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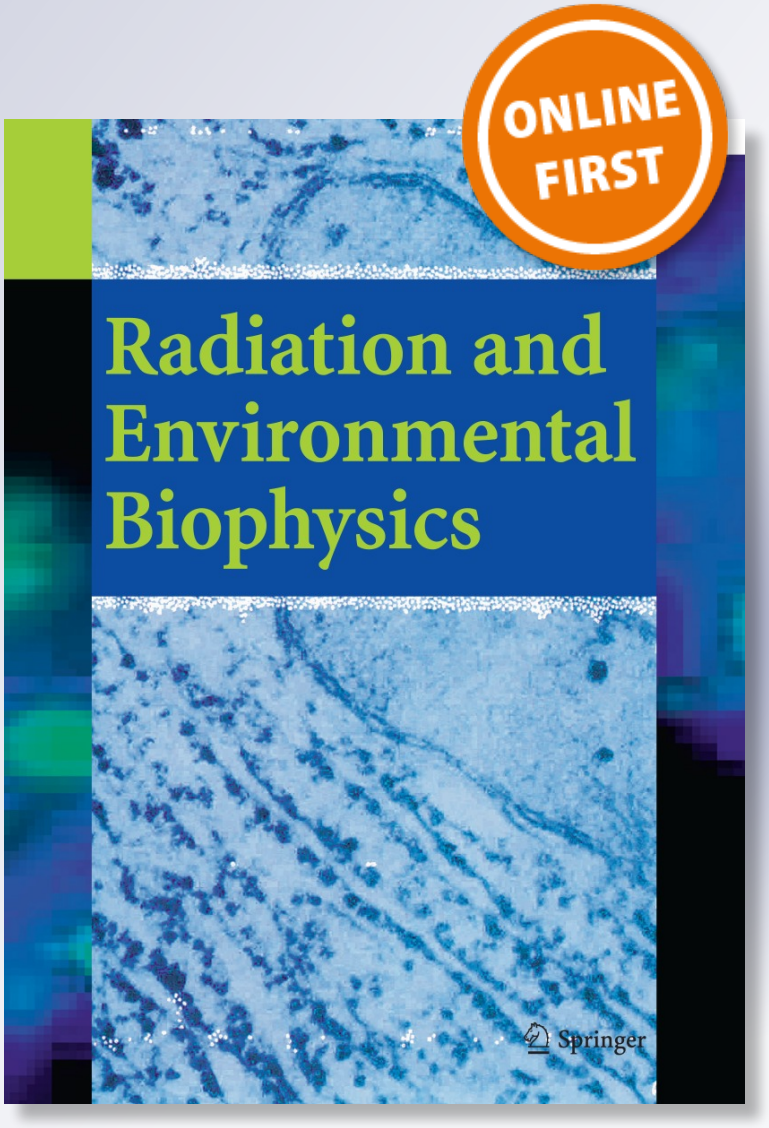

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ORIGINAL PAPER

Protective effect of an antithyroid compound against γ-radiation-induced damage in human colon cancer cells

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Abstract We have previously reported the radioprotective effect of propylthiouracil (PTU) on thyroid cells. The aim of the present study was to analyze whether tumor cells and normal cells demonstrate the same response to PTU. Human colon carcinoma cells were irradiated with γ-irradiation with or without PTU. We evaluated the clonogenic survival, intracellular reactive oxygen species levels, catalase, superoxide dismutase and glutathione peroxidase activities, and apoptosis by nuclear cell morphology and caspase-3 activity assays. Cyclic AMP (cAMP) levels were measured by radioimmunoassay. PTU treatment increased surviving cell fraction at 2 Gy (SF2) from 56.9 ± 3.6 in controls to 75.0 ± 3.5 ($p < 0.05$) and diminished radiation-induced apoptosis. In addition, we observed that the level of antioxidant enzymes' activity was increased in cells treated with PTU. Moreover, pretreatment with PTU increased intracellular levels of cAMP. Forskolin ($p < 0.01$) and dibutyryl cAMP ($p < 0.05$) mimicked the effect of PTU on SF2. Co-treatment with H89, an inhibitor of protein kinase A, abolished the radioprotective effect of PTU. PTU reduces the toxicity of ionizing radiation by increasing cAMP levels and also possibly through a reduction in apoptosis levels and in radiation-induced oxidative stress damage. We therefore conclude that PTU protects both normal and cancer cells during exposure to radiation in conditions mimicking the radiotherapy.

Keywords Ionizing radiation · Propylthiouracil · Radioprotection · Thyroid · Colon cancer

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Introduction

Radiation treatment is an effective therapeutic modality for a wide range of malignant conditions (American Cancer Society 2005). Approximately half of all cancer patients will receive radiotherapy at some point during their treatment, either for curative or palliative purposes (Nair et al. 2001). Nevertheless, the use of radiation therapy is often limited by the adverse effects on the normal tissue in the vicinity of the tumor (Stone et al. 2004). This could instigate undesirable side effects and an increase in the occurrence of secondary treatment-related cancers. Therefore, there is a constant effort to minimize the toxicities associated with the application of radiotherapy centered on technological improvements, in radiation delivery, and in developing chemical modifiers of radiation injury (Nair et al. 2001).

Agents that alter the response of normal tissues to irradiation have been categorized under the terms of radioprotectors, radiation mitigators, and treatment (Stone et al. 2004). Chemical radioprotectors are compounds that decrease the radiation damage when added to the culture medium before irradiation (Greenberger 2009; Grdina et al.

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2002). They exert their effects mainly through scavenging free radicals (Sonntag 1987). Different compounds have been described that function as chemical radioprotectors, such as different thiols (Yuhas 1970), tocols (Ghosh et al. 2009), superoxide dismutase (SOD) mimetics (Murley et al. 2007), nitroxides (Soule et al. 2007), melatonin, and its homologues (Blickenstaff et al. 1994). Despite the continued efforts of researchers, there are only a few radioprotectors in use today, although their clinical application is limited because of their toxicity (Grdina et al. 2002). Amifostine (WR-2721), a broad cytoprotective agent, is the only drug that has been approved by the Food and Drug Administration (FDA) for xerostomia in patients receiving radiotherapy for head and neck cancers (Sasse et al. 2006).

The thioureylene compounds methylmercaptoimidazole (MMI) and propylthiouracil (PTU) are antithyroid drugs that have been used to treat hyperthyroidism since 1940 (Ron et al. 1989). Both compounds share a relatively simple structure containing a sulfur atom commonly found in many chemical radioprotectors (Prasad 1995). Greig et al. (1965) have previously described that MMI protected rat thyroid glands from radiation-induced damage. Recently, it was also shown that MMI decreased DNA damage to 70 % in rat thyroid epithelial cells after external irradiation (Kahmann et al. 2010). On the other hand, a radioprotective effect of antithyroid drugs has been also described in patients treated with radioiodine that was controversially discussed (Mole et al. 1950; Sabri et al. 1999). Recently, we have reported that PTU protected rat normal thyroid cells from radiation damage through 3′-5′-adenosine monophosphate (cAMP) elevation and a reduction in radiation-induced oxidative stress damage (Perona et al. 2013).

The present study focuses on the evaluation of the response of tumor and normal cells to the treatment with PTU to further determine whether it would be useful in the radiotherapy clinic. Specifically, the experiments described here examine the ability of PTU to protect a human colon cancer cell line from the effects of gamma radiation. In addition, we studied the mechanisms associated with the radioprotective effect.

Materials and methods

Cell culture

The cell line ARO81-1, a subline derived from the human colon cancer cell line HT29 provided by Dr. G. Juillard (University of California, Los Angeles), was maintained in RPMI 1640 (GIBCO, Invitrogen Corporation, Carlsbad, CA, USA) medium supplemented with 10 % FBS. Cells were kept at 37 °C in 5 % CO_2–95 % air in a humidified atmosphere.

Irradiation characteristics

Cells were irradiated with a cobalt gamma ray source at a dose rate of 1 Gy/min ± 5 % (Roffo Institute, Buenos Aires, Argentina) at different times in order to obtain radiation-absorbed doses that ranged between 1 and 8 Gy.

Clonogenic assay

Exponentially growing cells were seeded in 25-cm^2 flasks (Nunc, Fisherbrand, UK) 24 h before the irradiation and divided into two groups: (1) irradiated without treatment and (2) incubated with the drug before irradiation (1 mM PTU; 1 mM dibutyryl cyclic AMP (dbcAMP) (Sigma-Aldrich, St Louis, MO, USA); 0.01 mM Forskolin (Sigma-Aldrich) or 1 mM PTU + 0.01 mM H-89). Following irradiation, cells were trypsinized, counted, seeded in 60-mm culture dishes, and further incubated for 9–14 days to allow colony formation. At that time, colonies were fixed with 5 % glutaraldehyde and stained with 0.5 % crystal violet. Isolated clusters of more than 50 cells were counted as a single colony. The surviving fraction (SF) of each radiation dose was normalized to that of the control to obtain a radiation survival curve. Curves were fitted according to the linear-quadratic model (surviving fraction $= \exp^{-\alpha(D)-\beta(D)2}$) using Origin 7.5 (OriginLab, Northampton, MA, USA). SF at 2 Gy (SF2) was calculated for each curve. Each point in the curve is the result of three independent experiments.

Cyclic AMP measurement

Cells were seeded in 24-well plates and incubated with different concentrations of PTU (0, 0.5, 1 mM) or the combination of PTU and 100 μM of the phosphodiesterase inhibitor 1-methyl-3-isobutylxanthine (IBMX) (Sigma-Aldrich) for 5, 24, 48, and 72 h. Cells were rinsed twice with ice-cold phosphate-buffered saline 1X (PBS) and incubated with absolute ethanol at 4 °C for 30 min. cAMP was measured in the supernatant using a method described previously (Del Punta et al. 1996). The antibody used was kindly provided by the NIH (National Hormone and peptide program, Dr A. F. Parlow). Results are expressed in fmol cAMP/μg protein.

Intracellular ROS levels

Intracellular reactive oxygen species (ROS) production was measured 1 and 24 h after irradiation (3 and 5 Gy) using the fluorescent probe 2′,7′-dichlorofluorescein-diacetate

(DCFH-DA) (Sigma-Aldrich) that reacts with intracellular ROS. Briefly, cells were incubated with 10 μM of DCFH-DA at 37 °C for 20 min, washed twice with PBS 1X and immediately after ROS levels were determined by fluorescence at $\lambda_{excitation}$ 485/20 nm and $\lambda_{emission}$ 530/25 nm using a plate reader (Beckman Coulter DTX 880 Multimode detector; Beckman Coulter, Inc., CA, USA). Results are expressed as relative absorbance units/mg protein.

Enzymatic assay

Cells were collected at a time point of 1 and 24 h after irradiation (3 and 5 Gy) and spun at 1,200×*g* for 10 min at 4 °C. Pellets were resuspended in sodium phosphate buffer (pH 7.8 50 mM) and sonicated for two 15-s bursts. The supernatants were collected after centrifugation at 15000×*g* for 10 min at 4 °C for enzymatic determinations.

Catalase

The activity was measured by monitoring the enzyme-catalyzed decomposition of H_2O_2 at 240 nm by the method described by Aebi (1984). Activity is expressed as nmol of H_2O_2 consumed per minute per mg protein.

Superoxide dismutase

Superoxide dismutase activity was measured using a SOD Assay Kit according to the manufacturer's instructions (Cayman Chemical, Ann Arbor, MI, USA). Absorbance was measured at 450 nm. Results are expressed as percentage of unirradiated controls.

Glutathione peroxidase

Glutathione peroxidase activity was determined using the Glutathione Peroxidase Cellular Activity Assay Kit according to the manufacturer's instructions (SIGMA CGP-1; Sigma-Aldrich). A unit of glutathione peroxidase is defined as the amount of enzyme that will cause the formation of 1.0 μmol of $NADP^+$ from NADPH per minute at pH 8.0 at 25 °C in a coupled reaction in the presence of reduced glutathione, glutathione reductase, and *tert*-butyl hydroperoxide.

Apoptosis assessment by nuclear morphology

Apoptosis was assessed using fluorescence staining 6, 24, 48, and 72 h after irradiation (3 and 5 Gy). Cells were incubated with 2 μl of a MIX buffer containing Hoechst 33258 (0.59 mg/mL), 4,5-diaminofluorescein (DAF) (0.12 mg/mL), and propidium iodide (PI) (0.59 mg/mL) (Sigma-Aldrich) at room temperature for 5 min. Hoechst 33258 was used to visualize nucleus morphology and evaluate the differences between normal and apoptotic nuclei. PI and DAF were use to stain dead cells and cytoplasm of viable cells, respectively. Fluorescent microscopy was used to count propidium iodide-impermeable cells having condensed/fragmented nuclei (apoptotic). The percentage of apoptotic cells was determined by identifying those cells with chromatin condensation and fragmentation characteristics of apoptosis over at least 200 observed cells.

Caspase-3 activity

Cells were collected in PBS and centrifuged at 600×*g* for 5 min at 4 °C. The pellet was resuspended in 1X lysis buffer [50 mm HEPES, 5 mM dithiothreitol (DTT), 5 mM CHAPS, 10 μg/mL pepstatin, benzamidine 2.5 mM, aprotinin 10 μg/mL, pepstatin 1 μg/mL, 0.5 mM phenylmethylsulfonyl fluoride (PMSF), pH 7.4] and incubated in ice for 15 min. Lysates were centrifuged at 15,000×*g* for 20 min at 4 °C. Clear lysates containing 200 μg proteins were incubated with caspase-3 substrate, at 37 °C for 3 h. Caspase-3 activity was measured in the supernatant 6, 24, 48, and 72 h after irradiation (3 and 5 Gy) with the Caspase-3 Colorimetric Assay Kit (CASP-3-C, Sigma-Aldrich). This assay is based on the spectrophotometric detection of the Ac-DEVD-pNA substrate after cleavage. The activity (μmol pNA released per minute per ml) was calculated with a *p*-nitroaniline calibration curve. A positive control of caspase-3 and an inhibitor of caspase-3 (200 μM Ac-DEVD-CHO) were added to the plate.

Protein concentration was determined by the method of Lowry (Lowry et al. 1951). All reagents were obtained from Sigma Chemical.

Statistical analysis

All data are expressed as the mean ± standard error of the mean (SEM) of at least three independent experiments. Differences between SFs were calculated with Student's two-tailed *t* test. Statistical analysis among experimental groups was determined using one-way ANOVA, followed by Tukey–Kramer multiple comparison test. A p value of <0.05 was considered statistically significant.

Results

Radioprotective effect of PTU

To evaluate the effect of PTU on cell radioprotection, clonogenic survival assays were performed. Cell pretreatment with 1 mM PTU alone for 24 h had no effect on cell proliferation (results not shown). As shown in Fig. 1a, PTU

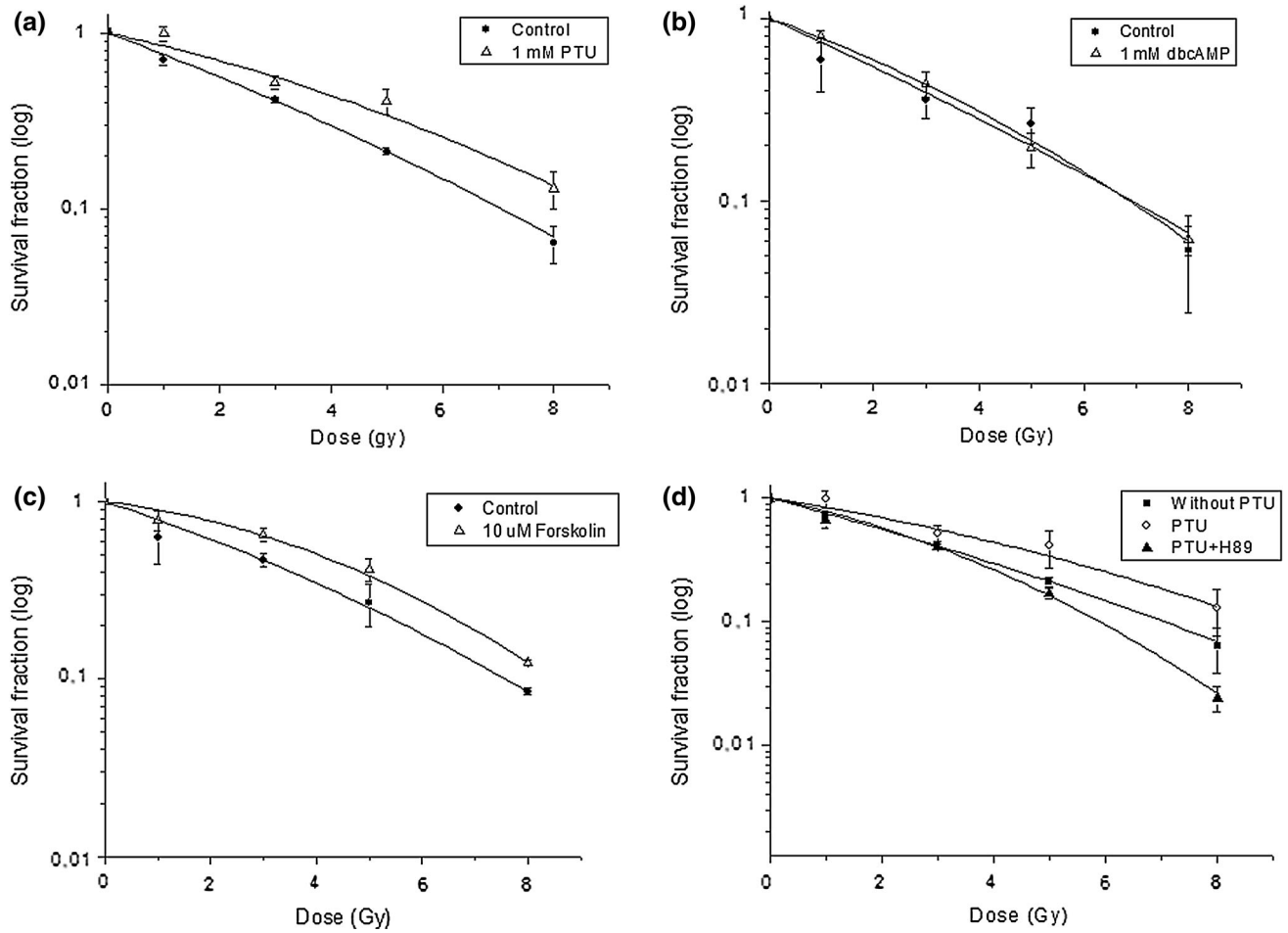

Fig. 1 Survival curves of cells untreated or treated with **a** propylthiouracil, **b** dibutyryl cAMP, **c** forskolin, or **d** propylthiouracil *plus* H89, before irradiation. Survival fraction at 2 Gy was significantly elevated in cells incubated with PTU, dibutyryl cAMP, and forskolin while the radioprotective effect of PTU was eliminated when co-incubated with H89. Curves were fitted according to the linear-quadratic model. *Error bars* indicate the standard error of the mean of three independent experiments

Table 1 Effect of PTU on cAMP intracellular levels

Time (h)	Treatment					
	Control	C + IBMX	PTU 0.5 mM	PTU 0.5 mM + IBMX	PTU 1 mM	PTU 1 mM +IBMX
5	1.08 ± 0.13	3.52 ± 0.47	1.45 ± 0.13	3.52 ± 0.30	8.50 ± 0.33***	13.12 ± 0.75$^{\circ\circ\circ}$
24	4.42 ± 0.24	17.53 ± 1.74	6.01 ± 0.75	16.55 ± 0.94	24.57 ± 2.85***	39.69 ± 2.75$^{\circ\circ\circ}$
48	0.39 ± 0.05	4.39 ± 0.44	3.24 ± 0.48	6.40 ± 0.67	21.43 ± 1.7***	43.59 ± 2.67$^{\circ\circ\circ}$
72	0.35 ± 0.02	11.75 ± 0.25	1.60 ± 0.07	7.50 ± 0.44	6.51 ± 1.00***	10.82 ± 0.58

Exponentially growing cells were incubated with different concentrations of PTU for 6, 24, 48, and 72 h. Each value is the average of four experimental determinations by quadruplicate. Results are expressed as the mean ± SEM of each group

*** $p < 0.001$ versus control

$^{\circ\circ\circ}$ $p < 0.001$ versus control + IBMX

induced an increase in post-irradiation survival at 2 Gy (*C*: 56.9 ± 3.6, PTU: 75.0 ± 3.5; $p < 0.05$). When PTU was added to the culture medium after irradiation, no protection was observed compared to the group with pre-irradiation treatment (results not shown).

Effects of PTU on cyclic AMP levels

To further investigate the cellular mechanism of PTU radioprotection, cAMP levels were determined at different times after incubation. cAMP levels (Table 1) were

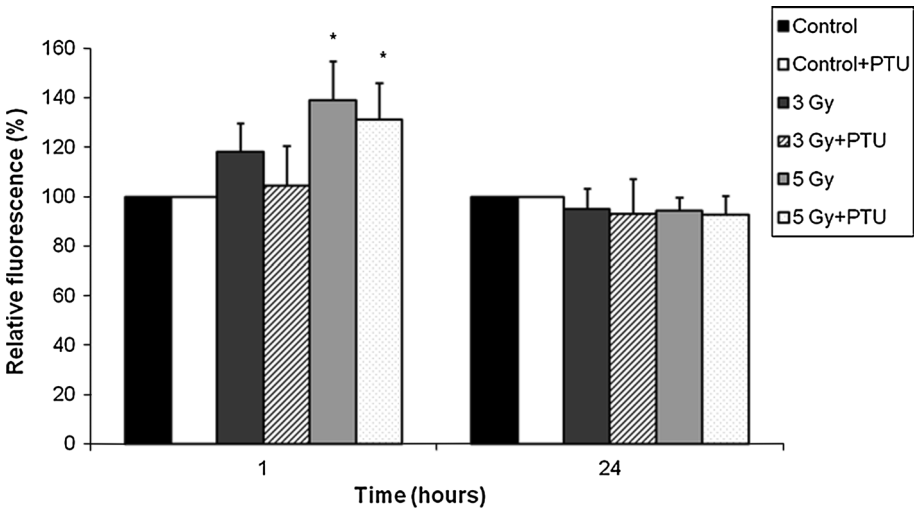

Fig. 2 Effect of PTU on intracellular ROS levels with or without irradiation. Cells were irradiated with or without the addition of PTU, and ROS levels were measured 1 and 24 h after irradiation. PTU reduced the increase in ROS levels induced by irradiation only after 1 h. *Error bars* indicate the standard error of the mean of three independent experiments. *$p < 0.05$ versus untreated cells

increased when cells were incubated with 1 mM PTU, reaching a peak at 24 h.

Since PTU increased cAMP levels, we then investigated the effect of different cAMP elevating agents on cellular radioprotection. Incubation with 1 mM dibutyryl cAMP resulted in an increase in SF2 from 55.2 ± 5.8 to 65.1 ± 3.3 ($p < 0.05$) (Fig. 1b). At higher doses, there were no significant changes in survival fraction compared to the irradiation-only group. Forskolin enhanced cell's SF2 from 67.8 ± 5.1 to 83.3 ± 7.5 ($p < 0.01$) (Fig. 1c). This effect was significant also for higher doses. Moreover, when cells were incubated with the protein kinase A inhibitor H-89, there was a reduction in the radioprotective effect of PTU (SF2, *C*: 53.3 ± 3.9; PTU: 74.3 ± 6.5; PTU + H-89: 57.9 ± 5.3) (Fig. 1d).

Effect of PTU on radiation-induced oxidative stress and apoptosis

Some compounds exert their radioprotective effect by scavenging free radicals and thereby reducing the indirect DNA damage. No changes in ROS levels were observed when cells were incubated with PTU alone compared to untreated controls (data not shown). Exposure to irradiation led to increased production of intracellular ROS levels at 1 h in cells irradiated with and without PTU. No differences were observed in ROS levels 24 h after irradiation between the treatments (Fig. 2).

Next, we examined the effect of the radiation dose on the activities of antioxidant enzymes (catalase, SOD and GPx) at 1 and 24 h after irradiation in treated and untreated cells. Catalase activity was significantly increased by PTU addition 1 h after irradiation at both doses ($p < 0.05$), while no significant effects were observed at 24 h (Fig. 3a). Treatment with PTU resulted in a significant increase in SOD activity 1 h after irradiation ($p < 0.01$ for 3 Gy and $p < 0.05$ for 5 Gy) (Fig. 3b). At 24 h, there was a significant increase in the enzyme's activity at the dose of 3 Gy ($p < 0.01$) (Fig. 3b). GPx activity was increased as a function of the dose in ARO81-1-irradiated cells compared to unirradiated controls, and there were no significant changes with PTU at both times studied (Fig. 3c).

To study the relevance of the different mechanisms of cell death following radiation, we analyzed cell death morphologic characteristics in irradiated ARO81-1 cells. Irradiation alone led to substantial increase in apoptosis, as evidenced by morphological characteristics of programmed cell death like karyorrhexis, cell shrinkage, pyknosis and cell blebbing, and necrosis percentages. The percentage of necrotic cells was higher than that of apoptotic cells. The dead/apoptotic cells ratio decreased with higher doses of radiation except at early times (6 h). PTU pretreatment reduced the percentage of apoptotic and dead cells observed after irradiation (Fig. 4). Measurement of caspase-3 activity indicated that the enzyme's activity was not significantly modified except at longer times by irradiation (Fig. 5). No changes were observed in cells incubated with PTU before irradiation.

Discussion

Radiotherapy is one of the most effective treatments for cancer. It is frequently used to achieve local or regional control of malignancies, either alone or in combination with other treatment modalities such as surgery or chemotherapy. Despite its effectiveness and even with the use of localized delivery techniques, the radiosensitivity of normal tissues adjacent to the tumor limits the therapeutic gain (Betzen and Overgaard 1997). Irradiation of noncancerous tissues can result in different side effects that include self-limited acute toxicities, mild chronic

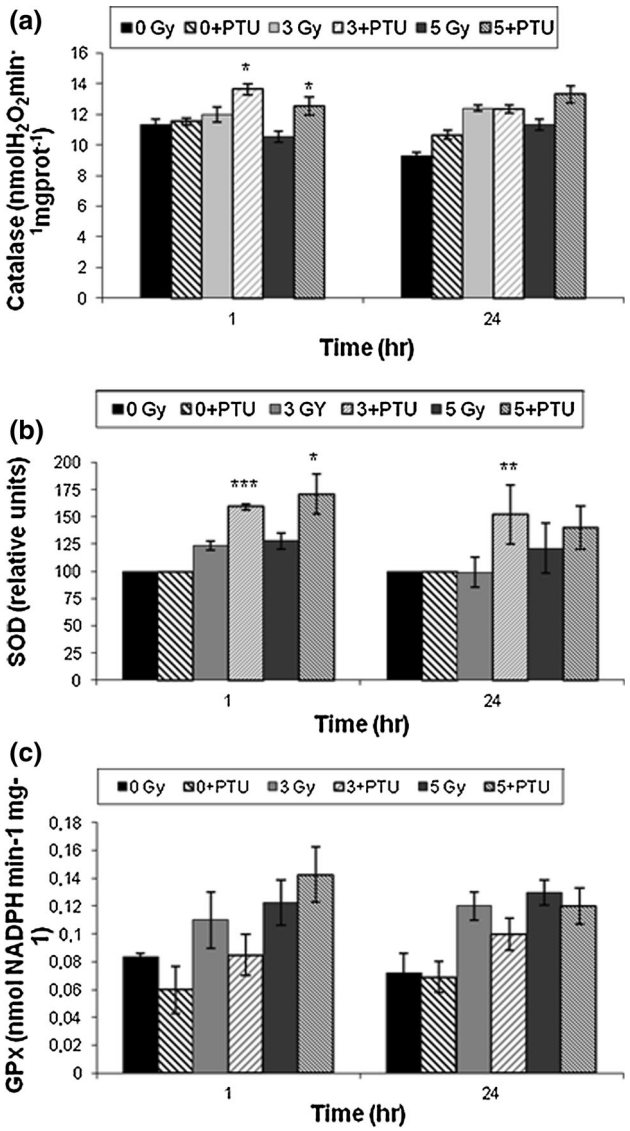

Fig. 3 Effect of PTU on antioxidant enzyme activities with or without irradiation. Cells were irradiated with or without the addition of PTU, and enzyme activities were measured 1 and 24 h after irradiation. **a** Catalase activity. **b** SOD activity. **c** Glutathione peroxidase activity. PTU increased the activity of antioxidant enzymes especially at the earlier time after irradiation. *Error bars* indicate the standard error of the mean of three independent experiments. $*p < 0.05$ versus irradiated without PTU cells. $**p < 0.01$ versus irradiated without PTU cells. $***p < 0.001$ versus irradiated without PTU cells

symptoms, or severe organ dysfunction. Therefore, the identification and development of radiation modifiers to protect normal tissue is of considerable importance in the context of possible applications in clinical radiotherapy or after accidental exposure to radiation. Several compounds have been tested as radioprotectors but their side effects at the doses required for radioprotection excluded them from clinical use (Greenberger 2009; Grdina et al. 2002). The application of antithyroid drugs as radioprotectors for the thyroid gland has already been studied. It has been demonstrated that pretreatment with methylthiouracil before irradiation protected thyroid cells from radiation-induced damage in both rat (Greig et al. 1965) and patients (Crooks et al. 1960). Sener et al. (2006) found that pretreating rats with PTU during 15 days prior to whole-body irradiation reduced irradiation-induced oxidative damage in several organs. They suggested that there could be a link between the radioprotective effect of PTU and the drug-induced hypothyroidism, which in turn reduced the production of reactive oxygen metabolites and enhanced the antioxidant mechanisms. A radioprotective effect of antithyroid drugs has also been described in patients undergoing radioiodine treatment, although the effect could be related to a reduction of intrathyroid iodine half-life (Bonnema et al. 2006; Sabri et al. 1999). On the other hand, Kahmann et al. (2010) found that following external irradiation, DNA damage was decreased by MMI to 70 % in rat epithelial thyroid cells. It has also been recently shown that perchlorate, iodide, and thiocyanate protected rat thyroid epithelial cells against DNA double-strand breaks induced by radioiodine (Hershman et al. 2011). In agreement with these results, we have demonstrated that PTU protected rat thyroid epithelial cells from radiation-induced damage. We found that the radioprotective effect could be mediated in part by an increase in cyclic AMP levels induced by the drug. We also demonstrated that PTU decreased the oxidative damage induced by irradiation (Perona et al. 2013).

Considering our previous results, this study aimed to evaluate whether tumor cells and normal cells demonstrate the same response to PTU. Cell treatment with PTU before γ-irradiation resulted in a significant increase in cell survival. SF2 was increased by about 31 % when compared to control irradiated cells. In contrast, there was no enhancement in survival fraction after irradiation when PTU was added to the culture medium after irradiation (data not shown). This could be suggesting that the exposure during irradiation is critical for protection.

Cyclic AMP regulates many physiological phenomena (Robison and Sutherland 1971). It has been reported that cell radiosensitivity could be related to intracellular cAMP level (Prasad 1972). Many reports have shown that increased cAMP levels were significantly associated with radioprotective effects (Lehnert 1975; Ojeda et al. 1980). Scaife (1971) demonstrated the mitogenic activity of cAMP under different conditions in irradiated cells with a radiation-induced G_2 mitotic delay diminution. A similar effect was described in S-180 ascites tumor cells treated prior to irradiation with an injection of theophylline, caffeine, or dibutyryl cAMP (Boynton and Evans 1973). To study whether PTU could be acting through modifications in intracellular cAMP levels, we incubated cells with different concentrations of PTU. We observed a significant

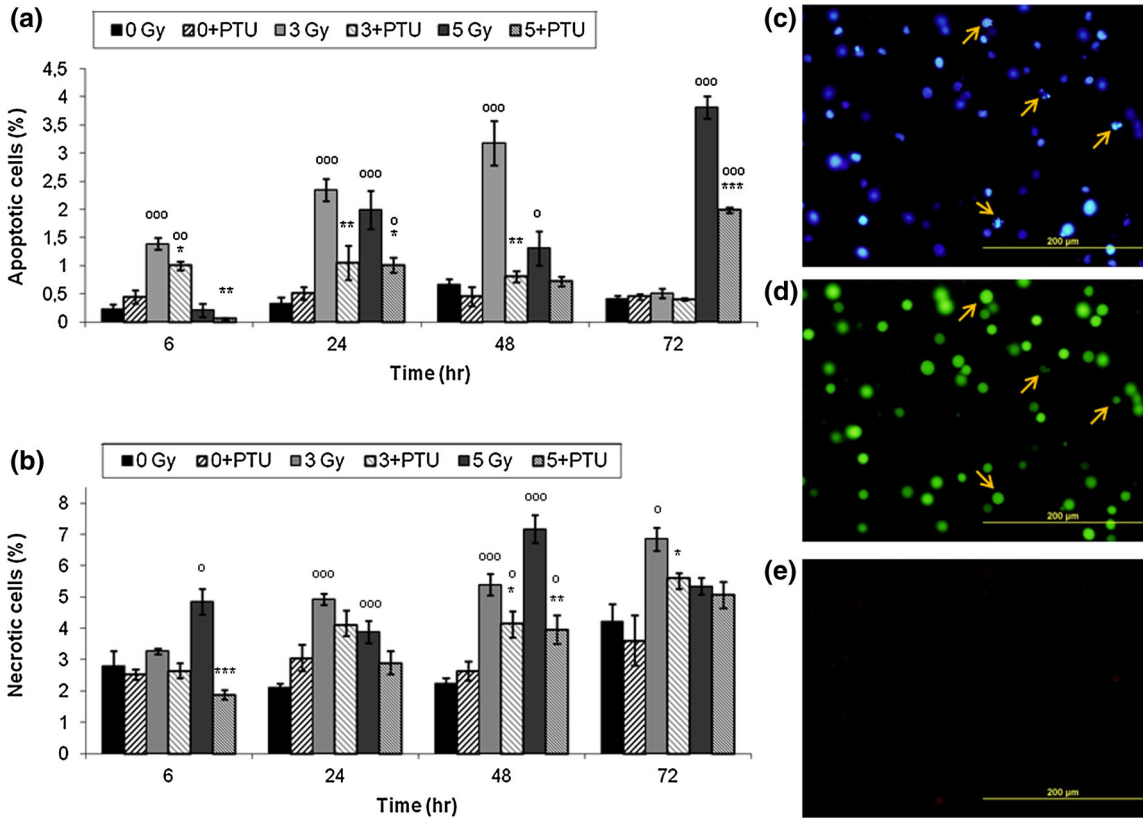

Fig. 4 Effect of PTU on radiation-induced apoptosis. Cell death was assessed at different times by fluorescence staining in cells irradiated with or without PTU. **a** Percentage of apoptotic cells. **b** Percentage of necrotic cells. **c**–**e** Representative images from stained cells 24 h after irradiation. Apoptotic nuclei labelled with Hoechst 33258 (*arrowheads*) exhibited peripheral chromatin clumping, blebbing, and fragmentation (**c**), cytoplasm of living cells was labelled with DAF, (**d**) and necrotic cells were labelled with IP (**e**). Irradiation alone induced an increase in cell death. PTU reduced the percentage of apoptotic and necrotic cells. Error bars indicate the standard error of three independent experiments. $^{\circ}p < 0.05$; $^{\circ\circ}p < 0.01$; $^{\circ\circ\circ}p < 0.001$ versus unirradiated cells. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ versus irradiated without PTU cells

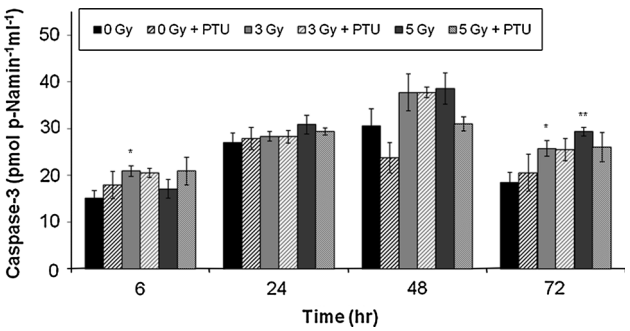

Fig. 5 Effects of PTU on radiation-induced caspase-3 activity. Caspase-3 activity was measured in irradiated cells with or without PTU at different times. No changes in the enzyme's activity were observed in irradiated cells except at longer times. PTU did not modify caspase-3 activity. *Error bars* indicate the standard error of three independent experiments. $^{*}p < 0.05$; $^{**}p < 0.01$ versus unirradiated cells

elevation in intracellular cAMP levels in cells treated with 1 mM PTU with a peak at 24 h. When we incubated cells with agents that raised intracellular cAMP levels prior to irradiation, we observed an increase in the clonogenic survival. Furthermore, pretreatment with the selective inhibitor of cAMP-dependent protein kinase, H-89, abolished the radioprotective effect described earlier. These results suggested that the mechanism for the radioprotection could be associated, in part, with the elevation of intracellular cAMP levels.

Propylthiouracil (PTU) blocks the conversion of L-thyroxine (T_4) to 3,5,3′-triiodo-L-thyronine (T_3). A role of thyroid hormone metabolites in the radiation response could not be ruled out since it has been shown that tetraiodothyroacetic acid (Tetrac) confers radiosensitivity on brain tumor cells (Hercbergs et al. 2011). Tetrac acts in part by blocking the access of T_4 and of T_3 to the plasma membrane thyroid hormone receptor site on αvβ3 integrin (Davis et al. 2006, Cheng et al. 2010). Moreover, photon radiotherapy enhances $\alpha v\beta_3$ integrin, a receptor involved in nongenomic thyroid hormone action (Rieken et al. 2012). However, these receptors transduce the hormone signal by the phosphatidylinositol 3-kinase (PI3K) or ERK1/2

pathways and not by the cAMP second messenger pathway, which may be involved in PTU action.

Ionizing radiation causes damage to living tissues by both direct and indirect mechanisms. The direct action disrupts sensitive molecules while the indirect action occurs when ionizing radiation interacts with water molecules in the cell. This causes the generation of highly reactive ROS such as O_2^-, OH, and H_2O_2. These free radicals can immediately react with any biomolecules in the vicinity and induce highly site-specific oxidative damage. This interaction can disrupt cell function and lead to cell death or neoplastic transformation if not repaired efficiently (Cerutti 1985). Antithyroid drugs like PTU and MMI share a simple chemical structure containing a sulfhydryl group and a thiourea component within a heterocyclic structure like other described radioprotective agents (Nair et al. 2001). PTU has been shown to have antioxidant effects acting as an efficient scavenger of OH radicals, as a powerful inhibitor of lipid peroxidation in model membranes (Hicks et al. 1992) and inhibiting H_2O_2 production in neutrophils (Imamura et al. 1986) or Graves' retroocular fibroblasts (Heufelder et al. 1992). We observed that ROS levels increase 1 h after irradiation in a radiation dose-dependent manner. No statistically significant diminution of ROS levels was found when cells were pretreated with PTU prior to irradiation. We next analyzed radiation-induced disturbances in the antioxidant enzymes activities. Antioxidant systems include low-molecular-weight antioxidants and antioxidant enzymes that contribute to detoxify the reactive oxygen radicals produced by oxygen metabolism processes or after the exposure to ionizing radiation (Matés et al. 1999). We observed an increase in the activity of catalase and SOD enzymes at both studied time points and doses of irradiation, while no such effect was observed in GPx activity. A similar effect in SOD and GPx activities was described in the skin of mice pretreated with the free radical scavenger ascorbic acid prior to irradiation (Chandra et al. 2003). These results suggest that PTU might protect cells from the cytotoxic action of ROS by preventing the decrease in catalase and SOD activities after irradiation.

Exposure to ionizing radiation can induce damage to essential molecules for cell survival. As a consequence, after irradiation, different mechanisms of cell death can be triggered. To further investigate the cellular processes through which PTU protects from ionizing radiation, we studied cell death mechanism. We analyzed morphological nuclei alterations and observed a decrease in the percentage of apoptotic and necrotic cells. This could be associated with the radioprotective effect of the drug against oxidative damage induced by irradiation described previously. Besides, the results could be also in agreement with the partial prevention in the loss of the ability to divide and form recognizable colonies that has been related with growth arrest and cell senescence after irradiation (Roninson et al. 2001).

The present results would indicate a lack of preferential protection of normal cells in comparison with the tumor cells exposed to gamma radiation after the administration of PTU.

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Conflict of interest The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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