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Original Article

An organotin indomethacin derivative inhibits cancer cell proliferation and synergizes the antiproliferative effects of lapatinib in breast cancer cells

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Abstract: It is known that an inflammatory condition in different types of cancer provides a sustained microenvironment that favors tumor growth, invasion, and metastasis. Non-steroidal anti-inflammatory drugs such as indomethacin have demonstrated chemo-preventive, anti-proliferative and cytotoxic effects in a variety of tumors. The aim of this study was to investigate the effects of an organotin indomethacin derivative (OID) on the proliferation of breast and prostate cancer cell lines and the possible mechanisms of action of this compound. Different cancer cell lines were treated in the presence of OID and cell proliferation was measured by quantification of the DNA content, changes in the cell cycle profile and the activation of caspase 3 were evaluated by flow cytometry, interleukin 6 (IL-6) gene expression was evaluated by qPCR and protein expression was analyzed by ELISA and Western blot assays. OID inhibited the cell proliferation of a panel of cancer cell lines in a concentration-dependent manner. Moreover, the addition of OID to lapatinib treatment, targeted therapy for breast cancer, significantly enhanced its antiproliferative response. The effects on cell proliferation of these compounds involved, among others, the induction of apoptosis, the downregulation of IL-6 and a decrease of the MAPK activation pathway. Our results suggest that the use of OID alone or in combination with tyrosine kinase inhibitors could be considered as adjuvants in the treatment of cancer.

Keywords: Organotin derivative indomethacin, lapatinib, cancer cell proliferation, apoptosis, IL-6

Introduction

Cancer is a major public health problem worldwide and specifically, those from breast and prostate are among the most prevalent [1-3]. Breast cancer is principally classified into luminal A/B, human epidermal growth factor receptor type 2 (HER2) and triple negative [4]. The approved therapies for these types of cancers include inhibitors of molecular signaling targets such as tamoxifen, fulvestrant, lapatinib, trastuzumab, or chemo and radiotherapy [5-8]. Lapatinib, a tyrosine kinase inhibitor that selectively targets the epidermal growth factor receptor type 1 and 2 (EGFR and HER2), is indicated for patients with metastatic HER2 positive breast cancer. Currently, it is under investigation in combination with others therapeutic agents, particularly in triple negative breast cancer [9, 10]. On the other hand, prostate cancer in early stages respond to androgen deprivation therapy, however depending on the stages and the androgen response, prostatectomy, as well, chemo and radiotherapy are other ther-

apy options [11]. Breast and prostate cancer have remarkable underlying biological similarities, including sustenance of proliferative signaling, like PI3K/AKT and MAPK pathway, activation and dependence of hormonal and growth factor receptors, evasion of growth suppressors signals, deregulation of apoptotic pathways, metastasis and modulation of the immune response, among others [12-14]. In addition, the participation of pro-inflammatory microenvironment as part of the physiopathology of cancer has not been carefully considered for cancer treatment; however, different cytokines that are related with this condition can be good targets for therapy [15]. For instance, proinflammatory cytokines such as interleukin (IL)-6 have been related with cancer progression, poor outcome and relapse in both breast and prostate cancer [16, 17], suggesting that this biological immune-mediator could be also considered as target for cancer treatment. In line with that, the use of nonsteroidal anti-inflammatory drugs has been associated with reduced risk of some types of human tumors, including breast, lung, colon, head, and neck [18-20]. Several studies have shown that organotin compounds derived from indomethacin, a non-steroidal anti-inflammatory drug, have shown antineoplastic effects against different human and murine cancer cells [21-23]. Apoptosis induction and modulation of adhesion proteins are involved in the inhibitory effects of these compounds [24]. In addition, these compounds have the ability to potentiate the effects of several anti-cancer drugs [25, 26]; however, their effect on MAPK activation or the modulation of inflammatory cytokines such as IL-6 has not been thoroughly evaluated.

Camacho-Camacho, C. et al; previously reported the synthesis and cytotoxic activity of the organotin indomethacin derivative (tri(n-butyl) tin (IV) 2-[1-(4-chlorbenzoyl)-5-methoxy-2-methylindo-3-yl] acetate) in cervical and lung cancer cell lines [27]. However, no biological interactions involved in the mechanism of action of this compound were evaluated. In this study, we evaluated the anti-proliferative effects and the mechanism of action of this organotin indomethacin derivative (OID) in different proteins related with proliferation, apoptosis and chronic inflammation on cancer cell lines, including their effects when combined with lapatinib, a tyrosine kinase inhibitor.

Materials and methods

Reagents

Cell culture media were obtained from Life Technologies (Grand Island, NY, USA). Fetal bovine serum (FBS) was purchased from Hyclone Laboratories Inc (Logan, UT, USA). OID was synthesized at Departamento de Sistemas Biológicos [27]. Universidad Autónoma Metropolitana-Xochimilco, Ciudad de México, México. RNase A solution was from Promega (Madison, WI, USA). Lapatinib was acquired from Sequoia Research Products (United Kingdom). Propidium iodide was purchased from Sigma (St. Louis, MO, USA). Trizol and all oligonucleotides for real time polymerase chain reaction (qPCR) were obtained from Invitrogen (Carlsbad, CA, USA). IL-6 secretion in culture media was measured by ELISA.

Cell culture

SUM-229PE cell line was acquired from Asterand (San Francisco, CA), SK-BR-3, HCC1937, HeLa, DU-145 and PC-3 cell lines were obtained from ATCC (Manassas, VA. USA). The cells were seeded in their specific media following instructions from the supplier. The media were supplemented with 5% heat-inactivated-FBS, 100 units/mL penicillin plus 100 µg/mL streptomycin and maintained at 37°C with a 5% atmosphere of CO_2 and 95% humidity.

Proliferation and drug combination treatment

The cells were seeded in 96-well culture plates at a density of 1000-1500 cells per well depending on each line, then, the cells were incubated in the absence (0.1% v/v dimethyl sulfoxide) or presence of increasing concentrations of OID during 5 days. Subsequently, the DNA concentration was quantified using CyQUANT kit (Invitrogen) as described above [28]. The values of the inhibitory concentrations (IC) were calculated by non-linear regression sigmoidal fitting with a dose-response curve by means of a scientific graphing software (OriginLab Corporation, Northampton, MA, version 8.0). Experiments were performed in sextuplicate on at least 3 different occasions. Combination of OID (IC_{50} value) with the IC_{50} value of lapatinib (3.5×10^{-7} M, [29]) was performed. Pharmacological effect of combination studies was calculated with the combination index (CI) multi-drug

equation of Chou Talalay [30]. For this analysis, the parameters are as follows: IC<1 synergistic effect, IC>1 antagonistic effect and CI=1 additive effect.

Cell cycle distribution

SUM-229PE and DU-145 cells were incubated with the drug alone or in combination for 72 h. After treatment, the cells were collected and washed with phosphate buffer (PBS) pH 7.2, fixed in 70% v/v ethanol and stored at -20°C. For cell cycle analysis, the samples were washed and incubated in RNAse (10 µg/ml), 0.1% v/v triton X-100 and propidium iodide (1 µg/ml) solution in the dark at room temperature during 20 min. The DNA content was determined using a FACsCanto II flow cytometer (Becton Dickinson, San Jose CA, USA). For cell cycle analysis and detection of SubG0 peak, a total of 35,000 events from PI-area vs. PI-wide gate were acquired. The results were analyzed using Flow-Jo software (Tree Star Inc version 9.3.2.).

Detection of active form of caspase 3

SUM-229PE and DU-145 cells were incubated with the drugs independently or in combination for 48 h. Positive cells for the cleaved caspase 3 were labeled with a commercial apoptosis kit (BD Pahrmingen, CA, USA). Cells were collected, washed and resuspended in the BD Cytofix/Cytoperm buffer and incubated for 20 min at 4°C. The cell suspension was centrifuged and washed with the BD Perm/Wash buffer. Subsequently, the cells were incubated with the fluorescein isothiocyanate (FITC) anti-active caspase 3 antibody for 30 min. Cell pellets were washed, and resuspended with BD Perm/Wash buffer and analyzed with the FACsCanto II flow cytometer. A total of 20,000 events were acquired. Then, the percentage of active caspase 3 positive cells was obtained from FSC-A vs. FITC active caspase 3 pseudo-color plot.

Reverse transcriptase-qPCR amplifications

For IL-6 gene expression analysis, breast and prostate cancer cells were incubated in the presence of different drugs alone or in combination during 24 h. RNA was extracted with TRIzol reagent and then subjected to reverse transcription. Primers for qPCR amplification were designed with the Universal Probe Library Assay Design Center from Roche. Real-time PCR was carried out using the LightCycler® 480 II from Roche (Roche Diagnostics, Mannheim, Germany), according to the following protocol: activation of Taq DNA polymerase and DNA denaturation at 95°C for 10 min, proceeded by 45 amplification cycles consisting of 10 s at 95°C, 30 s at 60°C, and 1 s at 72°C. The following oligonucleotides were used: IL-6-L, 50-GATGAGTACAAAAGTCCTGATCCA-30; IL-6-R, CTGCAGCCACTGGTTCTGT; The gene expression of the housekeeping gene β-actin-L, CCAACCGCGAGAAGATGA; β-actin-R, CCAGAGGCGTACAGGGATAG, was used as an internal control. Data obtained from qPCR were analyzed using the ΔΔCt method.

Measurement of IL-6 secretion in cell cultures

Secretion of IL-6 was measured in the culture media using specific enzyme-linked immunosorbent assay (ELISA) following manufacturer's instructions. Briefly, 96-well plates were coated with capture antibody washed and blocked (1% bovine serum albumin). Standards and samples were incubated for 2 h at room temperature. Afterwards, plates were washed and coated with a detection antibody. Developing was carried out with streptavidin-HRP and further substrate addition. Optical density was determined using a microplate reader (Multiskan MS photometer type 352, Labsystems, Helsinki, Finland) set to 450 nm. Final IL-6 concentrations were calculated based on a standard curve using recombinant IL-6 standards. The detection limits of the assays were 15.6-1000 pg/mL.

Western blot analyses

ERK protein phosphorylation was analyzed in the SUM-229PE and DU-145 cells treated with vehicle, OID alone or in combination with lapatinib. After 48 h of treatment, the cells were lysed with a buffer containing HEPES 50 mM pH 7.4, NaCl 250 mM, EDTA 5 mM, Nonidet P-40 0.1%, NaF 10 mM, β-glycerophosphate 50 mM, Na_3VO_4 1 mM and complete EDTA-free protease inhibitor cocktail (Sigma, St. Louis, MO, USA). Total protein concentration was evaluated using the Bradford assay (BIO-RAD, Hercules, CA, USA) according to the manufacturer's instructions. For each sample, 25 µg of total protein were separated in 8% SDS gels and transferred to polyvinylidene difluoride

(PVDF) membranes previously activated with methanol. Then, membranes were blocked with 5% skim milk in Tris-Buffered Saline Tween-20 (TBST) for 1 h and washed with TBST. The following monoclonal antibodies: anti-pERK, total-ERK (1:1000 Cell Signaling, Boston, Massachusetts) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:1000 Santa Cruz Biotechnology Inc.) were used. The membranes were incubated in the presence of secondary antibody conjugated with horseradish peroxidase (Santa Cruz Biotechnology) respective for 2 h at room temperature. The detection of the bands was done by chemiluminescence using the ECL Plus (Amersham Pharmacia, UK). Densitometry was done using Image J software (NIH, USA). The normalization of the values of each treatment was performed with respect to the total protein of ERK.

Statistical analyses

The results were expressed as the mean ± standard deviation (S.D.). The statistical significance of the data was determined by one-way ANOVA followed by the Holm-Sidak method, using a specialized software package (SigmaStat, Jandel Scientific). A p value <0.05 was considered as significant.

Results

The organotin indomethacin derivative (OID) alone or in combination with lapatinib inhibited cancer cell proliferation

The effect of OID on the proliferation of the different cancer cell lines was evaluated using a DNA quantification assay. As shown in **Figure 1**, OID inhibited in a dose dependent manner the proliferation of breast (SUM-229PE, SK-BR-3 and HCC1937), prostate (DU-145 and PC-3) and cervical (HeLa) cancer cells (**Figure 1**). The IC_{50} values obtained with OID treatment are given in **Table 1**. Broadly, the majority of cell lines showed similar IC_{50} values, and the following sensitivity gradient was observed: HCC1937>HeLa>SK-BR-3>SUM-229PE>DU-145>PC-3. These results indicate that the antiproliferative effectivity of the OID is independent of the type of cancer. Additionally, we also tested the effects of this compound in mononuclear cells, as a control of toxicity in a non-tumor cell model. In these cells, the inhibitory effect of OID was only at high concentrations (1×10^{-6} and 1×10^{-5} M), indicating that the inhibition of growth was essentially nontoxic (**Figure 1D**). In addition, indomethacin, the parental compound, was also evaluated in SUM-229PE and DU-145 cell lines, showing a slightly inhibitory effect on cell proliferation only in DU-145 cells, without effect in SUM-229PE cells (data not shown). These results indicate that the modifications performed on the chemical structure of parental compound improved OID´s antiproliferative properties.

In order to test if OID could improve the antiproliferative effect of lapatinib, a tyrosine kinase inhibitor actually used in breast cancer treatment [5]. We chose SUM-229PE cells due to their sensitivity to lapatinib and aggressive phenotype [29]. The cells were incubated in the presence of lapatinib alone or in combination with OID. As shown, lapatinib alone significantly inhibited cell growth, however, a higher inhibitory effect of lapatinib was observed when cells were coincubated in the presence of OID (**Figure 2A**). The pharmacological effect of combined treatment obtained showed a synergistic nature according to combination index (CI) multi-drug equation of Chou Talalay [30].

OID induced changes in cell cycle profile and apoptosis

To get an insight into the antiproliferative mechanisms of OID, we analyzed the cell cycle distribution in breast and prostate cancer cells. The SUM-229PE cells treated with OID significantly increased the percentage of cells in subG1 phase and diminished S phase compared to non-treated cells (**Figure 2B**). Similar cell cycle distribution was observed with lapatinib. Moreover, the tyrosine kinase inhibitor significantly decreased G2/M phase. Similar results were obtained in cells with the combined treatment (**Figure 2B**).

In prostate cancer cells, the OID treatment resulted in a significant reduction of G1, S and G2/M phases with an increase in the percentage of cells in SubG1 phase. Only in the PC-3 cell line the S phase percentage was not modified (**Figure 2C** and **2D**).

We next investigated if the increment of DNA fragmentation, corresponding to the increase in subG1 peak, correlates with apoptosis induction. **Figure 3A** and **3C** in the gate is shown the presence of active caspase 3, in the absence or presence of the compounds in cancer cells.

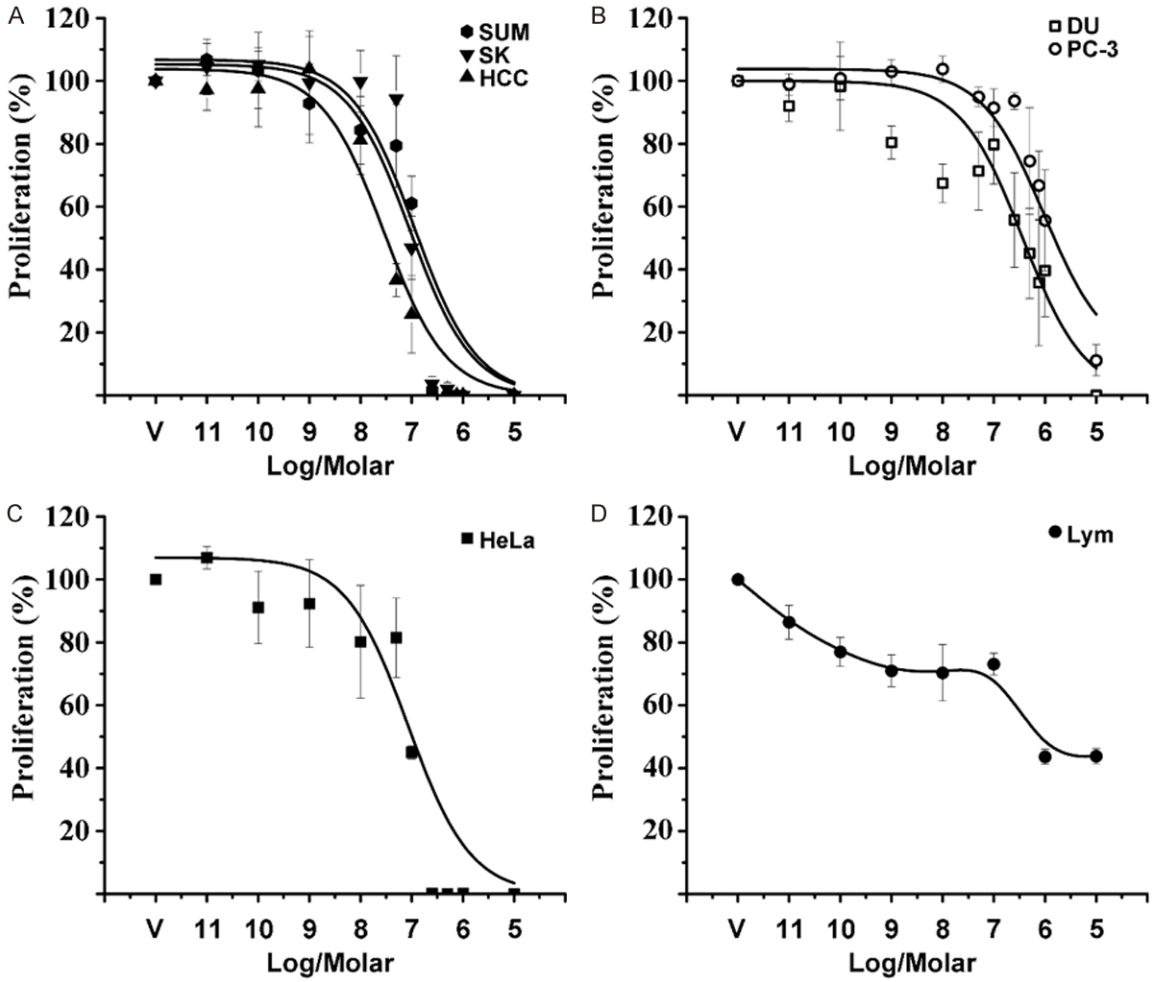

Figure 1. Antiproliferative effect of OID in cancer cells. (A) Breast (SUM-229PE (SUM), SK-BR3 (SK), HCC1937 (HCC)), (B) prostate (DU-145 (DU), PC-3), (C) cervical (HeLa) cancer cells, and (D) lymphocytes (lym) stimulated with PHA (phytohemagglutinin) were incubated in the presence of different OID concentrations during 5 days. Cell proliferation was evaluated by quantification of DNA. Results are shown as the mean ± S.D. of sextuplicate determinations of three independent experiments. Data from vehicle-treated cells (V) were normalized to 100%.

Table 1. Inhibitory concentration (IC) 50 values of organotin indomethacin derivative on cancer cells proliferation

Cell Line	IC_{50} value [Log Molar]
SUM-229PE	1.3×10^{-7}
SK-BR-3	9.2×10^{-8}
HCC1937	2.8×10^{-8}
DU-145	3.6×10^{-7}
PC-3	9.5×10^{-7}
HeLa	8.5×10^{-8}

The OID treatment significantly induced the active form of caspase 3 in both SUM-229PE and DU-145 cells (**Figure 3B** and **3D**), suggesting that the compound induces apoptosis in both tumor cell types. Additionally, OID or lapatinib significantly increased the activation of this pro-apoptotic protein in breast cancer cells (**Figure 3B**). Furthermore, caspase 3 active was increased in a greater extent in co-treated cells than in cells treated with the compounds alone indicating that OID enhanced the apoptotic activity of lapatinib.

OID diminished MAPK phosphorylation

Considering that OID inhibited proliferation and induced cell death, we evaluated its effects on the phosphorylation status of MAPK pathway. SUM-229PE and DU-145 cell lines were cultured in the absence or presence of compounds alone or in a combined fashion and Western blot analyses were performed. The results demonstrated that OID alone did not decrease

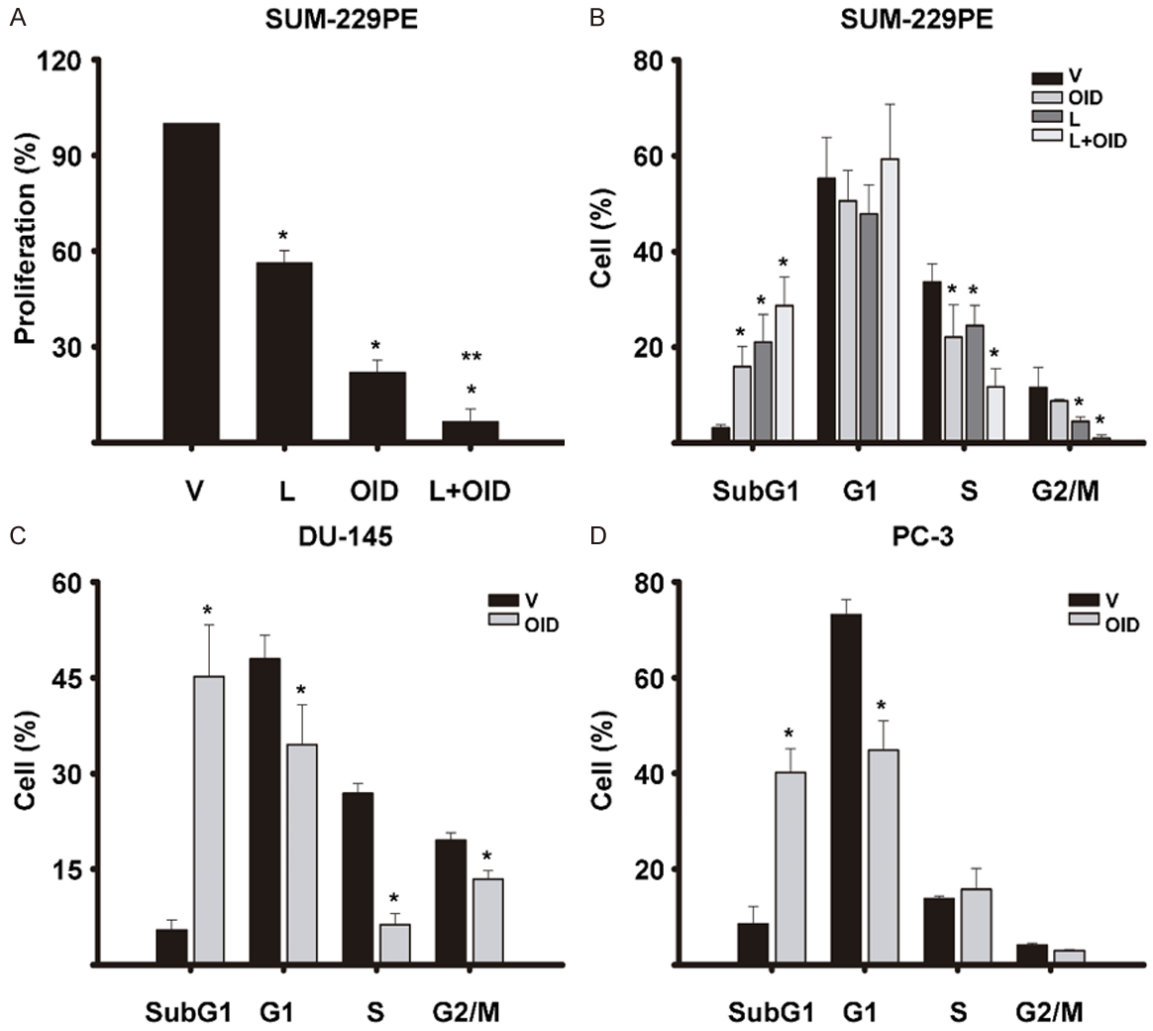

Figure 2. OID improved lapatinib antiproliferative effect in breast cancer cells and altered cell cycle profile in breast and prostate cancer cells. (A and B) Breast cancer cells were treated in the absence (V) or presence of OID, lapatinib (L) or their combination during 72 hrs. Also, (C and D) prostate cancer cells were incubated with OID or its vehicle. After that, cell proliferation and cell cycle profile were evaluated. Bars represent the mean ± S.D. of three independent experiments per triplicate. *$P \leq 0.05$ *vs*. vehicle, **$P \leq 0.05$ *vs*. each compound alone.

ERK phosphorylation in breast cancer cells (**Figure 4A**); however, with the combined treatment a significant inhibition of ERK phosphorylation was observed as compared with non-treated cells (**Figure 4B**). In contrast, in prostate cells ERK phosphorylation was significantly diminished by the OID treatment (**Figure 4B** and **4C**).

OID inhibited the expression of IL-6, a pro-inflammatory regulator

Since inflammatory microenvironment has a critical role in tumor progression and metastasis of breast and prostate cancer, we decided to examine in the cancer cells the expression of a key inflammatory cytokine, IL-6. The cell lines were incubated in the presence of compounds alone or in combination and qPCR amplifications or ELISA analyses were performed. As shown, OID inhibited significantly IL-6 gene expression in breast (**Figure 5A**) and prostate (**Figure 5B**) cancer cells. In addition, treatment with OID did not modify IL-6 levels on breast cancer cells (**Figure 5C**), however, it decreased significantly levels of this cytokine compared with non-treated prostate cancer cells (**Figure 5D**). Interestingly, OID in combination with lapatinib further decreased gene expression and levels of IL-6 than that obtained with either of the compounds alone in breast cancer cells (**Figure 5A** and **5C**).

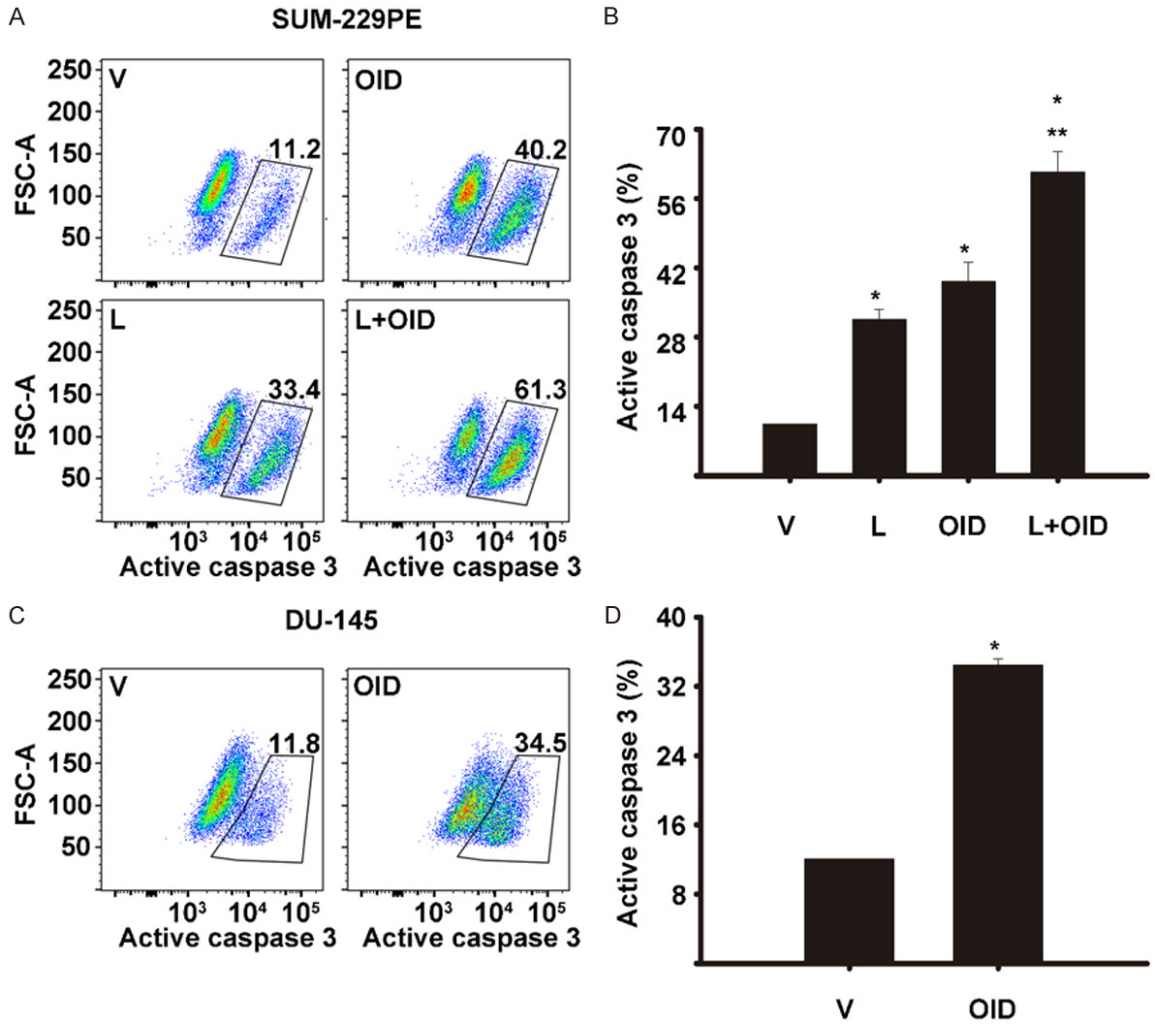

Figure 3. OID induced apoptosis in cancer cells. (A) breast and (C) prostate cancer cells were incubated in the absence (V) or presence of OID alone or in combination with lapatinib (L) during 48 h and active caspase 3-positive cells analysis was performed. The cells positive to caspase 3 are shown in the gate. Quantification of percentage of cells positive to caspase 3 of (B) breast and (D) prostate cancer cells obtained of three experiments independently. *$P \leq 0.05$ *vs*. V, **$P \leq 0.05$ *vs*. each compound alone.

Discussion

As breast and prostate cancer depend on the activation of hormone-driven intracellular signaling pathways, as well as on the establishment of a chronic inflammatory microenvironment [17, 31], the development of strategies targeting in these conditions is nowadays a key issue in cancer treatment research. Accordingly, we studied the effects of an indomethacin derivative on breast and prostate cancer cell proliferation. In addition, proteins involved in cell proliferation, death and inflammatory processes were studied in order to offer a mechanistically explanation for the action of this compound. Our results indicated that tri (n-butyl) tin (IV) 2-[1-(4-chlorbenzoyl)-5-methoxy-2-methylindo-3-yl] acetate, (OID), was effective for inhibiting cancer cell proliferation in contrast to the parental compound, indomethacin. These results indicate that the modifications performed on the chemical structure of the parental compound confer it anti-proliferative properties. Moreover, the fact that OID did not inhibit the growth, at the IC_{50} values observed for cancer cells, of normal lymphocytes cells, allowed us to rule out a potential toxic effect on cell growth. Indeed, the IC_{50} values of OID were lower than those reported for other indomethacin derivatives representing an advantage in clinical treatment [32].

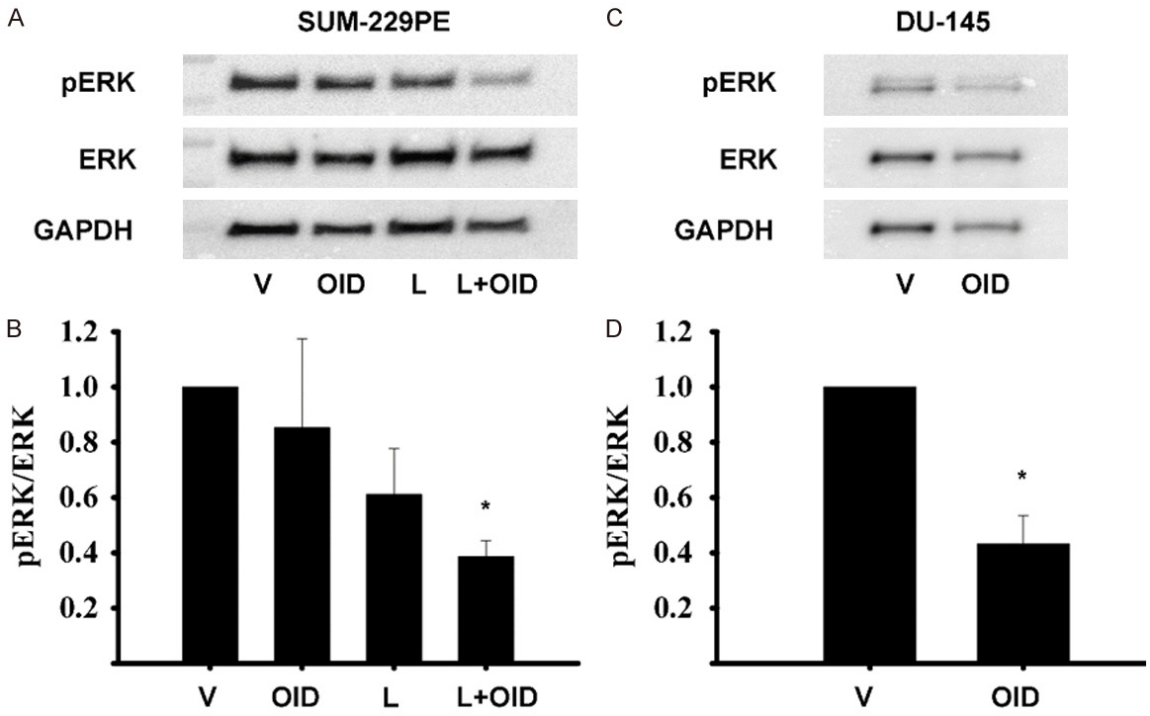

Figure 4. OID alone or in combination with lapatinib treatment inhibited the MAPK signaling pathway in cancer cells. (A) Breast or (C) prostate cancer cells were incubated in the absence (V) or presence of OID alone or with lapatinib (L) during 48 h. The phosphorylated form of ERK1/2 (Thr202/Tyr204) protein was determined by Western blot. Glyceraldehyde 3-phosphate dehydrogenase protein (GAPDH) was used as the loading control. Representative image from three independent experiments is shown. Normalization was performed against to total form of ERK protein in (B) SUM-229PE and (D) DU-145 cancer cells. Densitometry of three independent experiments is shown. *$P \leq 0.05$ *vs*. V.

Interestingly, OID induced changes in cell cycle profile and cell fragments, in a remarkable way, in both breast and prostate cancer cells. This fact is interesting since indomethacin or another of its derivatives only inhibit cancer cell proliferation and induce cell cycle arrest [32-34]. Unlike these derivatives, with structural modifications based on phenolic rings addition, OID has a metal group on its chemical structure that could be responsible for cell death effects observed in our results. In this study, OID induced apoptosis in breast and prostate cells through the activation of caspase 3. Similarly, it has been reported that indomethacin induces apoptosis in colon, renal and esophageal cancer cells [25, 35, 36] with scarce information in breast and prostate cancer, which suggest that OID share some of the indomethacin-related inhibitory and apoptotic pathways. Of note, OID study in both apoptotic and side effects context should be accomplished on in vivo studies.

Previous studies have shown that indomethacin inhibits the proliferation of gastric cancer cells by blocking the MAPK signaling pathway [37], therefore, we evaluated the OID effects on ERK1/2 activation [38]. In this study, the observation that OID alone or in lapatinib combination significantly diminished MAPK phosphorylation, suggested a potential new promising cell strategy for OID-like effects on cell growth, as well as to offer the bases for the development of new compounds with antiproliferative activity. In this regard, OID also inhibited the expression of IL-6, a pro-inflammatory cytokine; an effect that was more evident in prostate compared with breast cancer cells. This is relevant since this cytokine is associated with cancer promotion and a worse prognosis [17]. Interestingly, indomethacin inhibits IL-6 expression in a tumor mice model where inhibition of this cytokine results in the induction of apoptosis in xenografts prostate tumors [29, 39]. In addition, the expression of it in breast cancer is importantly associated with drug resistance, stem/progenitor cells formation and metastasis induction [40-42]. Supporting the fact that the blockage of this cytokine by OID could be beneficial in breast and prostate cancer patients.

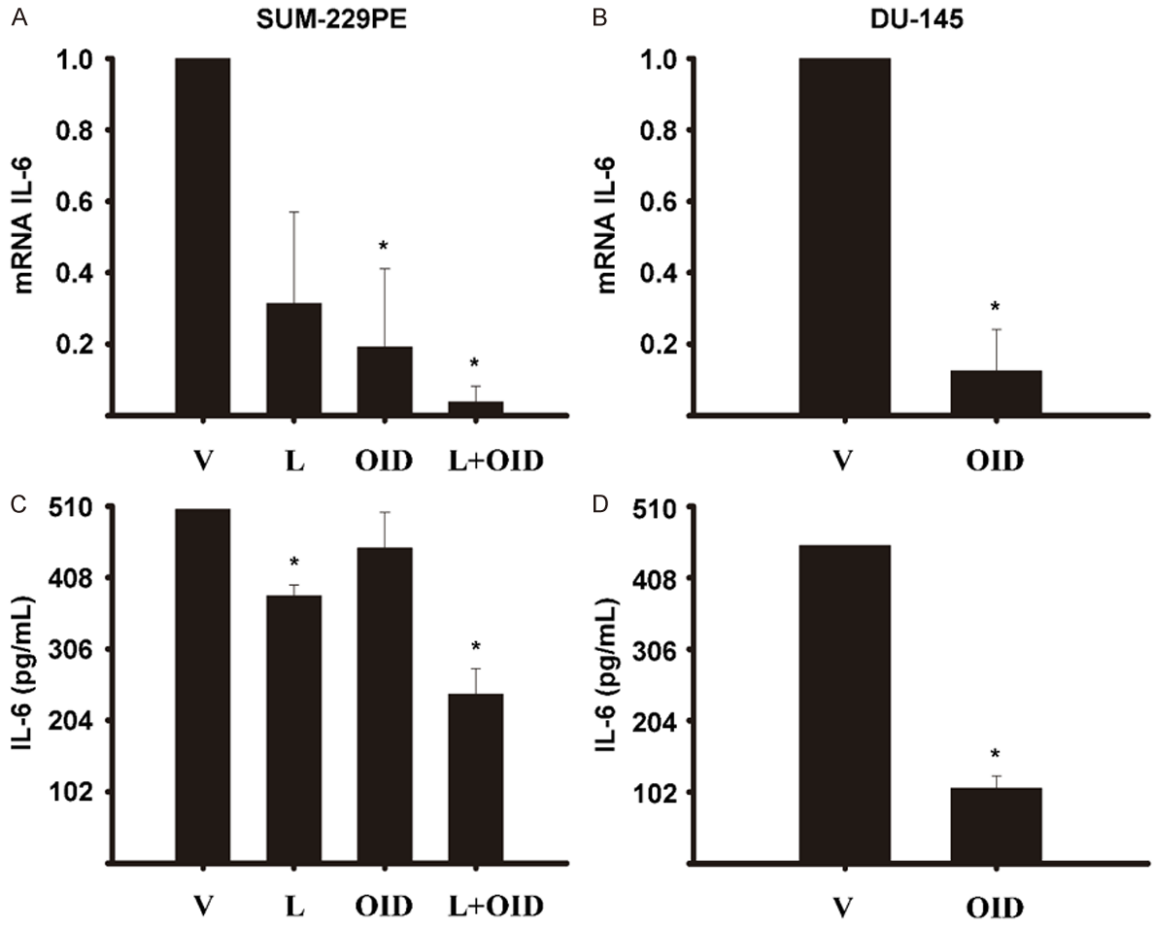

Figure 5. OID inhibited the expression of pro-inflammatory cytokines. Breast (SUM-229PE) and prostate (DU-145) cancer cells were incubated in the absence (V) or presence of OID alone or with lapatinib (L) during 24 or 48 h. Relative gene expression of IL-6 (A and B) were determined by RT-PCR. Glyceraldehyde 3-phosphate dehydrogenase gene (GAPDH) was considered as constitutive reference. For IL-6 (C and D) protein expression, the supernatants of cell cultures treated were quantified by ELISA. *$P \leq 0.05$ *vs*. V.

The combined therapy schemes have been successfully used for improving cancer responses to therapy. For instance, the combination of lapatinib with other drugs such as capecitabine has proved to be of significant benefit in breast cancer patients [26]. We previously reported in breast cancer that the addition of calcitriol, a hormone with important antineoplastic activity, to lapatinib treatment showed synergistic interactions resulting in a significant inhibition in cell proliferation and induction of apoptosis activation [29]. In this study, the combination of lapatinib and OID resulted also in a major significant inhibitory effect on cell proliferation and cell death induced by lapatinib, including down regulation of IL-6. In addition, MAPK phosphorylation was markedly decreased by the combination in breast cancer cells. Supporting this fact, a recent study reported that the combination of indomethacin with tyrosine kinase inhibitors of c-Kit and PDGFRα receptors resulted a higher inhibitory effect compared with the drugs alone on bladder cancer cells. This effect was mediated by the inhibition of AKT pathway and cyclooxygenase 1 expression [43].

Interestingly, some reports mention that IL-6 production was increased in acquired lapatinib-resistant breast cancer cells and its blockade contributes to overcoming lapatinib resistance [44, 45]. Of note, OID in combination with lapatinib inhibited the IL-6 gene expression and secretion in breast cancer cells, suggesting that this compound could counteract the resistance of this tyrosine kinase inhibitor in breast cancer cells. Altogether, the data described above support the use of OID in adjuvant thera-

py breast and prostate cancer or in combination schemes with lapatinib in breast cancer, however, *in vivo* studies are needed in order to confirm this fact.

Conclusion

The OID treatment inhibited significantly cell proliferation and activated apoptosis by regulating MAPK, activating caspase 3 and the cytokine immune mediator, IL-6, in breast and prostate cancer cells. Besides, the addition of lapatinib to OID treatment enhanced these molecular and cellular events in breast cancer cells.

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Disclosure of conflict of interest

None.

Abbreviations

PI3K/AKT, Phosphatidylinositol 3-kinase and protein kinase B pathway; EGFR, Epidermal growth factor receptor; HER2, Epidermal growth factor receptor type II; IL-6, Interleukin 6; MAP, Mitogen activated phosphor kinase signaling; OID, Organotin indomethacin derivative.

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