Molecular Medicine

# Iodine Uptake and Prostate Cancer in the TRAMP Mouse Model

*Paloma Olvera-Caltzontzin, Guadalupe Delgado, Carmen Aceves, and Brenda Anguiano*

Departamento de Neurobiología Celular y Molecular, Instituto de Neurobiología, Universidad Nacional Autónoma de México, Campus Juriquilla, Querétaro, México

Iodine supplementation exerts antitumor effects in several types of cancer. Iodide ($I^-$) and iodine ($I_2$) reduce cell proliferation and induce apoptosis in human prostate cancer cells (LNCaP and DU-145). Both chemical species decrease tumor growth in athymic mice xenografted with DU-145 cells. The aim of this study was to analyze the uptake and effects of iodine in a preclinical model of prostate cancer (transgenic adenocarcinoma of the mouse prostate (TRAMP) mice/*SV40*-TAG antigens), which develops cancer by 12 wks of age. $^{125}I^-$ and $^{125}I_2$ uptake was analyzed in prostates from wild-type and TRAMP mice of 12 and 24 wks in the presence of perchlorate (inhibitor of the $Na^+/I^-$ symporter (NIS)). *NIS* expression was quantified by quantitative polymerase chain reaction (qPCR). Mice (6 wks old) were supplemented with 0.125 mg $I^-$ plus 0.062 mg $I_2$/mouse/day for 12 or 24 wks. The weight of the genitourinary tract (GUT), the number of acini with lesions, cell proliferation (levels of proliferating cell nuclear antigen (PCNA) by immunohistochemistry), *p53* and *p21* expression (by qPCR) and apoptosis (relative amount of nucleosomes by enzyme-linked immunosorbent assay) were evaluated. In both age-groups, normal and tumoral prostates take up both forms of iodine, but only $I^-$ uptake was blocked by perchlorate. Iodine supplementation prevented the overexpression of *NIS* in the TRAMP mice, but had no effect on the GUT weight, cell phenotype, proliferation or apoptosis. In TRAMP mice, iodine increased *p53* expression but had no effect on *p21* (a p53-dependent gene). Our data corroborate NIS involvement in $I^-$ uptake and support the notion that another transporter mediates $I_2$ uptake. Iodine did not prevent cancer progression. This result could be explained by a strong inactivation of the p53 pathway by TAG antigens.

**Online address: http://www.molmed.org**
**doi: 10.2119/molmed.2013.00093**

## INTRODUCTION

Prostate cancer is the second most frequently diagnosed cancer in men worldwide, with an incidence significantly lower in Japan than in the United States (22.7 versus 83.8/100,000 per year, respectively) (1). This lower risk has been associated with lifestyle factors such as diet. The traditional Japanese diet is high in fish (rich in omega-3 fatty acids), soybeans (isoflavones) and seaweeds (iodine) (2). Epidemiological studies show a possible association between high iodine intake and low risk of prostate cancer, given that the daily intake of iodine in Japan is 25-fold higher than in Western countries (5,280 µg in Japan versus 209 µg in the US) (3,4).

This chemoprotection by iodine is also evident in thyroid and breast cancer, and it seems to be related to the ability of these tissues to take up and metabolize iodide ($I^-$) and/or molecular iodine ($I_2$) (5). It is well established that $I^-$ uptake depends on the $Na^+/I^-$ symporter (NIS), whereas the uptake of $I_2$ is probably mediated by facilitated diffusion (6–8). $I_2$ supplementation exerts antitumor effects in thyroid, breast, neuroblastoma and lung carcinoma by arresting the cell cycle (increases p53 and p21 proteins), inducing apoptosis (dissipates mitochondrial membrane potential and increases Bax, caspases 3 and 7) and reducing mRNA expression of invasion genes (vascular endothelial growth factor [*VEGF*] and urokinase-type plasminogen activator [*uPA*]) (9–14). For $I^-$ to exert some of these effects, it must be converted to an oxidized species of iodine by specific peroxidases (thyro-, lacto- or myeloperoxidase) (15–17).

The pathways by which $I_2$ inhibits the carcinogenic process are not fully understood, but contributions of both direct (anti- or prooxidant) and indirect mechanisms (generation of iodolipids, iodinated derivatives of arachidonic acid) have been proposed (5). 6-Iodolactone (6-IL) is an iodolipid that reproduces some of the antitumorogenic effects mentioned above, and it has been suggested that 6-IL could function as a ligand/activator

***Address correspondence to*** *Brenda Anguiano, Instituto de Neurobiología, UNAM Campus Juriquilla, Boulevard Juriquilla, #3001, Querétaro, 76230, México. Phone and Fax: +52-442-2381067; E-mail: anguianoo@unam.mx.*
*Submitted August 22, 2013; Accepted for publication November 7, 2013; Epub (www.molmed.org) ahead of print November 8, 2013.*

The Feinstein Institute for Medical Research North Shore LIJ

for peroxisome proliferator–activated receptors (12,13,18–20).

There is evidence that prostate epithelium is an iodine-responsive target. In several species, *NIS* mRNA was detected in normal and cancerous prostate (21–23). In humans, NIS protein is present in 50–70% of adenocarcinomas (24). With regard to the effects of iodine, *in vitro* studies show that LNCaP and DU-145 prostate cancer cells take up both $I^-$ and $I_2$, and treatment with either chemical species reduces cell proliferation and induces apoptosis (Bax capsases). Consistent with these results, supplementation in nude mice with both chemical species consistently reduces tumor growth of DU-145 xenografts (25). The aims of this study were to analyze the uptake and potential antineoplasic effects of a mixture of iodine and iodide in transgenic adenocarcinoma of the mouse prostate (TRAMP) mice, a preclinical model of prostate cancer.

## MATERIALS AND METHODS

### Transgenic Animals

The TRAMP mice were obtained from The Jackson Laboratory (Bar Harbor, ME, USA). Heterozygous ($TRAMP^{+/-}$) females were crossed with nontransgenic C57BL/6 males to generate heterozygous transgenic male offspring. In the TRAMP model, expression of *SV40* early genes (*TAG*) is driven by the prostate-specific promoter probasin. Long and short antigens inactivate p53 and Rb proteins as well as protein phosphatase 2, respectively, leading to the development of intraepithelial neoplasia (prostatic intraepithelial neoplasia [PIN]) and both well-differentiated and poorly differentiated cancer. Tail deoxyribonucleic acid (DNA) was used to determine the presence of the transgene by polymerase chain reaction (PCR). Experiments were approved by the Committee on Bioethics from the Universidad Nacional Autónoma de México, and procedures were carried out according to guidelines of the Norma Oficial Mexicana NOM-062-ZOO-1999 (26).

### Radio-Iodine Uptake Assay

Prostate tissue from wild-type and TRAMP mice of 12 and 24 wks was used to characterize the uptake of $I^-$ and $I_2$ by using $^{125}I$ as a tracer ($Na^{125}I$; PerkinElmer, Waltham, MA, USA; specific activity: ~17 Ci [629 GBq]/mg). Oxidation of $^{125}I^-$ to $^{125}I_2$ was carried out according to the method described by McAlpine (27). Mice received intraperitoneal doses of $^{125}I^-$ or $^{125}I_2$ (0.001 Ci/kg body weight) and were euthanized after 5, 10, 15, 30, 60, 120 and 180 min. Thyroid gland was used as an internal positive control.

To analyze NIS involvement in iodine uptake, one group each of wild-type and TRAMP mice received intraperitoneal physiological saline solution or 25 mg perchlorate ($ClO_4^-$) (specific inhibitor of NIS) per kilogram body weight. Two hours after the $ClO_4^-$ injection, mice were injected with $^{125}I^-$ or $^{125}I_2$, and after another 2 h, they were killed. This dose of $ClO_4^-$ inhibits >80% of iodide uptake in thyroid gland (positive control) (28). Thyroid, prostate and liver were collected, and the radiolabel was measured in counts per minute (cpm) in a γ-counter (Packard, Palo Alto, CA, USA). Radioactive iodine uptake by thyroid and prostate was normalized to liver uptake (negative control). Data are expressed as cpm per milligram thyroid or cpm per milligram prostate/cpm per milligram liver (23).

### Effects of Iodine Supplementation on Prostate Cancer Development

Male wild-type and TRAMP mice (6 wks old) were given drinking water supplemented with a mixture of iodine (0.125 mg $I^-$ plus 0.062 mg $I_2$/day/mouse) for 12 or 24 wks. Body weight was registered every 2 wks. At the end of the experiments, mice were anesthetized with a ketamine and xylazine mixture (Cheminova, Mexico City, Mexico; 8 and 0.6 mg, respectively, per 100-g body weight) and killed by decapitation. The genitourinary tract (GUT) (prostate, bladder, seminal vesicles and urethra) was weighed, and the prostate was dissected. Prostate tissue was fixed in 10% neutral buffered formalin (Sigma-Aldrich, St. Louis, MO, USA) for histopathology and immunohistochemistry or frozen and stored at –70°C to measure expression of the *NIS*, *p53* and *p21* genes by reverse transcriptase–quantitative polymerase chain reaction (RT-qPCR). Blood was collected to determine the circulating levels of triiodothyronine (T3). At the time of necropsy, all organs were examined for gross abnormalities.

### Histopathology and Immunohistochemistry

The 5-μm, paraffin-embedded sections of the dorso-lateral lobes were stained with hematoxylin-eosin for histopathology or were processed for immunohistochemical analysis. The pathological grading system used was that proposed by Kaplan-Lefko, which considers several criteria such as epithelial cytoarchitecture and the presence of mitosis, apoptosis and inflammation (28). The percentages of normal and pathological acini were registered. The analysis was carried out by quantifying the whole field of three animals per group at 20× magnification.

Immunodetection of proliferating cell nuclear antigen (PCNA) was used as an indicator of cell proliferation. Briefly, paraffin tissue sections were deparaffinized, rehydrated and heated in citrate buffer (pH 6.0). Sections were blocked with peroxidase block solution from DakoCytomation (Dako, Glostrup, Denmark), followed by bovine serum albumin and then incubated overnight at 4°C with the anti-PCNA rabbit polyclonal primary antibody (1:50; Santa Cruz Biotechnology, Santa Cruz, CA, USA). Slides were then reacted with biotin-labeled anti-rabbit IgG/anti-mouse IgG and developed in diaminobenzidine (DAB) (DakoCytomation). Sections were counterstained with hematoxylin, dehydrated and mounted. Tissue sections without primary antibody were used as negative control. The analysis was carried out under light microscopy (Leica DM 2500), and images were acquired with a Leica DFC 420 camera. The percentage of PCNA-positive cells was defined as the number of brown-stained

**Table 1.** Primers used in qPCR.

| Gene | GeneBank | Sequence |
|---|---|---|
| *NIS* | NM_053248 | CCGGATCAACCTGATGGACT<br>CCTGAGGGTGCCACTGTAAG |
| *p53* | NM_001127233 | TGAACCGCCGACCTATCCTTA<br>GGCACAAACACGAACCTCAAA |
| *p21* | NM_001111099 | ATGTCCAATCCTGGTGATGT<br>TGCAGCAGGGCAGAGGAAGT |
| *β-Actin* | NM_031144 | GTCCCAGTATGCCTCTGGTCGTAC<br>CCACGCTCGGTCAGGATCTTCATG |

cells divided by the total number of cells in three randomly selected fields at 40× magnification. The quantification of cells was performed with the Leica Application Suite (version 2.8.1).

### Circulating T3 Levels

To verify thyroid status, serum T3 levels were measured by a homologous radioimmunoassay previously standardized in our laboratory (30).

### Apoptosis

Apoptosis was measured by enzyme-linked immunosorbent assay (ELISA) by using a cell death detection kit (Roche Diagnostics, Mannheim, Germany). This assay detects the relative amounts of mono- and oligo-nucleosomes. Data were normalized to DNA concentration. All samples exhibited at least twice the absorbance of the blank. The percent change of apoptosis was calculated, considering the prostatic levels of the non-supplemented wild-type mice as 100%.

### RT-qPCR (Real Time)

Total RNA was extracted by using the SV Total Isolation System (Promega, Madison, WI, USA) and was reverse-transcribed by using oligo-deoxythymine. The sequence detector system Rotor-Gene 3000 (Corbett Research, Mortlake, Australia) was used to perform qPCR with SYBRgreen. The reaction contained 2 μg cDNA template and the Maxima SYBR Green/ROX qPCR Master Mix (Fermentas, CA, USA), using 40 cycles of three-step amplification (95°C for 30 s, 55–60°C for 30 s and 72°C for 30 s). Table 1 shows the primers used in this study. PCR generated only the expected specific amplicon, which was demonstrated in each case by the melting temperature profile and by electrophoresis of the PCR product through a 2% agarose gel containing ethidium bromide in Tris-acetate-EDTA buffer. No PCR products were observed in the absence of template. Gene expression was calculated by using a standard curve and normalized to the content of *β-actin*. The coefficient of variation for this gene was <15%.

### Statistical Analysis

The data are shown as mean ± standard error of the mean (SEM). Differences between groups were analyzed by using a two-way analysis of variance (ANOVA) with a Bonferroni *post hoc* test and Student *t* test. Results were analyzed by using the statistical package GraphPad Prism 4 (GraphPad, San Diego, CA, USA).

## RESULTS

### Time Course of Iodine Uptake

Figure 1 shows that normal prostate of 12- and 24-wk-old mice takes up $^{125}I^-$ and $^{125}I_2$ within the first 5 min. For both iodine types, sustained but not statistically significant increases were observed in 24-wk-old mice after 10 min.

### Expression of NIS and Its Involvement in Iodide Uptake

Figure 2A shows that both chemical species of iodine are taken up by normal and cancerous prostate of 12- and 24-wk-old mice. In all groups, $^{125}I^-$ uptake was significantly inhibited by $ClO_4^-$. In contrast, $ClO_4^-$ administration did not modify $^{125}I_2$ uptake in any group. Figure 2B shows *NIS* expression in prostates from wild-type and TRAMP mice of 30 wks. TRAMP mice exhibited high *NIS* expression compared to wild-type mice.

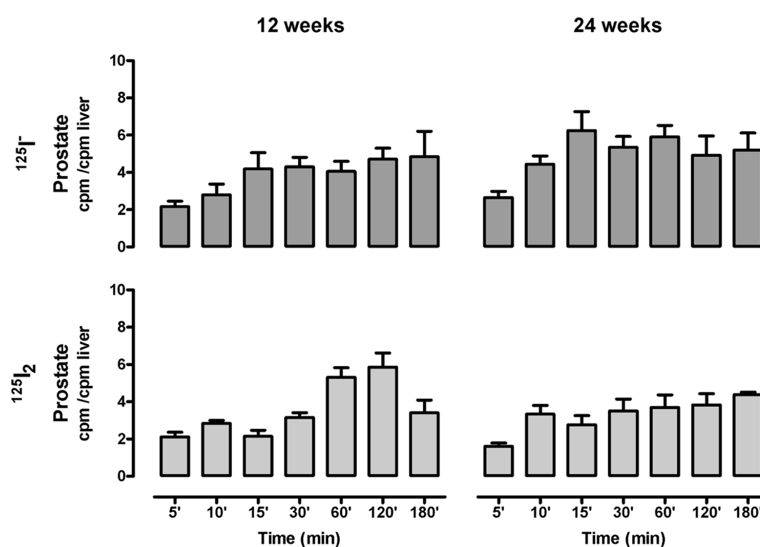


**Figure 1.** Time course for the uptake of iodide ($^{125}I^-$) or iodine ($^{125}I_2$) in normal prostate. Mice of 12 or 24 wks of age were intraperitoneally injected with either $^{125}I^-$ or $^{125}I_2$ (0.001 Ci/kg body weight) and were killed from 5 to 180 min after tracer administration. Tissues were weighed and the radioactivity measured (cpm). Prostate iodine uptake was expressed as cpm per mg prostate/cpm per mg liver (nonuptake organ). No significant differences were found. n = 3–6 per group.

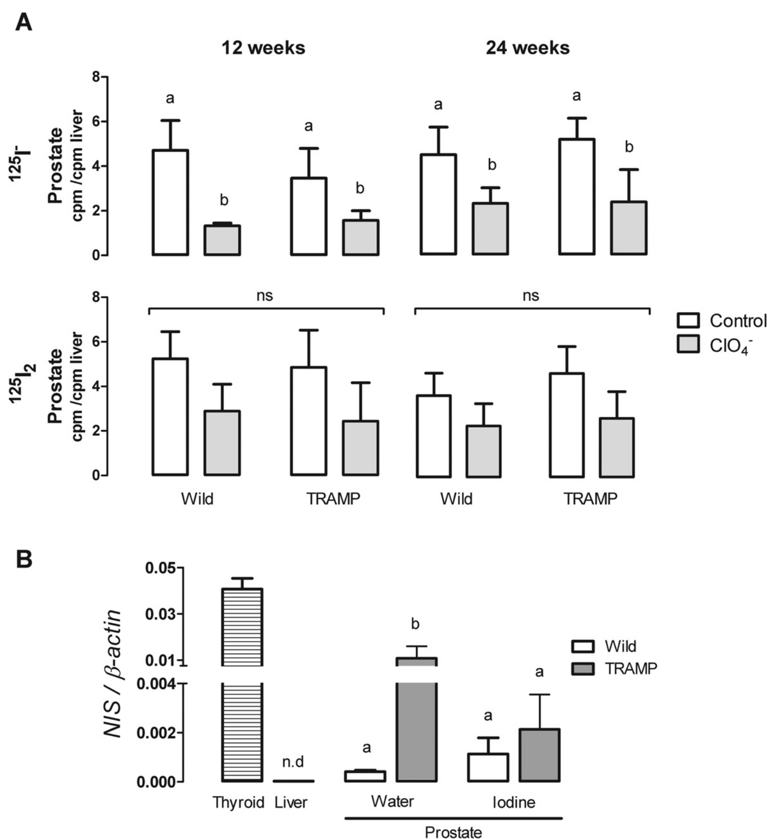


**Figure 2.** (A) $^{125}$I uptake in normal (wild type) or tumoral (TRAMP) prostate. Mice of 12 or 24 wks received an injection of saline (control) or perchlorate ($ClO_4^-$); 2 h later, they were intraperitoneally injected with 0.001 Ci/kg body weight of either $^{125}I^-$ or $^{125}I_2$. Mice were killed 2 h after tracer administration, and iodine uptake was analyzed as described in Figure 1. (B) Effect of iodine on *NIS* mRNA expression in normal and tumoral prostate. Iodine was supplemented for 24 wks (starting at 6 wks old). Thyroid and liver were used as positive and negative controls, respectively. *NIS* expression was analyzed by RT-qPCR. Data were analyzed using a two-way ANOVA, followed by the Bonferroni *post hoc* test. Different letters indicate significant differences between groups ($p < 0.05$). n.d., Not detected; n = 4–6 per group.

Chronic iodine supplementation (24 wks) decreased TRAMP *NIS* overexpression to values similar to those of the wild-type mice.

### Effect of Iodine Supplementation on the Development of Prostate Cancer

Figure 3 shows that iodine supplementation for 12 or 24 wks had no effect on body weight. The data show a significant increase in the GUT weight of TRAMP mice in comparison to wild-type mice at both ages. Iodine supplementation had no effect on the GUT weight or circulating levels of T3 (data not shown).

As expected, wild-type mice showed a normal histological phenotype, whereas TRAMP mice exhibited a well-differentiated cancer (Figure 4). A detailed analysis in TRAMP mice showed that around 40% of acini had a normal phenotype, whereas 5–10% of acini exhibited a high-grade PIN (epithelial stratification, cribiform structures, nuclear pleomorphism, hypercromatic nuclei), and around 50% exhibited a well-differentiated cancer (loss of intraductal spaces and invasion of basal membrane). However, iodine supplementation for 12 or 24 wks did not modify the normal (wild type) or tumor phenotype (TRAMP).

In relation to indicators of cell proliferation, our data showed a higher number of PCNA-positive cells in prostate tumors (TRAMP) than in normal prostate (wild type) (Figure 5). However, iodine supplementation for 12 or 24 wks did not modify the PCNA levels in any group. Figure 6 shows that iodine supplementation significantly increased *p53* mRNA expression but had no effect on *p21* expression in tumors from 24-wk-old TRAMP mice. We found no differences in apoptosis between control and iodine-supplemented groups of normal and cancerous prostate (Figure 7).

## DISCUSSION

The present study was designed to explore if continued supplementation with iodine inhibits or delays the development of prostate cancer in the TRAMP model. Our data showed that, although cancerous prostate takes up both $I^-$ and $I_2$, supplementation with a mixture of the two species of iodine for 12 or 24 wks does not reproduce the antitumor effects previously reported in an *in vitro* model of prostate cancer (25).

This study shows the ability of cancerous prostate to take up $I^-$ and $I_2$. *NIS* expression and the inhibition of $I^-$ uptake by $ClO_4^-$ (specific inhibitor of NIS symporter) in normal and cancerous prostate confirm that iodide uptake is NIS dependent. These data are in accord with a clinical study indicating that around 50% of human prostate tumors express NIS protein (24), but they differ with studies in LNCaP and DU-145 cells showing that $I^-$ uptake is NIS independent (25). This mechanistic difference between *in vivo* and *in vitro* models could be explained by the tumor heterogeneity. Our data show that cancerous prostate overexpresses *NIS*, but this increase is not functionally associated with higher $I^-$ influx. This dissociation between *NIS* transcription and iodide uptake has also been reported to occur in other cancers as part of the tumorogenic process. Well-differentiated thyroid and breast cancers

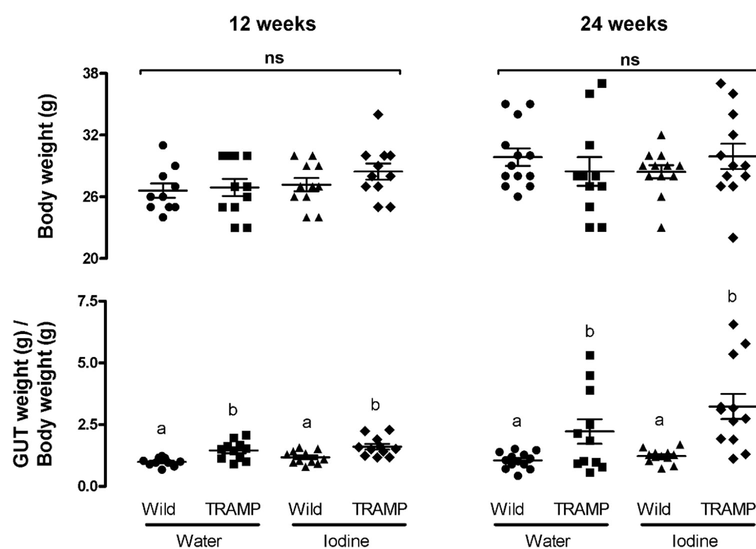


**Figure 3.** Effect of iodine on body and GUT weight of wild-type or TRAMP mice. Iodine was supplemented for 12 or 24 wks. Control groups received drinking water without iodine. Data were analyzed by using a two-way ANOVA, followed by Bonferroni *post hoc* test. Different letters indicate significant differences between groups ($p < 0.05$). n = 10–13 per group.

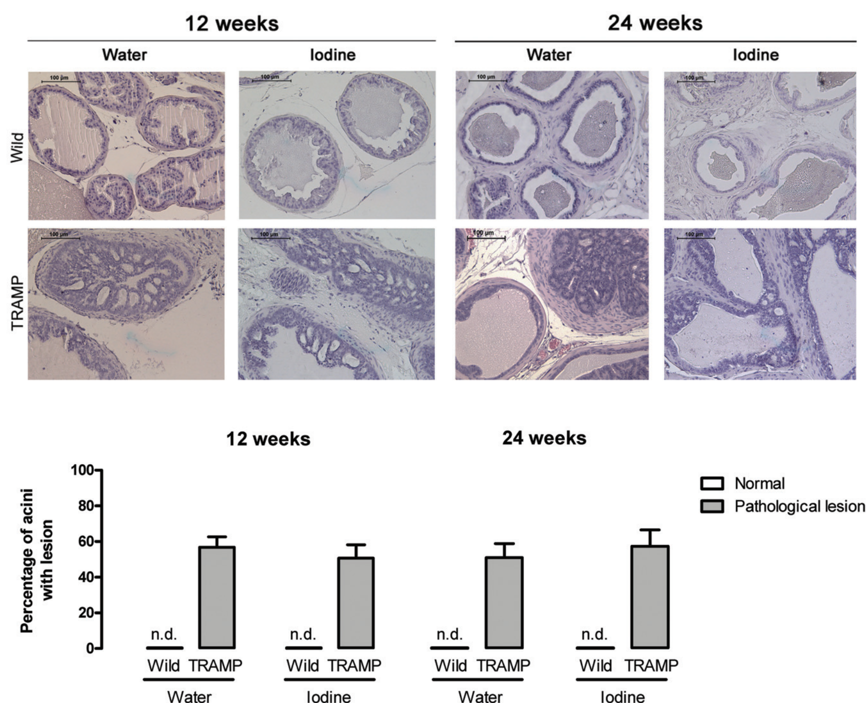


**Figure 4.** Effect of iodine on histology of normal or tumoral prostate. Iodine was supplemented for 12 or 24 wks. Representative images of prostates stained with hematoxylin-eosin are shown (magnification 20×). Histopathology analysis was made according to the criteria proposed by Kaplan-Lefko *et al.* (29). The percentage of acini with pathological lesions (PIN and well-differentiated cancer) is shown. Data were analyzed by using a two-way ANOVA, followed by a Bonferroni *post hoc* test. No significant differences were found between groups. n.d., Not detected; n = 4–6 per group.

express high levels of *NIS* compared with their normal counterparts; however, this expression decreases to undetectable levels when the cancer becomes undifferentiated (16,31–34). Our data show that iodine supplementation for 24 wks does not modify the basal expression of *NIS* in normal prostate, but it prevents the overexpression in cancerous prostate. In contrast, studies in breast tumors have shown that iodine supplementation induces *NIS* expression. These studies discussed the possibility that *NIS* upregulation by iodine might be associated with tumor redifferentiation (16,30).

With regard to $I_2$ uptake, no significant blockade by $ClO_4^-$ was observed in any group, corroborating an NIS-independent mechanism. These data agree with studies in human cell lines of prostate and breast cancer (8,25). There is evidence that $I_2$ uptake depends on protein synthesis, independent of ATP and $Na^+/K^-$ ATPase (8). A NIS-independent mechanism for $I_2$ uptake has also been demonstrated in sea urchin larvae and seaweeds (7,35), suggesting that this mechanism could be widespread in nature.

The functional relevance of iodine for the prostate gland is still unknown, but data from our group have shown antigrowth effects in models of prostatic diseases (25,36). The present study showed that iodine supplementation for 12 or 24 wks did not modify the GUT weight, incidence of lesions, cell proliferation or apoptosis in wild-type or TRAMP mice. This insensitivity to iodine contrasts with studies in benign prostate hyperplasia or cancer prostate cells that show (a) $I^-$ or $I_2$ supplementation prevents prostate hyperplasia induced by sexual hormones in rats (36); (b) $I^-$ or $I_2$ treatments reduce cell proliferation and induce apoptosis by a BAX caspase pathway in LNCaP and DU-145 cells; and (c) iodine supplementation (same schedule of administration used in the current study) reduces the tumor growth in nude mice xenografted with DU-145 cells (25). These data support the notion that the null response observed in TRAMP mice is not associated with the chemical species or schedule of

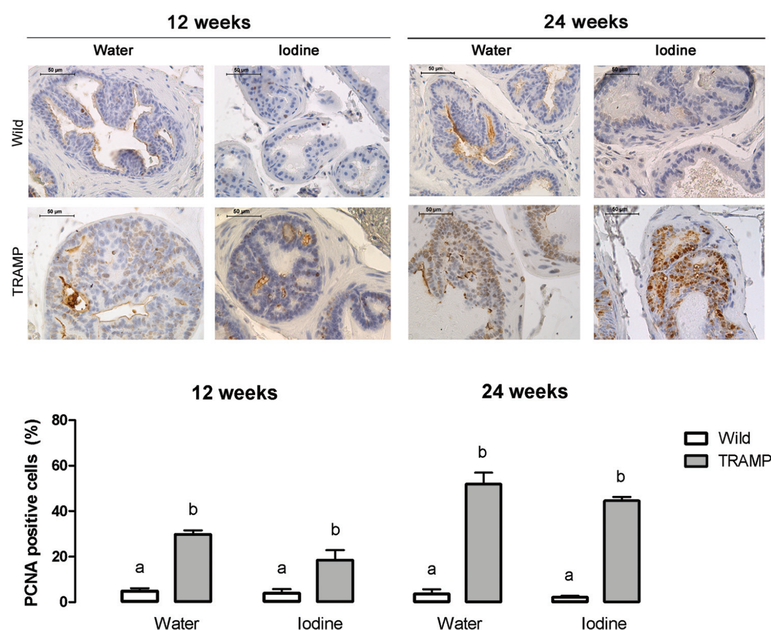


**Figure 5.** Effect of iodine on the percentage of PCNA-positive cells in normal or tumoral prostate. Iodine was supplemented for 12 or 24 wks. Representative immunohistochemical images from each group are shown (magnification 40×). Slides are counterstained with hematoxylin. Data were analyzed by using a two-way ANOVA followed by the Bonferroni *post hoc* test. Different letters indicate significant differences between groups ($p < 0.05$). n = 4–7 per group.

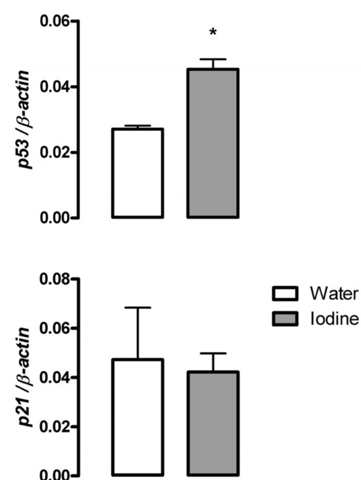


**Figure 6.** Effect of iodine on the expression of *p53* and *p21* mRNA in tumoral prostate. Iodine was supplemented for 24 wks. Gene expression was analyzed by RT-qPCR. Data were analyzed using a Student *t* test. *$p < 0.05$. n = 3 per group.

administration of iodine or with the origin of prostatic cells (rodents versus human). Instead, the insensitivity to iodine seems to be related to the mechanisms underlying the carcinogenic process in the TRAMP model. Studies in breast cancer cells (MCF-7) show a significant increase of p53 protein and, consequently, increased levels of p21 and Bax proteins as part of the anti-tumor mechanism of $I_2$ (11,12). In the current study we found an increase of *p53* expression but no increase of its target gene *p21*. The functional inhibition of p53 has been described in the TRAMP model as a consequence of the strong constitutive expression of the oncoprotein TAG, which directly inactivates antitumoral proteins such as p53 and Rb. There are controversies related to the sensitivity of prostate toward chemotherapeutic agents in the TRAMP model; some studies have validated its efficacy (37), but others have concluded that it represents an aggressive model of carcinogenesis (diffuse tumor, invasion of seminal vesicles and fast transition from low- to high-grade invasion) (38). Some antiinflammatory drugs such as celecoxib or exisulind exhibit important protection against development of early lesions (PIN), but they do not prevent the transition to adenocarcinoma. This partial protection has been explained as a coordinated activation of p53-dependent (p21/Bax) and p53-independent pathways (inhibition of nuclear factor κ–light-chain enhancer of activated B cells [NF-κB], cyclooxygenase-2, phosphatidylinositide 3-kinase [PI3K]/serine threonine kinase [AKT]/p27). Moreover, in studies using different compounds or diet conditions (low fat, energy restriction) whose action mechanisms involve the inhibition of p53-independent pathways (androgen receptor, NF-κB, PI3K/AKT, protein kinase Cε, and so on), these drugs have only a modest antitumor effect on this model (39–41).

On the other hand, there is evidence that iodine could also act against cancer progression, by preventing or delaying the acquisition of invasion mechanisms. In breast cancer models, it has been shown that iodine supplementation inhibits expression of the *VEGF* and *uPA* genes (13,14). Our study shows that none of the TRAMP mice (control or iodine supplemented) developed a phenotype of

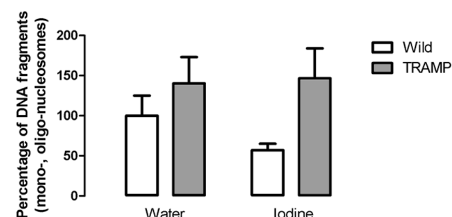


**Figure 7.** Effect of iodine in the induction of apoptosis (percentage of DNA fragments) in normal or tumoral prostate. Iodine was supplemented for 24 wks. The amount of mono- and oligo-nucleosomes was determined by ELISA. Data were normalized with respect to DNA concentration. The percentage of change of apoptosis was calculated considering the levels of the nonsupplemented wild-type prostate as 100%. Data were analyzed by using a two-way ANOVA followed by Bonferroni *post hoc* test. No significant differences were found between groups ($p < 0.05$). n = 4–5 per group.

undifferentiated cancer at 18 or 30 wks. Nevertheless, in the TRAMP model, it was found that prostate cancer could progress to an invasive neuroendocrine phenotype (42,43). It would be interesting to analyze the role of iodine in the acquisition of the neuroendocrine phenotype, since recent studies in neuroblastoma cells show that $I_2$ induces antiproliferative effects and sensitizes these cells to the differentiating effects of retinoic acid (9,44).

## CONCLUSION

Our data show that although normal and cancerous prostate take up both $I^-$ and $I_2$, the supplementation with a mixture of these two forms of iodine does not modify the physiopathology of the prostate. The inability of iodine to induce cell arrest or apoptosis could be explained by continued and exacerbated expression of TAG-oncoprotein (inhibitor of p53 and Rb) in the TRAMP model. Studies in less aggressive preclinical models, whose action mechanism does not involve a direct inactivation of p53, will be necessary to better understand the iodine effects on prostate tumor biology.

## ACKNOWLEDGMENTS

This study was supported by grants from Programa de Apoyo a Proyectos de Investigación e Inovación Tecnológica. Universidad Nacional Autónoma de México (PAPIIT-UNAM; IN202513, IN200813) and Consejo Nacional de Ciencia y Tecnología (CONACYT; 127368, 176911). P Olvera-Caltzontzin was a graduate student of the Programa de Doctorado en Ciencias Biomédicas, UNAM, and received a scholarship from CONACYT (215709). The authors thank Martín García, Alejandra Castilla, and Moisés Pérez-Mendoza for technical assistance; Francisco Valles, Leonor Casanova and Lourdes Lara for academic support; Ramon Martínez, Alberto Lara and Omar González for computer assistance; and Dr. Dorothy Pless for proofreading.

## DISCLOSURE

The authors declare that they have no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

## REFERENCES


1. Ferlay J, *et al*. (2010) GLOBOCAN 2008 v2.0, cancer incidence and mortality worldwide: IARC CancerBase no. 10 [Internet]. Lyon (France): International Agency for Research on Cancer; [cited 2013 Dec 4]. Available from: http://globocan.iarc.fr.
2. Sonoda T, *et al.* (1997) A case-control study of diet and prostate cancer in Japan: possible protective effect of traditional Japanese diet. *Cancer Sci.* 95:238–42.
3. Key TJ, Silcocks PB, Davey GK, Appleby PN, Bishop DT. (1997) A case-control study of diet and prostate cancer. *Br. J. Cancer.* 76:678–87.
4. Hoption Cann SA, Qiu Z, van Netten C. (2007) A prospective study of iodine status, thyroid function, and prostate cancer risk: follow-up of the First National Health and Nutrition Examination Survey. *Nutr. Cancer.* 58:28–34.
5. Aceves C, Anguiano B, Delgado G. (2013) The extrathyronine actions of iodine: antioxidant, apoptotic and differentiator factor in iodine-uptake tissues. *Thyroid.* 23:938–46.
6. Bizhanova A, Kopp P. (2009) Minireview: the sodium-iodide symporter *NIS* and pendrin in iodide homeostasis of the thyroid. *Endocrinology.* 150:1084–90.
7. Küpper FC, *et al.* (1998) Iodine uptake in laminariales involves extracellular, haloperoxidase-mediated oxidation of iodide. *Planta.* 207:163–71.
8. Arroyo-Helguera O, Anguiano B, Delgado G, Aceves C. (2006) Uptake and antiproliferative effect of molecular iodine in the MCF-7 breast cancer cell line. *Endocr. Relat. Cancer.* 13:1147–58.
9. Rösner H, Torremante P, Möller W, Gärtner R. (2010) Antiproliferative/cytotoxic activity of molecular iodine and iodolactones in various human carcinoma cell lines: no interfering with EGF-signaling, but evidence for apoptosis. *Exp. Clin. Endocrinol. Diabetes.* 8:410–9.
10. Liu XH, Chen GG, Vlantis AC, Tse GM, van Hasselt CA. (2010) Iodine induces apoptosis via regulating MAPKs-related *p53*, *p21*, and Bcl-xL in thyroid cancer cells. *Mol. Cell. Endocrinol.* 320:128–35.
11. Shrivastava A, *et al.* (2006) Molecular iodine induces caspase-independent apoptosis in human breast carcinoma cells involving the mitochondria-mediated pathway. *J. Biol. Chem.* 281:19762–71.
12. Arroyo-Helguera O, Rojas E, Delgado G, Aceves C. (2008) Signaling pathways involved in the antiproliferative effect of molecular iodine in normal and tumoral breast cells: evidence that 6-iodolactone mediates apoptotic effects. *Endocr. Relat. Cancer.* 15:1003–11.
13. Aceves C, *et al.* (2009) Antineoplastic effect of iodine in mammary cancer: participation of 6-iodolactone (6-IL) and peroxisome proliferator-activated receptors (PPAR). *Mol. Cancer.* 8:33–41.
14. Mendieta I, Nunez-Anita E, Delgado G, Aceves C. (2011) Differential effect of iodine on the implantation and metastatic potential of xenografts from two different human breast cancer cell lines [abstract]. *Cancer Res.* 71(8 Suppl):Abstract nr 4224.
15. Huang M, *et al.* (2001) Ectopic expression of the thyroperoxidase gene augments radioiodide uptake and retention mediated by the sodium iodide symporter in non-small cell lung cancer. *Cancer Gene Ther.* 8:612–8.
16. Soriano O, *et al.* (2011) Antineoplastic effect of iodine and iodide in dimethylbenz[a]anthracene-induced mammary tumors: association between lactoperoxidase and estrogen-adduct production. *Endocr. Relat. Cancer.* 18:529–39.
17. Vitale M, *et al.* (2000) Iodide excess induces apoptosis in thyroid cells through a *p53*-independent mechanism involving oxidative stress. *Endocrinology.* 141:598–605.
18. Langer R, Burzler C, Bechtner G, Gärtner R. (2003) Influence of iodide and iodolactones on thyroid apoptosis: evidence that apoptosis induced by iodide is mediated by iodolactones in intact porcine thyroid follicles. *Exp. Clin. Endocrinol. Diabetes.* 111:325–9.
19. Thomasz L, *et al.* (2010) 6 Iodo-delta-lactone reproduces many but not all the effects of iodide. *Mol. Cell. Endocrinol.* 323:161–6.
20. Nuñez-Anita RE, Arroyo-Helguera O, Cajero-Juárez M, López-Bojorquez L, Aceves C. (2009) A complex between 6-iodolactone and the peroxisome proliferator-activated receptor type gamma may mediate the antineoplastic effect of iodine in mammary cancer. *Prostaglandins Other Lipid Mediat.* 89:34–42.
21. Spitzweg C, Joba W, Eisenmenger W, Heufelder AE. (1998) Analysis of human sodium iodide symporter gene expression in extrathyroidal tissues and cloning of its complementary deoxyribonucleic acids from salivary gland, mammary gland, and gastric mucosa. *J. Clin. Endocrinol. Metab.* 83:1746–51.
22. Lacroix L, *et al.* (2001) Na(+)/I(–) symporter and Pendred syndrome gene and protein expressions in human extra-thyroidal tissues. *Eur. J. Endocrinol.* 144:297–302.
23. Perron B, Rodriguez AM, Leblanc G, Pourcher T. (2001) Cloning of the mouse sodium iodide symporter and its expression in the mammary gland and other tissues. *J. Endocrinol.* 170:185–96.
24. Navarra M, *et al.* (2010) Expression of the sodium/iodide symporter in human prostate adenocarcinoma. *Urology.* 75:773–8.
25. Aranda N, Sosa S, Delgado G, Aceves C, Anguiano B. (2013) Uptake and antitumoral effects of iodine and 6-iodolactone in differentiated and undifferentiated human prostate cancer cell lines. *Prostate.* 73:31–41.
26. (2001 Aug 22) Norma Oficial Mexicana NOM-062-ZOO-1999, Especificaciones técnicas para la producción, cuidado y uso de los animales de laboratorio. Mexico City: Diario Oficial de la Federación; [cited 2013 Dec 4]. Available from: http://www.senasica.gob.mx/?doc=743.

27. McAlpine RK. (1945) The rate of oxidation of iodide ion by hydrogen peroxide. *J. Chem. Educ.* 22:387–90.
28. York RG, *et al.* (2005) Refining the effects observed in a developmental neurobehavioral study of ammonium perchlorate administered orally in drinking water to rats. I. Thyroid and reproductive effects. *Int. J. Toxicol.* 24:403–18.
29. Kaplan-Lefko PJ, *et al.* (2003) Pathobiology of autochthonous prostate cancer in a pre-clinical transgenic mouse model. *Prostate.* 55:219–37.
30. Anguiano B, García-Solís P, Delgado G, Aceves Velasco C. (2007) Uptake and gene expression with antitumoral doses of iodine in thyroid and mammary gland: evidence that chronic administration has no harmful effects. *Thyroid.* 17:851–9.
31. Dohán O, Baloch Z, Bánrévi Z, Livolsi V, Carrasco N. (2001) Rapid communication: predominant intracellular overexpression of the Na(+)/I(-) symporter (*NIS*) in a large sampling of thyroid cancer cases. *J. Clin. Endocrinol. Metab.* 86:2697–700.
32. Wapnir IL, *et al.* (2003) Immunohistochemical profile of the sodium/iodide symporter in thyroid, breast, and other carcinomas using high density tissue microarrays and conventional sections. *J. Clin. Endocrinol. Metab*. 88:1880–8.
33. Wapnir IL, *et al.* (2004) The Na+/I– symporter mediates iodide uptake in breast cancer metastases and can be selectively down-regulated in the thyroid. *Clin. Cancer Res.* 10:4294–302.
34. Kollecker I, *et al.* (2012) Subcellular distribution of the sodium iodide symporter in benign and malignant thyroid tissues. *Thyroid*. 22:529–35.
35. Miller AE, Heyland A. (2013) Iodine accumulation in sea urchin larvae is dependent on peroxide. *J. Exp. Biol.* 216:915–26.
36. Aceves C, Anguiano B. (2009) Is Iodine an Antioxidant and Antiproliferative Agent for the Mammary and Prostate Glands? In: *Comprehensive Handbook of Iodine: Nutritional, Endocrine and Pathological Aspects.* Preedy VR, Burrow GN, Watson RR (eds.) London, Academic Press (Elsevier), pp. 249–57.
37. Nguewa PA, Calvo A. (2010) Use of transgenic mice as models for prostate cancer chemoprevention. *Curr. Mol. Med.* 10:705–18.
38. Gupta S, *et al.* (2004) Suppression of prostate carcinogenesis by dietary supplementation of celecoxib in transgenic adenocarcinoma of the mouse prostate model. *Cancer Res.* 64:3334–43.
39. Narayanan BA, Narayanan NK, Pittman B, Reddy BS. (2004) Regression of mouse prostatic intraepithelial neoplasia by nonsteroidal anti-inflammatory drugs in the transgenic adenocarcinoma mouse prostate model. *Clin. Cancer Res.* 10:7727–37.
40. Bonorden MJ, *et al.* (2012) Growth and progression of TRAMP prostate tumors in relationship to diet and obesity. *Prostate Cancer.* 2012:543970.
41. Berman-Booty LD, *et al.* (2013) Suppression of prostate epithelial proliferation and intraprostatic progrowth signaling in transgenic mice by a new energy restriction-mimetic agent. *Cancer Prev. Res. (Phila)*. 6:232–41.
42. Huss WJ, *et al.* (2007) Origin of androgen-insensitive poorly differentiated tumors in the transgenic adenocarcinoma of mouse prostate model. *Neoplasia.* 9:938–50.
43. Chiaverotti T, *et al.* (2008) Dissociation of epithelial and neuroendocrine carcinoma lineages in the transgenic adenocarcinoma of mouse prostate model of prostate cancer. *Am. J. Pathol.* 172:236–46.
44. Godoy-García L, Nava-Villalba M, Delgado G, Aceves C. (2013) Molecular iodine adjuvancy in the antineoplastic effect of retinoic acid on neuroblastoma cells [abstract]. *Eur. J. Cancer*. 49 Suppl 2:S363.