glands (B). (n = 3 per condition). \* p < 0.05; \*\*\* p < 0.001. % IA: percentage of the injected activity.



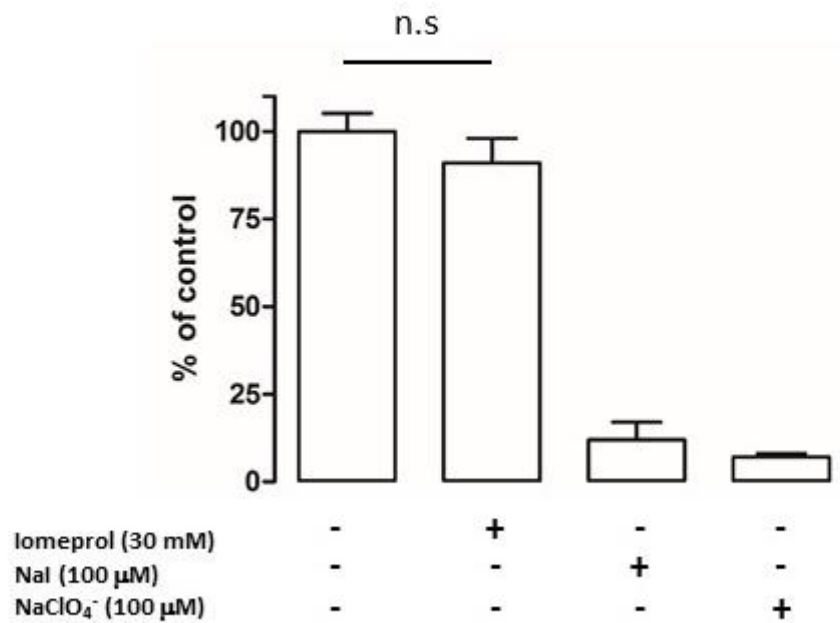
**Figure 5:** Analysis of NIS expression in the thyroid and salivary glands in response to Iomeron. Saline buffer (A, C) or Iomeron (B, D) were administered intravenously. Four days later, animals were culled and NIS expression in the thyroid glands (A, B) and salivary glands (C, D) were analyzed by immunohistochemistry (n = 3 per group). Thyroids and salivary glands were also processed for Western blot analysis of NIS and  $\beta$ -actin expression (n = 2 per group).

**A****B**

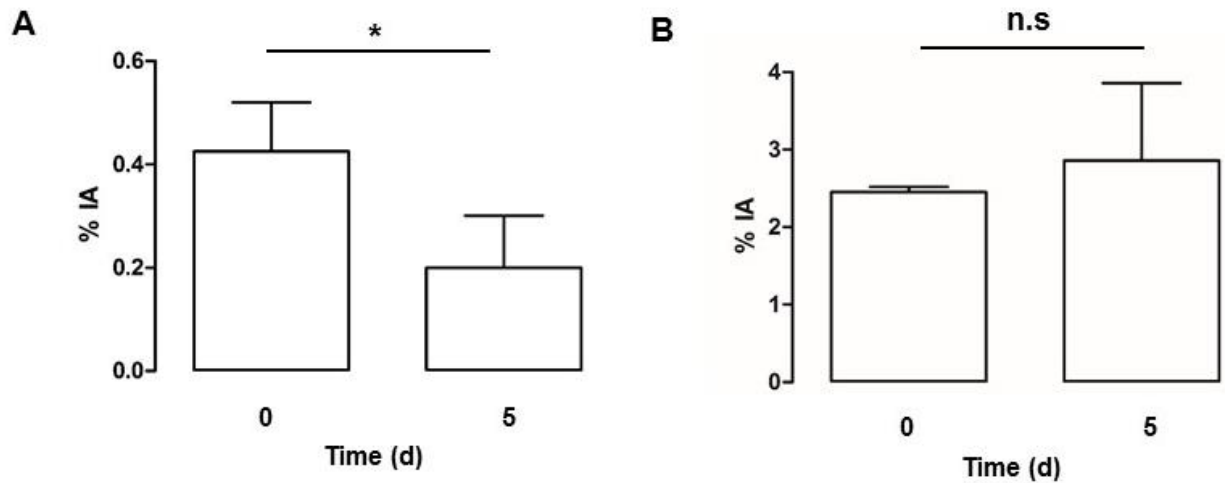
**Figure 6:** Evaluation of the effect of different modes of administration of Iomeron on the uptake of  $^{99m}\text{Tc}$ pertechnetate by the thyroid and salivary glands. SPECT/CT imaging of mice administered 20 MBq  $^{99m}\text{Tc}$ pertechnetate was performed (day 0). At the end of the scan, intravenous, sublingual, enteral, and intraperitoneal administrations of Iomeron were performed. Four days later, animals were injected with 20 MBq  $^{99m}\text{Tc}$ pertechnetate and new scans were performed. The data presented represent the percentage of radiotracer injected taken up by the thyroid (A) and salivary glands (B). (n = 2 per condition). \*\*\* p < 0.001. % IA: percentage of the injected activity.



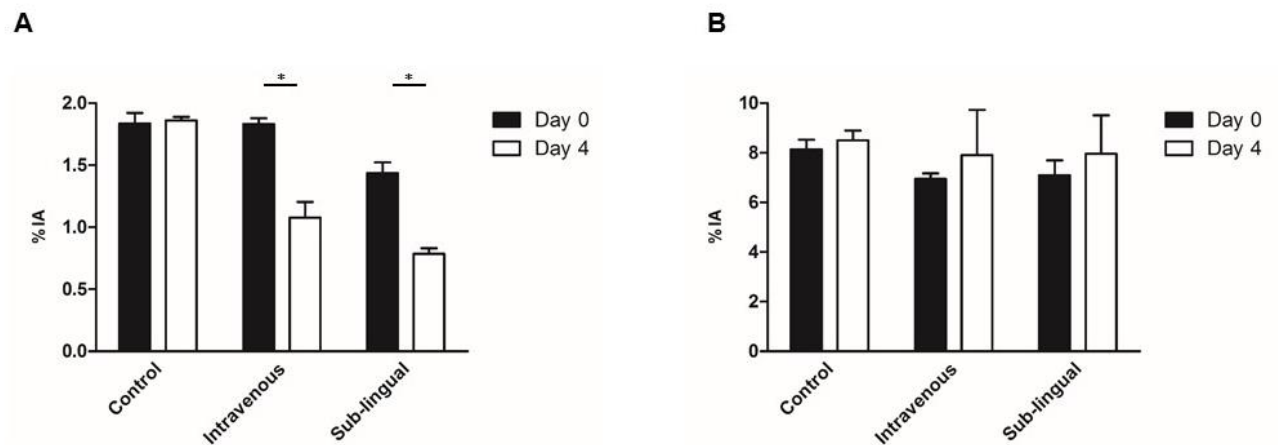
**Supplemental Figure 1: Determination of the free iodide content of Iomeron and Visipaque.** Standard solutions of NaI ranging from 1mM to 25 µM were analyzed and the area under the peak corresponding to iodide was calculated. Samples of Iomeron and Visipaque were analyzed in the same experimental conditions and the area under the peak of iodide (retention time between 1.08 min and 2.11 min) was calculated. Comparison with the NaI standard values led to the determination of the free iodide concentration in Iomeron (25 to 30 µM) and Visipaque (95 to 100 µM).



**Supplemental Figure 2: Effect of Iomeron on iodide uptake by HT29-NIS cells.** HT29-NIS cells were incubated for one hour with  $^{125}\text{I}$  in the presence of an equal volume of either saline, Iomeron (100  $\mu\text{L}$  corresponding to 71.4 mg of iomeprol in a total of 3 mL medium), NaI or perchlorate. Cells were then rapidly washed with saline buffer and lysed. Aliquots of lysates were counted in a  $\gamma$  counter. The results are expressed as percentage of uptake in the control condition. The data presented are the mean  $\pm$  SEM of triplicates and are representative of three independent experiments. n.s : not statistically significant.



**Supplemental Figure 3:** Effect of Iomeron on the uptake of  $^{123}\text{I}$  by the mouse thyroid and salivary glands. SPECT/CT imaging of mice administered 10 MBq  $^{123}\text{I}$  was performed (day 0). At the end of the scan, Iomeron was administered intravenously. Five days later, animals were injected with 10 MBq  $^{123}\text{I}$  and new scans were performed. The data presented represent the percentage of radiotracer injected taken up by the thyroid (A) and salivary glands (B). (n = 4 per condition). % IA: percentage of the injected activity. \* : p < 0.05; n.s : non statistically significant.



**Supplemental Figure 4: Evaluation of the effect of different modes of administration of Visipaque on the uptake of  $^{99m}\text{Tc}$ -pertechnetate by the thyroid and salivary glands.** SPECT/CT imaging of mice administered 20 MBq  $^{99m}\text{Tc}$ -pertechnetate was performed (day 0). At the end of the scan, intravenous or sublingual administrations of Visipaque were performed. Four days later, animals were injected with 20 MBq  $^{99m}\text{Tc}$ -pertechnetate and new scans were performed. The data presented represent the percentage of radiotracer injected taken up by the thyroid (A) and salivary glands (B). (n = 2 per condition). \*: p < 0.05. % IA: percentage of the injected activity.