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Original article

Iodine prevents the increase of testosterone-induced oxidative stress in a model of rat prostatic hyperplasia

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ABSTRACT

Oxidative stress and inflammation are involved in the development and/or progression of benign prostatic hyperplasia (BPH). Molecular iodine (I_2) induces antiproliferative and apoptotic effects in prostate cancer cells, but it is unknown if I_2 regulates oxidative stress in the normal and/or tumoral prostate. The purpose of this study was to analyze the effects of I_2 and celecoxib (Cxb) on oxidative stress and inflammation in a model of prostatic hyperplasia. Cxb was used as positive control of cyclooxygenase-2 (COX-2) inhibition. Prostatic hyperplasia was induced in male Wistar rats (170 g) with testosterone (5 mg/kg/week, for three weeks). One week before hyperplasia induction, I_2 (25 mg/day/rat) or Cxb (1.25 mg/day/rat) was supplied for four weeks in the drinking water. Prostatic hyperplasia was evaluated by histological analysis, DNA content, and/or proliferating cell nuclear antigen (PCNA) expression. Lipoperoxidation (malondialdehyde) and nitrite ($NO2^-$) levels were analyzed by colorimetric methods, while nitric oxide synthase (NOS), COX, and myeloperoxidase (MPO) enzymes were analyzed using RT-PCR, immunoblotting, and/or enzymatic assays. Levels of 15-F2t-isoprostanes, prostaglandins (PGE_2), leukotrienes (LTB_4), and tumor necrosis factor alpha (TNFα) were measured by ELISA. Control testosterone-treated animals exhibited hyperplasia in the dorsolateral prostate, as well as increments in almost all oxidative parameters except for COX-1, TNFα, or MPO. I_2 and Cxb prevented epithelial hyperplasia (DNA content) and oxidative stress induction generated by testosterone in almost the same intensity, and the minimum I_2 dose required was 2.5 mg/rat. The antioxidant capacity of I_2 was also analyzed in a cell-free system, showing that this element inhibited the conversion of nitrate (NO_3^-) to NO_2^-. I_2 did not modify the prostatic oxidative state in testosterone untreated rats. In summary, our data showed that antiproliferative and antioxidant effects of I_2 involve the inhibition of NOS and the COX-2 pathway. Further studies are necessary to analyze the therapeutic and/or adjuvant effects of I_2 with first-line medications used to treat BPH.

1. Introduction

Benign prostatic hyperplasia (BPH) is a chronic progressive disease of aging men, characterized by a non-malignant growth of stromal and epithelial cells in the prostate. The etiology is not well understood, but factors such as hormonal disruptions, inflammation, and oxidative stress are involved in the development and/or progression of this disease [1,2]. Clinical studies show that pathogenesis of BPH is accompanied by an overproduction of oxidant molecules and/or a deficiency in the antioxidant system [3,4]. Indeed, experimental studies show that activation of pro-oxidant systems, such as NADPH oxidase, cyclooxygenase, and lipoxygenase, induce prostatic hyperplasia and cell damage [5]. The factors that produce a more oxidative state in the prostate are unclear, but the hormonal environment (high or low testosterone and/or high estrogen ratio) and chronic inflammation could be relevant in this process. It has been shown that administration of non-steroid anti-inflammatory drugs (inhibitors of cyclooxygenase-2, COX-2) combined with conventional treatments for hyperplasia (α1-adrenergic antagonists and/or anti-androgens) improves lower urinary tract symptoms and/or reduces the levels of prostate-specific antigen (PSA) [6–8]. However, the use of these medications is usually accompanied by adverse effects, and new therapeutic approaches are trying to reduce them to the maximum.

Iodine is a micronutrient with a broad spectrum of actions that include redox capacity. This halogen can mediate anti- or prooxidant effects depending on the chemical species (iodide, diiodine, iodate), concentration, and/or oxidative state of the target organ. Iodide (I^-) acts as an antioxidant (electron donor), and in the presence of the NADPH-

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oxidase/H_2O_2 system, the enzymes thyro-, lacto-, and myeloperoxidase catalyze the oxidation from I^- as part of the process to synthesize thyroid hormones, casein, and antimicrobial compounds [9–11]. Antioxidant actions were also reported in an acute model of heart infarction, which showed that exogenous administration of I^-, but not of iodate (IO_3^-), prevents the oxidative damage associated with reperfusion [12]. In contrast, high doses of I^- induce oxidizing effects. Doses 10–50 times higher than the daily intake induce lipoperoxidation (LPO) and apoptosis in thyroid and non-thyroid tissues [13,14]. These effects are more evident when I^- is administered under a prooxidant condition as hypothyroidism [15]. Similarly, an excess of I^- (200–500 times the daily intake) induces oxidative stress on testes, reduces prostate weight, and decreases testosterone circulating levels [16], but it does not induce LPO in liver or muscles [14,17–19], showing tissue-specific effects.

On the other hand, in vitro studies show that molecular iodine, or diiodine (I_2), induces cell arrest and apoptosis in cancerous cell lines of thyroid, neuroblastoma, breast, and prostate [20–25]. I_2 prevents the increase of carcinogen-induced LPO, as well as the cardiotoxic effects induced by antineoplastic drugs in breast cancer models [26,27]. In a model of skin damage induced with sulfur mustard, it was shown that topical administration of I_2 reduces the number of cells positive to nitric oxide synthase (NOS) and COX-2 [28]. The mechanisms behind antioxidant actions are not well understood, but a cell-free system showed that I_2 acts as a scavenger of H_2O_2, and it is more potent than I^- or IO_3^- [27].

In prostate cancer cells, we have shown that I^- and I_2 treatments decrease cell proliferation and induce apoptosis [25], and that continuous administration of a high dose of I_2 prevents the hyperplasia and epithelial hypertrophy induced by sex hormones in rat prostate [29].

The aim of this study was to analyze the effects of I_2 on hyperplasia and testosterone-induced oxidative stress, and compare them with an inhibitor of COX-2 (Celecoxib, Cxb). In addition, the minimum dose of I_2 required for maintaining the efficacy against hyperplasia and/or oxidative stress was evaluated. LPO and nitrite levels were measured as indicators of oxidative state, and the effects of I_2 and/or Cxb on pathways of nitric oxide, cyclooxygenase (COX), and lipoxygenase (LOX) were analyzed.

2. Materials and methods

2.1. Chemicals

Testosterone (Sostenon 250) was obtained from Organon (Mexico City, Mexico). Celecoxib (Celebrex) was obtained from Pfizer (Mexico City, Mexico). Assay kits for lipid peroxidation (malondialdehyde, MDA), nitrate/nitrite colorimetric determination, myeloperoxidase activity, and COX-2 human enzyme (≥ 8000 units/mg protein) were obtained from Sigma-Aldrich (St Louis, MO). Cyclooxygenases activities kit was obtained from Cayman Chemical Company (Ann Arbor, MI). Ultrasensitive colorimetric assay for nitric oxide synthase activity and ELISA kit for 15-F2t-isoprostanes (15-F2t-IsoP) was obtained from Oxford Biomedical Research (Rochester, MI). ELISA kits for prostaglandin E2 and leukotriene B4 were obtained from Thermo Scientific (Waltham, MA). Rat TNFα sandwich-ELISA was obtained from Cusabio (Hubei, China). C18 reverse phase column and silica cartridges (500 mg) were purchased from Waters Corporation (Milford, MA). Mouse monoclonal antibodies against COX-2 (sc-376861), 5-LOX (sc-136195), goat polyclonal antibody against actin (sc-1616), goat anti mouse (sc-2060) and donkey anti goat (sc-2020) were obtained from Santa Cruz Biotechnology (Texas, USA). All other reagents were of the highest purity commercially available.

2.2. Animals

Male Wistar rats were housed in polypropylene cages under controlled temperature (22 ° ± 1 °C) and constant 12-h light/dark cycle. They had free access to standard rat pellet diet chow (Purina, Richmond, CA) and tap water. Procedures for handling and euthanasia of rats were reviewed and approved by the Ethics Committee of the Instituto de Neurobiología, UNAM, and they complied with guidelines from the Institute of Laboratory Animal Resources Commission on Life Sciences National Research Council, USA [30].

2.3. Prostatic hyperplasia induction and treatments

Prostatic hyperplasia was induced in rats by oily intramuscular injection of testosterone (5 mg/kg body weight, once a week, for three weeks). A high dose of I_2 was administered in the drinking water at 0.05%. The average consumption of water per rat per day was 50 ml, doses equivalent to a daily intake of 25 mg I_2/rat. Rats were randomly assigned to the following groups: intact and intact + I_2 and/or three of hyperplasia: water, I_2, and Cxb (n = 3–7 rats/group). Treatments with I_2 or Cxb started one week before hyperplasia induction. Both treatments were continuously administered in the drinking water for four weeks. Daily intake of Cxb was 1.25 mg/rat, which was equivalent to therapeutic doses [31]. Rats were sacrificed by decapitation, and prostate lobes were removed and weighed. The tissues were stored in neutral buffered formalin (10%) or frozen at −70 °C for histological and biochemical analysis. The hyperplastic phenotype was determined by histology and DNA quantification. Prostate oxidative state was analyzed on dorsolateral lobes by measuring LPO ratio: MDA and 15-F2t-IsoP and nitrite levels. The pathways of nitric oxide, cyclooxygenase and lipoxygenases were analyzed through measurement of mRNA, protein levels, and/or enzymatic activity of NOS, COX, and 5-LOX. The final products were also measured, such as prostaglandin E_2 (PGE_2) and leukotriene B4 (LTB_4), as well as levels of some mediators of inflammation, as myeloperoxidase (MPO) and tumor necrosis factor alpha (TNFα).

2.4. Minimum dose of iodine

I_2 was administered in the drinking water at different concentrations (0.0005%, 0.001%, 0.005%, and 0.05%), and intake was equivalent to daily doses of 0.25, 0.5, 2.5, and 25 mg of I_2/rat (n = 3–4 rats/group). The minimal effective dose of I_2 against hyperplasia and oxidative stress was validated by histological analysis, the number of proliferating cell nuclear antigen (PCNA)-positive cells, and LPO ratio (MDA).

2.5. Histology

Prostatic samples in formalin were embedded in paraffin, cut into 5-μm sections, and stained with hematoxylin and eosin. Prostate epithelium was examined by light microscope (Leica DM2500), and images were acquired with a Leica camera (DFC 420).

2.6. Immunohistochemistry for PCNA protein

Paraffin tissue sections were deparaffinized and rehydrated. Antigen retrieval was performed by adding 0.05% citraconic anhydride solution, pH 7.4, and heating in a pressure cooker for 25 min. Sections were blocked with peroxidase block solution and then incubated overnight at 4 °C with rabbit polyclonal IgG PCNA antibody (FL-261) 1:100 from Santa Cruz Biotechnology. Then, the sections were incubated with the secondary antibody goat anti-rabbit IgG-horseradish peroxidase 1:1000 from DAKO at 37 °C for 1 h. PCNA immunoreactivity was visualized with 3,3′-diaminobenzamine tetrahydrochloride and incubated for 5 min. Sections were counterstained with hematoxylin, dehydrated, and mounted in Entellan solution. Tissues incubated without primary antibodies were used as negative controls. Images were acquired with a camera (Leica DFC420) and analyzed under light microscopy (Leica DM 2500) at 40x. PCNA-positive cells were counted using the cell counter