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6 Iodo-δ-lactone: A derivative of arachidonic acid with antitumor effects in HT-29 colon cancer cells ☆

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ABSTRACT

Background: IL-δ (5-hydroxy-6 iodo-8,11,14-eicosatrienoic delta lactone) an iodinated arachidonic acid (AA) derivative, is one of the iodolipids biosynthesized by the thyroid. Although IL-δ regulates several thyroid parameters such as cell proliferation and goiter growth it was found that this iodolipid inhibits the growth of other non thyroid cell lines.
Objectives: To study the effect of IL-δ on cell proliferation and apoptosis in the colon cancer cell line HT-29.
Results: Treatment with IL-δ reduced cell viability in a concentration-dependent manner: 1 μM 20%, 5 μM 25%, 10 μM 31%, 50 μM 47% and caused a significant decrease of PCNA expression (25%). IL-δ had pro-apoptotic effects, evidenced by morphological features of programmed cell death such as pyknosis, karyorrhexis, cell shrinkage and cell blebbing observed by fluorescence microscopy, and an increase in caspase-3 activity and in Bax/Bcl-2 ratio (2.5 after 3 h of treatment). Furthermore, IL-δ increased ROS production (30%) and lipid peroxidation levels (19%), suggesting that apoptosis could be a result of increased oxidative stress. A maximum increase in c-fos and c-jun protein expression in response to IL-δ was observed 1 h after initiation of the treatment. IL-δ also induced a tumour growth delay of 70% compared to the control group in NIH nude mice implanted with HT-29 cells.
Conclusion: Our study shows that IL-δ inhibits cell growth and induces apoptosis in the colon cancer cell line, HT-29 and opens the possibility that IL-δ could be a potential useful chemotherapy agent.
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1. Introduction

Iodine excess inhibits thyroid proliferation and thyroid function through the synthesis of an organified compound [1,2]. Several iodinated lipids, biosynthesized by the thyroid, were postulated as intermediaries in the action of iodide. Boeynaems and Hubbard [3] and Dugrillon et al. [4] have reported the conversion of exogenous free arachidonic acid into 5-hydroxy-6 iodo-8,11,14-eicosatrienoic delta lactone (IL-δ) in rat thyroid and isolated porcine thyroid follicles when they were incubated in the presence of iodide. Pereira et al. [5] found 2-iodohexadecanal (2-IHDA) as the major iodolipid in horse thyroid. The formation of these iodolipids requires iodide uptake and its oxidation by a peroxidase.

IL-δ mimics the inhibitory effects of iodine on thyroid cell proliferation [6,7], goiter growth and cyclic AMP accumulation in the rat [8], iodide uptake, hormone synthesis and H_2O_2 production [9], cell membrane transport of glucose and aminoacids [10].

The synthesis of iodolipids is not only restricted to the thyroid, it has also been reported in the mammary gland, which organifies iodide as well. It was shown that I_2 inhibited proliferation and induced apoptosis on different breast cancer cell lines. This effect may be due to the synthesis of intracellular iodolipids [11,12]. Moreover, IL-δ had a 4-fold more potent antiproliferative effect on breast cancer cells than that of I_2 [12].

It was also shown an antiproliferative effect of I_2 and IL-δ in several human cancer cell lines through a mitochondrial mediated apoptosis mechanism [13–15].

Colon cancers often display perturbations in arachidonic acid metabolism, with elevated levels of cyclooxygenase (COX) expression which converts arachidonic acid into prostaglandins and thromboxanes, and lipoxigenase (LOX) expression which converts arachidonic acid into leukotrienes (LTs) and hydroxyeicosatetraenoic acids (HETEs) [16–22]. Whereas these enzymes and their products are associated with cancer cell survival and tumour angiogenesis, arachidonic acid itself is a strong apoptotic signal that may facilitate cancer cell death [23–25].

The aim of the present study was to study the effects of an iodinated AA derivative (IL-δ) on cell growth in the colon cancer

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cell line HT-29 and to study the cellular pathways involved in the antineoplasic effect of IL-δ.

2. Materials and methods

2.1. Cell culture

The HT-29 cell line was cultured in RPMI 1640 supplemented with 10% FBS and penicillin (100 U/ml) and were grown in a water-saturated atmosphere containing 5% CO_2 and 95% air at 37 °C.

2.2. Cell growth assay

Cell proliferation was measured by counting the cells and using the MTT assay. For the first method, cells were seeded in low density into 24-well plates. After 24 h, the cells were incubated with different compounds for 72 h. After this time, the cells were washed, trypsinised and counted with a microscope using trypan blue to assess cell survival. The viability of HT-29 cells was also evaluated using MTT assay. This assay is based on the cleavage of the tetrazolium salt MTT to a dark blue formazan product by mitochondrial dehydrogenase in viable cells. The absorbance of viable cells was measured in a Spectra Microplate Reader with a test wavelength of 570 nm.

2.3. Western blot analysis

Cells were seeded in 60-mm dishes and incubated with different compounds for the time indicated in the text. Proteins were extracted in lysis buffer RIPA (50 mM Tris–HCl pH 7.4, 150 mM NaCl, 1% Nonidet P40, 0.1% SDS, 0.5% deoxycholate), supplemented with PMSF 0.5 mM and protease inhibitor cocktail (Sigma-Aldrich).

Total proteins (30 μg) were electrophoresed on 10% polyacrylamide gels and transferred to nitrocellulose membranes. Membranes were blocked with 5% non fat dry milk in phosphate buffer saline solution (PBS) with 0.2% Tween 20 (Sigma) and 5% BSA for 1 h at RT and then incubated overnight at 4 °C with monoclonal anti c-fos antibodies (dilution 1:200, Calbiochem), and polyclonal anti c-jun (dilution 1:500, Calbiochem), anti PCNA antibodies (dilution 1:500, Santa Cruz Biotechnology), anti Bax (dilution 1:500, Calbiochem, USA) and anti Bcl-2 (dilution 1:500, Calbiochem, USA). Membranes were washed, incubated for 1 h at RT with peroxidase-labeled secondary antirabbit antibody or secondary antimouse antibody (1:3000; Amersham), and visualized with the enhanced chemiluminescence method. Densitometric analysis was performed using the NIH ImageJ analysis Software (1.40 g Wayne Rasband, National Institute of Health, USA) and results were corrected for protein loading by normalization for β-actin expression

2.4. Caspase-3 activity

Caspase-3 activity was determined with the caspase-3 Assay kit, according to the manufacturer's instructions (Sigma CASP-3-C, Sigma-Aldrich, St. Louis, Mo, USA). This assay is based on the spectrophotometric detection of the Ac-DEVD-pNA substrate after cleavage. Cells were harvested in lysis buffer [50 mm HEPES, 5 mM dithiothreitol (DTT), 5 mN CHAPS, 10 μg/ml pepstatin, benzamidine 2.5 mM, aprotinina 10 μg/ml, pepstatin 1 μg/ml, 0,5 mM phenylmethylsulfonylfluoride (PMSF), pH 7.4]. Lysates were clarified by centrifugation at 10,000 × *g* for 5 min, and clear lysates containing 100 μg proteins were incubated with caspase-3 substrate, at 37 °C for 3 h. The concentration of the *p*-nitroaniline (pNA) released from the substrate is calculated from the absorbance values at 405 nm. The activity, expressed as micromoles of *p*-nitroaniline per minute per milliliter, was calculated with a *p*-nitroaniline calibration curve. A positive control of caspase-3 and an inhibitor of caspase-3 (200 m mol/l Inhibitor Acetyl–Asp–Glu–Val–Asp–al [Ac–DEVD–CHO]) were added to the plate.

2.5. Apoptosis assay by nuclear morphology

Apoptosis was assessed using fluorescence staining. Cells were incubated with 2 μl of MIX buffer containing 12 μl of propidium iodide (1 mg/ml), 2 μl of Hoechst 33342 (1 mg/ml) and 30 μl of fluorescein diacetate (1.5 mg/ml) at room temperature for 5 min. Fluorescein diacetate and propidium iodide were used to stain viable and dead cells, respectively. Hoechst 33342 was used to evaluate differences between normal and apoptotic nuclei. Fluorescent microscopy was used to identify the percentage of propidium iodide-impermeable cells having condensed/fragmented nuclei (apoptotic). The percentage of apoptotic cells was determined by counting the number of nuclei showing chromatin condensation and fragmentation characteristic of apoptosis after observing a total of at least 100 cells.

2.6. Peroxide levels determination

Peroxide content was determined with the PeroxiDetect Kit (Sigma-Aldrich, St. Louis, Mo, USA). This kit is based on the fact that peroxides will convert Fe^{2+} ion to Fe^{3+} ion at acid pH. The Fe^{3+} ion forms a coloured adduct with xylenol orange which is determined at 560 nm.

2.7. ROS assay

Intracellular production of ROS was assayed by fluorescence analysis using 2′-7′-dichlorofluorescein diacetate (DCFH–DA) which reacts with intracellular reactive oxygen species. Cells (1.0×10^6) were incubated with 10 μM of DCFH–DA for 20 min at 37 °C, and relative ROS units were determined by fluorescence at $\lambda_{excitation}$: 485/20 nm and $\lambda_{emission}$: 530/25 nm. The results were expressed as arbitrary absorbance units/mg protein.

2.8. In vivo tumorigenicity assay

Each experimental group included 7 female homozygous NIH-nude mice, 20–25 g b.w., 6–8 weeks of age, bred and maintained in laminar air-flow racks. HT-29 cells were harvested and injected subcutaneously into the flanks of the mice. The tumors were allowed to develop during the following 7 days. After the development of palpable tumors (approximately 0.02 cm^3), the mice were treated with IL-δ. The iodolipid was i.p. injected daily at a dose of 15 μg. The size of the tumors was measured with a caliper twice a week, and the volume was calculated according to the following formula: $A^2 \times B/2$ (where A is the width and B is the length). The studies were performed in accordance with International Helsinki Code and the NIH guidelines.

Protein was determined according to Lowry. All reagents were purchased from Sigma Chemical Co, Mo, USA).

2.9. Statistical analysis

All data are presented as mean ± SE. Statistical analysis of the results was made by one way ANOVA followed by Student–Newman–Keuls test. For all statistical analyzes, a probability value of < 0.05 was considered significant.

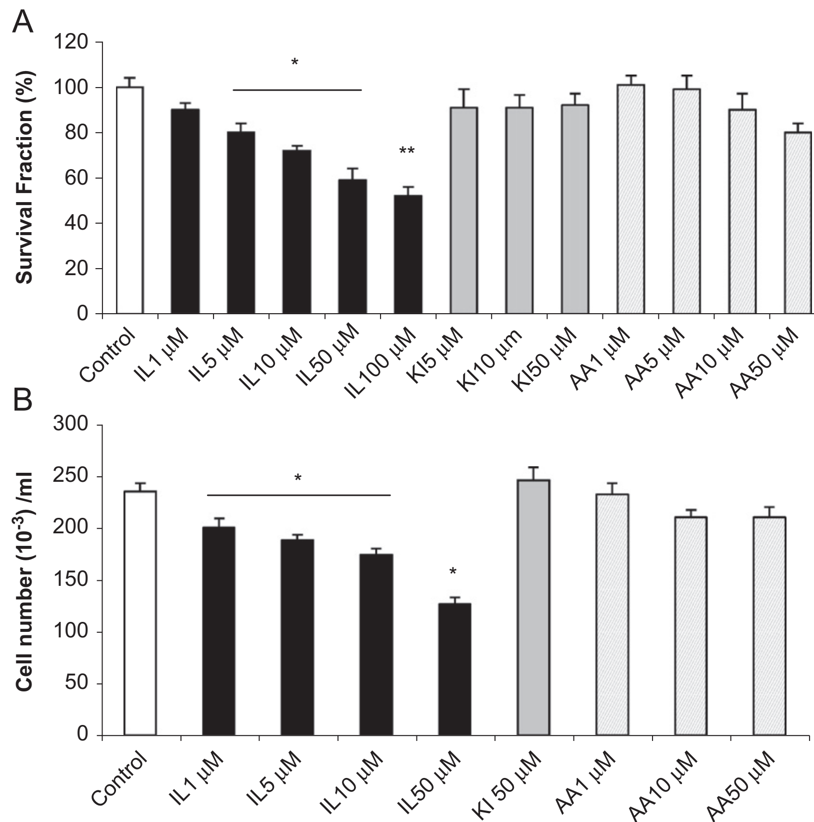

Fig. 1. Effect of IL-δ, arachidonic acid and KI on cell viability. Cells were plated and cultured in 10% FBS-RPMI. The next day, culture medium was removed and cells were incubated for 72 h with 10% FBS-RPMI containing IL-δ, arachidonic acid (AA) or KI at the indicated concentrations. (A) MTT assay after 3 days of treatment, (B) cells were scraped off and counted. Results are means ± SE from four independents experiments by quadriplicate $^{*}p<0.05$ versus control, $^{**}p<0.01$ versus control.

3. Results

3.1. Effect of IL-δ, KI and arachidonic acid on cell proliferation

As shown in Fig. 1A and B, IL-δ caused a significant inhibition of cell proliferation in a concentration dependent-manner. Its precursors, arachidonic acid or KI, did not produce any detectable effect indicating that the effect is independent of the iodide which could be released from its dehalogenation or the arachidonic acid originated during a possible metabolization. The minimum effective dose of IL-δ was 5 μM and cell proliferation was reduced to approximately 20%. For the following studies a concentration of 10 μM of IL-δ was employed. The effect of IL-δ was blocked by the pretreatment of cells with the antioxidant trolox (T) (100 μM) (*C*: 100 ± 2.6%; IL-δ: 68 ± 4.6% ($p<0.01$); *T*: 103 ± 7.8: *T*+IL-δ: 92 ± 11%).

As a marker of cell proliferation, PCNA was examined by Western blots; IL-δ caused a significant decrease of 35% after 3 days of treatment ($p<0.01$) (Fig. 2).

3.2. IL-δ induces apoptosis in HT-29 cells

IL-δ induced apoptosis in HT-29 cells, as evidenced by morphological features of programmed cell death such as pyknosis, karyorrhexis, cell shrinkage and cell blebbing (48 h: 6% and 76 h: 11%) ($p<0.05$) (Fig. 3A and B).

Caspase-3 activity, an indicator of apoptosis, was significantly increased by around 20% and 24% after 48 h and 72 h of treatment with IL-δ (Fig. 4A). The addition of trolox inhibited the effect of IL-δ (Fig. 4B) measured at 72 h.

3.3. Modulation of Bcl-2/Bax expression

No changes of Bcl-2 expression were detected after IL-δ treatment (Fig. 5A). However, IL-δ caused a significant increase

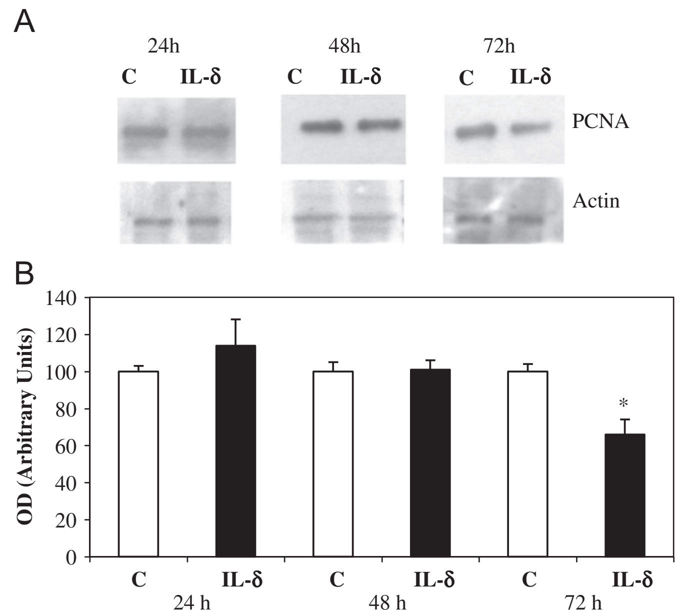

Fig. 2. Western blot of PCNA expression in cultured HT-29 cells. Cells were incubated with 10 μM of IL-δ for 24, 48 and 72 h. (A) Immunochemical detection of PCNA levels using a specific antibody. (B) Quantification of PCNA levels by densitometry scanning of the inmunoblots. Values were normalized with an anti-β-actin antibody. Each value is the average of 4–5 experimental determinations by quadruplicate, $^{*}p<0.05$ versus control.

of Bax level, an important pro-apoptotic protein, after 3 h of treatment. As a result, the ratio of Bax/Bcl-2 increased significantly: 2.5 after 3 h ($p<0.01$) and 1.9 after 6 and 24 h of

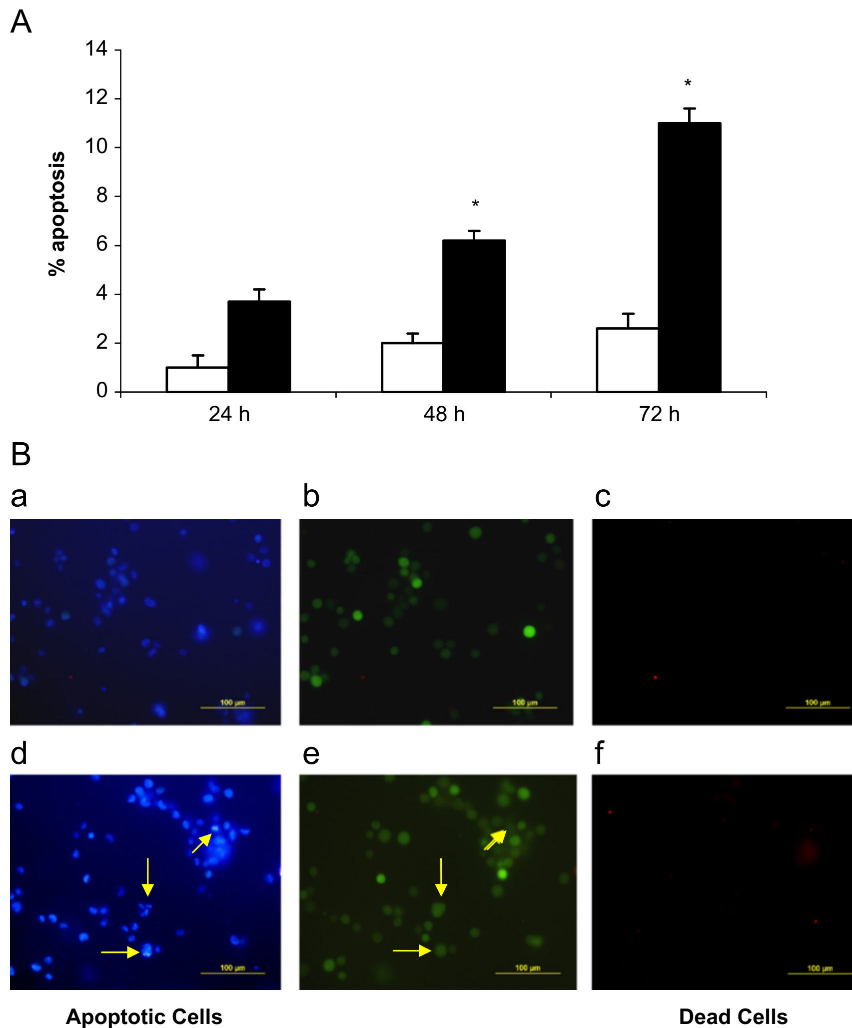

Fig. 3. Effects of IL-δ on Cell Apoptosis. (A) Time curse of IL-δ induced apoptosis. HT-29 cells were cultured for 24 h, and then were incubated with 10 μM of IL-δ for different times. Data are expressed as the number of apoptotic nucleus per 100 cells of three independent experiments ± SE by quadriplicate. $^*p<0.05$ versus control. (B) Representative micrographs of IL-δ induced changes of cell morphology after 72 h of treatment with IL-δ. Cells were staining with Hoechst ((a) and (d)), DAF ((b) and (e)) and PI ((c) and (f)) and observed by fluorescence microscopy. Apoptotic nuclei (arrowheads) exhibited peripheral chromatin clumping, blebbing, and fragmentation (d), cytoplasm of living cells were labelled with DAF ((b) and (e)) and necrotic cells were labelled with IP ((c) and (f)).

treatment ($p<0.05$) (Fig. 5B). These events might be involved in the apoptotic death induced by IL-δ. As it was expected, trolox inhibited the effect of IL-δ on Bax levels and Bax/Bcl-2 ratio (Fig. 5C and D).

3.4. Oxidative status

IL-δ increased the content of organic peroxide levels (19%, $p<0.05$) after 24, 48 and 72 h of IL-δ administration (Fig. 6A). A significant increase in ROS production was detected in a time-dependent fashion after IL-δ administration (Fig. 6B), with a peak reached 0.5 h after stimulation (30%, $p<0.01$). This effect was reversed by the addition of the antioxidant trolox (Fig. 6C).

3.5. c-fos and c-jun expression

As shown in Fig. 7, IL-δ stimulated the expression of c-fos and c-jun proteins in a time-dependent manner. A maximum fold increase in c-fos and c-jun protein expression in response to IL-δ was observed 1 h after initiation of treatment ($p<0.05$). Whereas c-jun response was transient and had almost decreased after 6 h, c-fos responses persisted for at least 24 h.

3.6. IL-δ decreases tumor growth

To determine whether these *in vitro* effects of IL-δ could be recapitulated *in vivo*, we established a xenograft model of HT-29 cells. When tumors reached around 0.02 cm^3, the mice were treated with vehicle or IL-δ for 30 consecutive days. As shown in Fig. 8, the tumors continued to grow in the mice of the control group. In contrast, there was a significant inhibitory effect on tumor growth in animals treated with IL-δ after 12 days (50% of their initial size, $p<0.05$). At the end of the 30-day treatment period, the mice treated with IL-δ showed 71% decrease in the mean estimated tumor volume compared with the control group ($p<0.01$). IL-δ was well tolerated by the animals without substantial adverse effects. Animals' weight remained constant throughout the treatment period (data not shown) and none of the animals required sacrifice before the end of the study.

4. Discussion

The biosynthesis of iodolipids has been observed in the thyroid gland of several species and their participation in thyroid regulation

has been suggested [1,2]. Iodinated derivatives from arachidonic acid, (IL-δ and omega lactone: IL-ω) inhibit several thyroid parameters such as cell proliferation [6,7], Iodide uptake [26], H_2O_2 production [9], goiter growth [8] and inositol-1,4,5-triphosphate (IP3) formation [27]. The occurrence *in vivo* was demonstrated for IL-δ in thyroid tissue from one patient with Graves' disease treated with iodide [7], although the formation of IL-δ in other species could not be detected unless exogenous arachidonic acid (AA) was added [3,4]. Recently, Gärtner et al. [13] showed that IL-δ inhibited the growth of B-CPAP (derived from papillary thyroid carcinoma) but not of FTC-133 (derived from a metastasis of a follicular carcinoma) and 8505C (derived from anaplastic thyroid tissue).

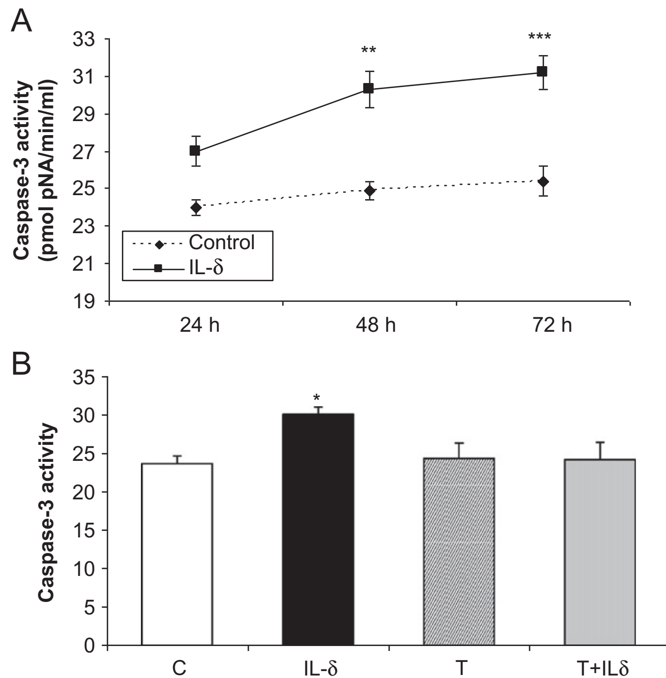

Fig. 4. Caspase-3 activity measure as pmol of pNA released/min/ml. (A) Treatments were carried on during 24 h, 48 h and 72 h. Caspase-3 activity shows a significant difference between the control and the IL-δ groups (**$p<0.01$ for values at 48 h; ***$p<0.01$ for values at 72 h). (B) Cells were treated with IL-δ (10 μM) only or in the presence of the antioxidant trolox (100 μM); Caspase-3 activity was measured at 72 h (**$p<0.01$). Results are means ± SE from four independent experiments by quadruplicate.

The synthesis of iodolipids is not only restricted to the thyroid. In fact, it has been demonstrated that in the mammary gland, which also organifies iodide [28], the synthesis of IL-δ takes place too. Moreover this iodolipid in μM concentrations inhibits proliferation of the breast cancer cell line MCF7 [12]. Rösner et al. [14] have described that IL-δ has antitumor properties in breast cancer, neuroblastoma, glioblastoma, melanoma and lung carcinoma cells, while Aranda et al. [15] showed apoptotic effects of 6 IL-δ in human prostate cells lines, indicating that the action of IL-δ is not restricted to the thyroid cell.

The objective of this study was to study the antiproliferative activity of IL-δ on a colon cancer cell line, HT-29.

It was shown that treatment with IL-δ resulted in loss of cell viability in a concentration-dependent manner, with any detectable effects of its precursors: AA and KI, suggesting a direct action of this compound. This inhibition on cell proliferation was correlated with a significant decrease in cell number and PCNA expression.

An increase in the percentage of apoptotic cells and caspase-3 activity was observed after 48 h of treatment with IL-δ. These results are consistent with previous reports which demonstrated that iodine and IL-δ induce apoptosis in *in vitro* studies [14,15,29,30]. Besides, Thomasz et al. showed in *in vivo* studies a stimulation of apoptosis in rat thyroid gland after 7 days of treatment [31].

Colon cancers often display perturbations in arachidonic acid metabolism with elevated levels of cyclooxygenase or lipoxigenase expression [20–22,32,33]. It was shown that mice expressing

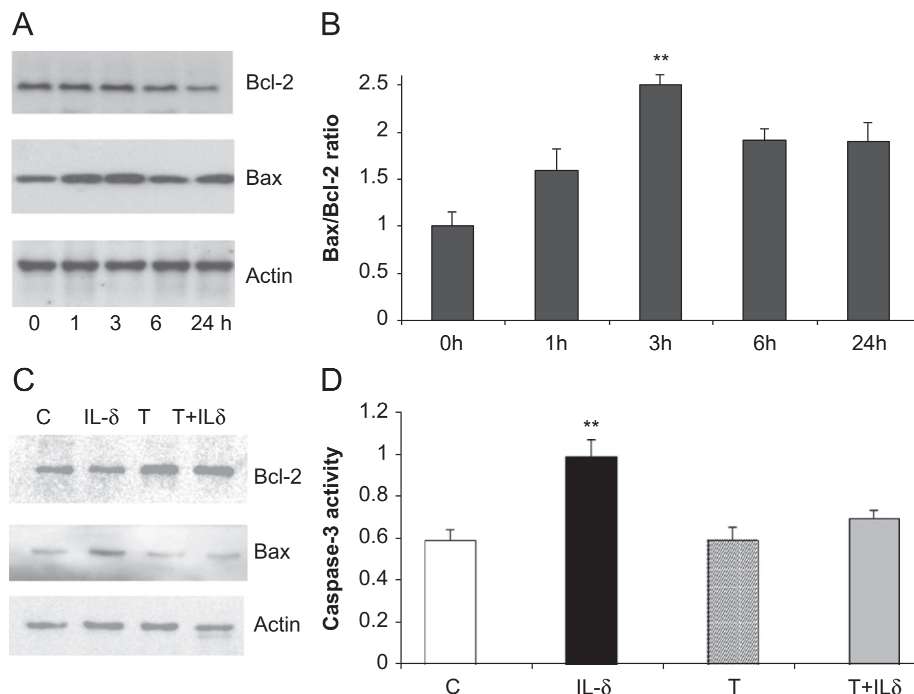

Fig. 5. Western blot analysis of Bax and bcl-2 expression in HT-29 cells. Cells were incubated with 10 μM of IL-δ for different times. (A) Immunochemical detection of Bax, Bcl-2 and actin levels using a specific antibody. (B) Quantification of Bax and Bcl-2 levels by densitometry scanning of the immunoblots, values were normalized and are expressed as Bax/Bcl-2 ratio. (C) and (D) Incubation with trolox (100 μM) inhibited the effect of IL-δ (10 μM) on Bax levels and Bax/Bcl-2 ratio after 3 h. Results are expressed as the mean ± SE from four independent experiments by quadruplicate.

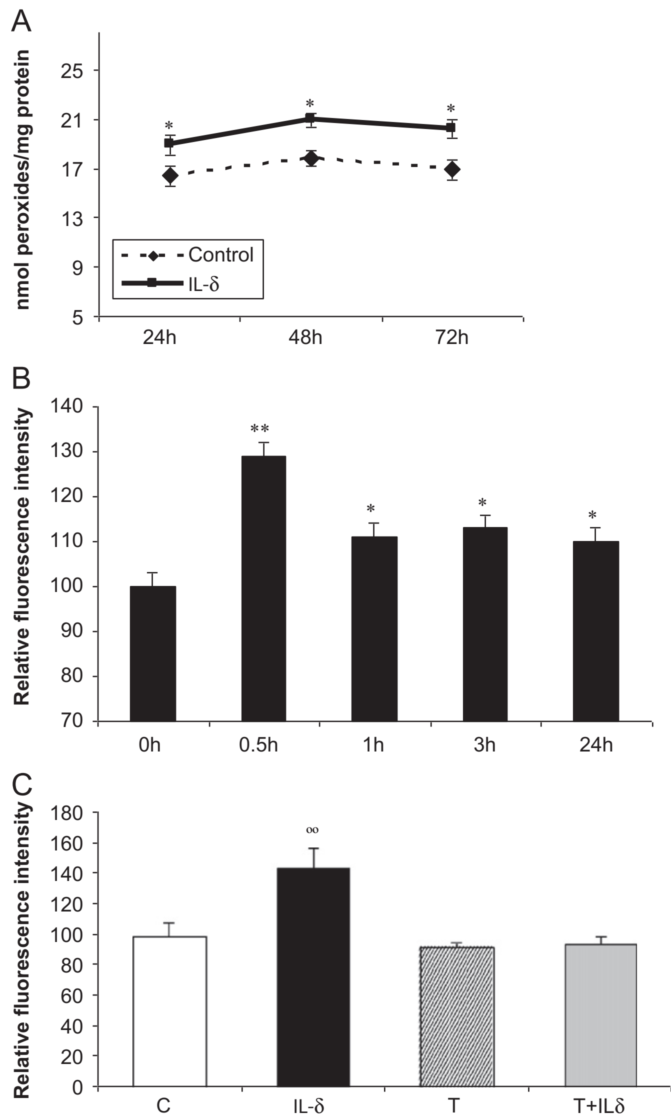

Fig. 6. Effects of IL-δ (10 μM) on induced organic peroxide levels (A) and intracellular ROS generation (B). (C) Cells were treated with IL-δ (10 μM) only or in the presence of the antioxidant trolox (100 μM). Relative fluorescence was measured as described in the method section. Data are expressed as mean ± SE of four independent experiments by quadriplicate. $^{*}p<0.05$, $^{**}p<0.01$ versus control, $^{\circ\circ}p<0.01$ versus control, *T* and *T*+IL-δ.

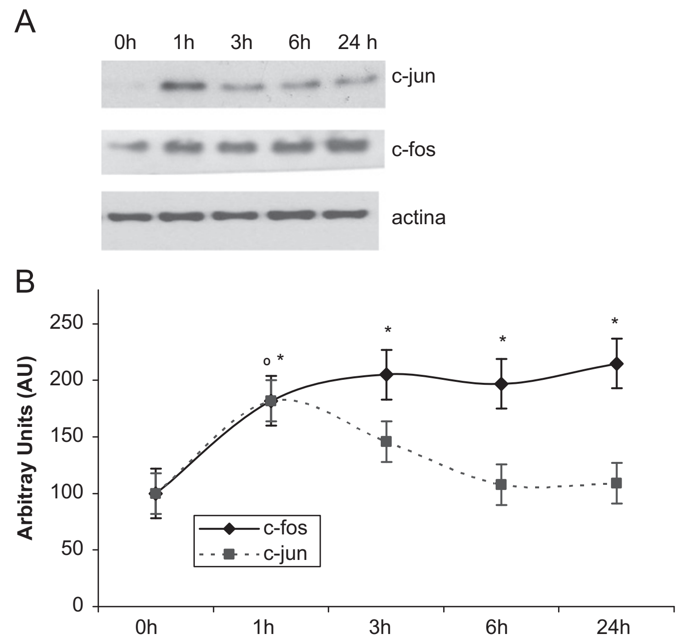

Fig. 7. Western blot of c-jun and c-fos expression in cultured HT-29 cells. Cells were incubated with 10 μM of IL-δ for 0, 1, 3, 6 and 24 h. Results are means ± SE from three independent experiments. (A) Immunochemical detection of c-jun, c-fos and actin levels using a specific antibody and anti-rabbit IgG antibody conjugated with peroxidase. (B) Quantification of c-jun and c-fos levels by densitometry scanning of the immunoblots. Values were normalized with an anti-β-actin antibody. Results are expressed as means ± SE from three independent experiments, $^{\circ}p<0.05$ c-jun versus control; $^{*}p<0.05$ c-fos versus control.

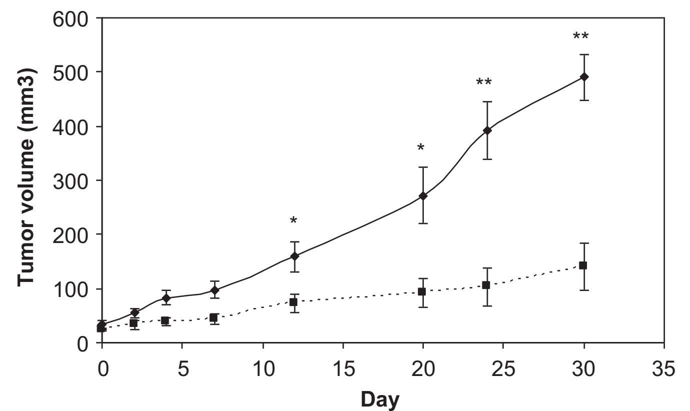

Fig. 8. IL-δ inhibits the growth of HT-29 xenografts in nude mice. HT-29 cells were injected into the right flank of nude mice. After tumor development, the mice (n=7) were treated with IL-δ (15 μg) daily (■) or vehicle (◆). Points: mean tumor size; bars: SE, $^{*}p<0.05$, $^{**}p<0.01$ versus control.

a COX-2 transgene in colon epithelium treated with azoxymethane, a colon carcinogen, had a higher tumor load compared to wild type mice [34]. The regular use of Nonsteroidal anti-inflammatory drugs (NSAIDs), diminish the incidence of colon cancer [35–37]. NSAIDs inhibits cyclooxygenases (COXs) and it was shown described that NSAIDs can induce apoptosis in colon cancer cells but it was suggested that the tumor suppressive effects of NSAIDs are not likely to be related to a reduction in prostaglandins but rather are due to an elevation of the prostaglandin precursor AA [38]. Moreover it was shown that AA induced apoptosis in colon cancer cells [23–25] but the doses used in these studies were between 20 and 30 fold higher than the dose of IL-δ used in this study. The AA induced apoptosis is mediated by ROS generation, although it was demonstrated in non colon cancer cells [39,40].

In this study, trolox a phenolic antioxidant, blocked the increase of ROS production and apoptotic parameters induced by IL-δ, suggesting that oxidative stress may play an important role in IL-δ-mediated apoptosis in HT-29 cells.

The Bcl-2 family proteins (pro-apoptotic and anti-apoptotic proteins) are critical regulator of the apoptotic pathway. The Bcl-2 family of anti-apoptotic proteins (e.g., Bcl-2) and pro-apoptotic proteins (e.g., Bax) are the central regulators of caspase activation and cellular life-and-death switch [41]. Alteration of the ratio of Bcl-2 to Bax is significant in determining whether apoptosis occurs [42]

We investigated the possible involvement of Bax/Bcl-2 in IL-δ action; Western blot analysis showed that Bax was up-regulated after IL-δ treatment and Bcl-2 expression was not modified, thereby an increased in the Bax/Bcl-2 ratio was observed. We conclude, therefore, that IL-δ increases oxidative stress, inhibits cell growth and induces cell death by apoptosis.

Multiple signalling pathways may be involved in oxidant stress-induced apoptosis and AP-1 pathway is a possible candidate. It was shown that increased expression of AP-1 complex is mediated by increased levels of H_2O_2 and ROS [43–45]. It was demonstrated that AA upregulated the expression of *JUN* and *FOS* among other genes involved in apoptotic signalling [46]. We observed that the treatment with IL-δ produced a modulation of AP1 expression. Whereas c-jun increase was transient and had almost decreased after 6 h, c-fos responses persisted for at least 24 h. This effect could be mediated by increased intracellular ROS levels [43–45].

The mechanisms involved in the antiproliferative effect and in apoptosis induction by IL-δ are still not well understood. The receptor activator of peroxisome proliferation (PPAR-γ) could be implicated in the observed effects. Polyunsaturated fatty acids including linoleic acid, eicosanoic and arachidonic acid (precursor of IL-δ) are endogenous ligands of PPAR-γ [47] and regulate cell differentiation, cell cycle and apoptosis [48].

Nuñez Anita et al. [49] and Aceves et al. [50] showed the antiproliferative and pro-apoptotic effect of IL-δ in a breast cancer cell line (MCF-7) and demonstrated that PPAR-γ pathway is involved in these processes. IL-δ is a specific ligand of PPARs with almost 6-fold higher affinity than AA, and activates specifically the PPAR gamma isoform. These data suggest that IL-δ/PPAR gamma could participate in the antiproliferative and proapoptotic effect of IL-δ, but further studies are needed to clarify the molecular mechanisms involved in proliferation and apoptosis regulated by IL-δ.

To further validate the potential therapeutic use of IL-δ, we analyzed the effects of iodolipid treatment on xenograft tumors. In the present study, we observed that IL-δ potently inhibited the *in vivo* growth of HT-29 xenografts resulting in tumor regression. No side effects were caused by the administration of IL-δ as it was demonstrated in previous studies [51].

In summary our results demonstrate that IL-δ inhibits cell proliferation and induced apoptosis cell death in the colon cancer cell line HT-29. The present study suggests that IL-δ could be use as a chemotherapeutic agent for the treatment of colon cancer, alone or in combination with another therapy.

Acknowledgments

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References

[1] M.A. Pisarev, R. Gärtner R, Autoregulatory action of iodine, in: L.E. Braverman, R.D. Uitger (Eds.), Werner & Ingbar's the Thyroid a Fundamental and Clinical Text, 2000, pp. 85–90 8/e.
[2] V. Panneels, G. Juvenal, J.M. Boeynaems, J.E. Dumont, J. Van Sande, Iodide effects on the thyroid: biochemical, physiological, pharmacological and clinical effects of iodide on the thyroid, in: V.R. Preedy, G.N. Burrow, R. Watson (Eds.), Comprehensive Handbook on Iodine: Nutritional, Endocrine and Pathological Aspects, Oxford Academic Press, Oxford, 2009, pp. 303–314.
[3] J.M. Boeynaems, W.C. Hubbard, Transformation of arachidonic acid into an iodolactone by the rat thyroid, J. Biol. Chem. 255 (1980) 9001–9004.
[4] A. Dugrillon, G. Bechtner, W.M. Uedelhoven, P.C. Weber, R. Gartner, Evidence that an iodolactone mediates the inhibitory effect of iodide on thyroid cell proliferation but not on adenosine 3′,5′-monophosphate formation, Endocrinology 127 (1990) 337–343.
[5] A. Pereira, J.C. Braekman, J.E. Dumont, J.M. Boeynaems, Identification of a major iodolipid from the horse thyroid gland as 2-iodohexadecanal, J. Biol. Chem. 265 (1990) 17018–17025.
[6] M.A. Pisarev, L.V. Bocanera, H.A. Chester, D.L. Kleiman de Pisarev, G.J. Juvenal, L.B. Pregliasco, L. Krawiec, Effect of iodoarachidonates on thyroid FRTL-5 cells growth, Horm. Metab. Res. 24 (1992) 558–561.
[7] A. Dugrillon, W.M. Uedelhoven, M.A. Pisarev, G. Bechtner, R. Gärtner, Identification of delta-iodolactone in iodide treated human goiter and its inhibitory effect on proliferation of human thyroid follicles, Horm. Metab. Res 26 (1994) 465–469.
[8] M.A. Pisarev, G.D. Chazenbalk, R.M. Valsecchi, G. Burton, L. Krawiec, E. Monteagudo, G.J. Juvenal, R. Boado, H.A. Chester, Thyroid autoregulation. Inhibition of goiter growth and of cyclic AMP formation in rat thyroid by iodinated derivatives of arachidonic acid, J. Endocrinol. Invest. 11 (1988) 669–674.
[9] L. Krawiec, G.D. Chazenbalk, S.A. Puntarulo, G. Burton, A. Boveris, R.M. Valsecchi, M.A. Pisarev, The inhibition of $PB^{125}I$ formation in calf thyroid caused by 14-iodo-15-hydroxy-eicosatrienoic acid is due to decreased H_2O_2 availability, Horm. Metab. Res. 20 (1988) 86–90.
[10] L. Thomasz, R. Oglio, M.A. Dagrosa, L. Krawiec, M.A. Pisarev, G.J. Juvenal, 6 Iodo-delta-lactone reproduces many but not all the effects of iodide, Mol. Cell. Endocrinol. 323 (2010) 161–166.
[11] A. Shrivastava, M. Tiwari, R.A. Sinha, A. Kumar, A.K. Balapure, V.K. Bajpai, R. Sharma, K. Mitra, A. Tandon, M.M. Godbole, Molecular iodine induces caspase-independent apoptosis in human breast carcinoma cells involving mitochondriamediated pathway, J. Biol. Chem. 281 (2006) 19762–19771.
[12] O. Arroyo-Helguera, B. Anguiano, G. Delgado, C. Aceves, Uptake and antiproliferative effect of molecular iodine in the MCF-7 breast cancer cell line, Endocr. Relat. Cancer 13 (2006) 1147–1158.
[13] R. Gärtner, P. Rank, B. Ander, The role of iodine and δ-iodolactone in growth and apoptosis of malignant thyroid epithelial cells and breast cancer cells, Hormones 9 (2010) 60–66.
[14] H. Rösner, P. Torremante, W. Möller, R. Gärtner, 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 118 (2010) 410–419.
[15] N. Aranda, S. Sosa, G. Delgado, C. Aceves, B. Anguiano, Uptake and antitumoral effects of iodine and 6-iodolactone in differentiated and undifferentiated human prostate cancer cell lines, Prostate 73 (2013) 31–41.
[16] C. Gustafson-Svärd, I. Lilja, O. Hallbook, R. Sjodahl, Cyclooxygenase-1 and cyclooxygenase-2 gene expression in human colorectal adenocarcinomas and in azoxymethane induced colonic tumours in rats, Gut 38 (1996) 79–84.
[17] M. Azumaya, M. Kobayashi, Y. Ajioka, T. Honma, Y. Suzuki, M. Takeuchi, R. Narisawa, Asakura, Size-dependent expression of cyclooxygenase-2 in sporadic colorectal adenomas relative to adenomas in patients with familial adenomatous polyposis, Pathol. Int. 52 (2002) 272–276.
[18] M. Dong, M. Johnson, A. Rezaie, J.N.M. Ilsley, M. Nakanishi, M.M. Sanders, F. Forouhar, J. Levine, D.C. Montrose, C. Giardina, D.W. Rosenberg, Cytoplasmic phospholipase A2 levels correlate with apoptosis in human colon tumorigenesis, Clin. Cancer Res. 11 (2005) 2265–2271.
[19] L.T. Soumaoro, S. Iida, H. Uetake, M. Ishiguro, Y. Takagi, T. Higuchi, M. Yasuno, M. Enomoto, K. Sugihara, Expression of 5-lipoxygenase in human colorectal cancer, World J. Gastroenterol. 12 (2006) 6355–6360.
[20] M.P. Wasilewicz, B. Kołodziej, T. Bojułko, M. Kaczmarczyk, V. Sulżyc-Bielicka, D. Bielicki, K. Ciepiela, Overexpression of 5-lipoxygenase in sporadic colonic adenomas and a possible new aspect of colon carcinogenesis, Int. J. Colorectal Dis. 25 (2010) 1079–1085.
[21] M.P. Wasilewicz, B. Kołodziej, T. Bojułko, M. Kaczmarczyk, V. Sulzyc-Bielicka, D. Bielicki, Expression of cyclooxygenase-2 in colonic polyps, Pol. Arch. Med. Wewn. 120 (2010) 313–320.
[22] A. Alexanian, B. Miller, R.J. Roman, A. Sorokin, 20-HETE-producing enzymes are up-regulated in human cancers, Cancer Genomics Proteomics 9 (2012) 163–169.
[23] T.A. Chan, P.J. Morin, B. Vogelstein, K.W. Kinzler, Mechanisms underlying nonsteroidal antiinflammatory drug-mediated apoptosis, Proc. Nat. Acad. Sci. U.S.A. 95 (1998) 681–686.
[24] Y. Cao, A. Terrece Pearman, G.A. Zimmerman, T.M. McIntyre, S.M. Prescott, Intracellular unesterified arachidonic acid signals apoptosis, Proc. Nat. Acad. Sci. U.S.A. 97 (2000) 11280–11285.
[25] A.M. Monjazeb, K.P. High, C. Koumenis, F.H. Chilton, Inhibitors of arachidonic acid metabolism act synergistically to signal apoptosis in neoplastic cells, Prostaglandins Leukot. Essent. Fatty Acids 73 (2005) 463–474.
[26] G.D. Chazenbalk, R.M. Valsecchi, L. Krawiec, G. Burton, G.J. Juvenal, E. Monteagudo, H.A. Chester, M.A. Pisarev, Thyroid autoregulation. Inhibitory effects of iodinated derivatives of arachidonic acid on iodine metabolism, Prostaglandins 36 (1988) 163–172.
[27] A. Dugrillon, R. Gärtner, Delta-iodolactones decrease epidermal growth factor-induced proliferation and inositol-1,4,5-trisphosphate generation in porcine thyroid follicles—a possible mechanism of growth inhibition by iodide, Eur. J. Endocrinol. 132 (1995) 735–743.
[28] N. Freinkel, S.H. Ingbar, The metabolism of ^{131}I by surviving slices of rat mammary tissue, Endocrinology 58 (1956) 51–56.
[29] R. Langer, C. Burzler, G. Bechtner, R. Gärtner, 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 (2003) 325–329.

[30] O. Arroyo-Helguera, E. Rojas, G. Delgado, C. Aceves, 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 (2008) 1003–1011.
[31] L. Thomasz, R. Oglio, A.S. Randi, M. Fernandez, M.A. Dagrosa, R.L. Cabrini, G.J. Juvenal, M.A. Pisarev, Biochemical changes during goiter induction by methylmercaptoimidazol and inhibition by delta-iodolactone in rat, Thyroid 20 (2010) 1003–1013.
[32] S. Bhattacharya, G. Mathew, D.G. Jayne, S. Pelengaris, M. Khan, 15-lipoxygenase-1 in colorectal cancer: a review, Tumour Biol. 30 (2009) 185–199.
[33] D. Wang D, R.N. Dubois, The role of COX-2 in intestinal inflammation and colorectal cancer, Oncogene 29 (2010) 781–788.
[34] M.A. Al-Salihi, A. Terrece Pearman, T. Doan, E.C. Reichert, D.W. Rosenberg, S.M. Prescott, D.M. Stafforini, M.K. Topham, Transgenic expression of cyclooxygenase-2 in mouse intestine epithelium is insufficient to initiate tumorigenesis but promotes tumor progression, Cancer Lett. 273 (2009) 225–232.
[35] S. Chell, A. Kaidi, A.C. Williams, C. Paraskeva, Mediators of PGE2 synthesis and signalling downstream of COX-2 represent potential targets for the prevention/treatment of colorectal cancer, Biochim. Biophys. Acta 1766 (2006) 104–119.
[36] F.V. Din, E. Theodoratou, S.M. Farrington, A. Tenesa, R.A. Barnetson, R. Cetnarskyj, L. Stark, M.E. Porteous, H. Campbell, M.G. Dunlop, Effect of aspirin and NSAIDs on risk and survival from colorectal cancer, Gut 59 (2010) 1670–1679.
[37] E.H. Ruder, A.O. Laiyemo, B.I. Graubard, A.R. Hollenbeck, A. Schatzkin, A.J. Cross, Non-steroidal anti-inflammatory drugs and colorectal cancer risk in a large, prospective Cohort, Am. J. Gastroenterol. 106 (2011) 1340–1350.
[38] S. Serini, E. Piccioni, N. Merendino, G. Calviello, Dietary polyunsaturated fatty acids as inducers of apoptosis: implications for cancer, Apoptosis 14 (2009) 135–152.
[39] K.C. Chen, L.S. Chang, Arachidonic acid-induced apoptosis of human neuroblastoma SK-N-SH cells is mediated through mitochondrial alteration elicited by ROS and Ca$^{(2+)}$-evoked activation of p38alpha MAPK and JNK1, Toxicology 262 (2009) 199–206.
[40] D. Dymkowska, L. Wojtczak, Arachidonic acid-induced apoptosis in rat hepatoma AS-30D cells is mediated by reactive oxygen species, Acta Biochim. Pol. 56 (2009) 711–715.
[41] S.G. Rolland, B. Conradt, New role of the BCL2 family of proteins in the regulation of mitochondrial dynamics, Curr. Opin. Cell Biol. 22 (2010) 852–858.
[42] S.W. Tait, D.R. Green, Mitochondria and cell death: outer membrane permeabilization and beyond, Nat. Rev. Mol. Cell Biol. 11 (2010) 621–632.
[43] M. Kitamura, Y. Ishikawa, V. Moreno-Manzano, Q. Xu, T. Konta, J. Lucio-Cazana, A. Furusu, K. Nakayama, Intervention by retinoic acid in oxidative stress-induced apoptosis, Nephrol. Dial. Transplant. 17 (Suppl 9) (2002) 84–87.
[44] P. Bécuwe, A. Bianchi, C. Didelot, M. Barberi-Heyob, M. Dauça, Arachidonic acid activates a functional AP-1 and an inactive NF-kappaB complex in human HepG2 hepatoma cells, Free Radical Biol. Med. 35 (2003) 636–647.
[45] J.Y. Zhang, H. Jiang, W. Gao, J. Wu, K. Peng, Y.F. Shi, X.J. Zhang, The JNK/AP1/ATF2 pathway is involved in H_2O_2-induced acetylcholinesterase expression during apoptosis, Cell. Mol. Life Sci. 65 (2008) 1435–1445.
[46] A.M. Monjazeb, K.P. High, A. Connoy, L.S. Hart, C. Koumenis, F.H. Chilton, Arachidonic acid-induced gene expression in colon cancer cells, Carcinogenesis 27 (2006) 1950–1960.
[47] S.A. Kliewer, S.S. Sundseth, S.A. Jones, P.J. Brown, G.B. Wisely, C.S. Koble, P. Devchand, W. Wahli, T.M. Willson, J.M. Lenhard, J.M. Lehmann, Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors alpha and gamma, Proc. Nat. Acad. Sci. U.S.A. 94 (1997) 4318–4323.
[48] E.D. Rosen, B.M. Spiegelman, PPARgamma: a nuclear regulator of metabolism, differentiation, and cell growth, J. Biol. Chem. 276 (2001) 37731–37734.
[49] R.E. Nuñez Anita, A.O. Arroyo-Helguera, M. Cajero-Juárez, L. López-Bojorquez, C. Aceves, Complex between 6-iodolactone and the peroxisome proliferator-activated receptor type gamma may mediate the antineoplasic effect of iodine in mammary cancer, Prostaglandins Other Lipid Mediat. 89 (2009) 34–42.
[50] C. Aceves, P. García-Solís, O. Arroyo-Helguera, L. Vega-Riveroll, G. Delgado, B. Anguiano, Antineoplastic effect of iodine in mammary cancer: participation of 6-iodolactone (6-IL) and peroxisome proliferator-activated receptors (PPAR), Mol. Cancer (2009) 33.
[51] M.A. Pisarev, L. Krawiec, G.J. Juvenal, L.V. Bocanera, L.B. Pregliasco, G. Sartorio, H.A. Chester, Studies on the goiter inhibiting action of iodolactones, Eur. J. Pharmacol. 258 (1994) 33–37.