NPC Natural Product Communications

2016
Vol. 11
No. 12
1871 - 1875

# Cytotoxic Activity of Microalgal-derived Oxylipins against Human Cancer Cell lines and their Impact on ATP Levels

**Javier Ávila-Román[a], Elena Talero[a], Carolina de los Reyes[b], Eva Zubía[b], Virginia Motilva[a] and Sofía García-Mauriño[c,*]**

[a]*Department of Pharmacology*, *University of Seville*, *Seville*, *Spain*
[b]*Departament of Organic Chemistry*, *University of Cádiz*, *Puerto Real*, *Spain*
[c]*Department of Plant Biology and Ecology*, *University of Seville*, *Seville*, *Spain*

*sgarma@us.es*

**Received: June 8th, 2016; Accepted: October 19th, 2016**

Oxylipins are metabolites derived from lipid peroxidation. The plant oxylipin methyl jasmonate (MJ) shows cytotoxic activity against cancer cell lines of various origins, with ATP-depletion being one of the mechanisms responsible for this effect. The cytotoxic activity of oxylipins (OXLs) isolated from the microalgae *Chlamydomonas debaryana* (13-HOTE) and *Nannochloropsis gaditana* (15-HEPE) was higher against UACC-62 (melanoma) than towards HT-29 (colon adenocarcinoma) cells. OXLs lowered the ATP levels of HT-29 and UACC-62 cells, but the effect was higher on the second cell line, which had higher basal ATP. This result proves a link between the cytotoxicity and the capability of these compounds to deplete ATP. In addition, the combination of 13-HOTE with the anticancer drug 5-fluorouracil (5-FU) induced a synergistic toxicity against HT-29 cells. These results highlight the therapeutic potential of oxylipins derived from microalgae.

**Keywords:** *Chlamydomonas debaryana*, *Nannochloropsis gaditana*, Oxylipin, Methyl jasmonate, ATP, Cancer.

Polyunsaturated fatty acids (PUFAs) and their derivatives play important roles as signaling molecules. Metabolites produced by the oxidative transformation of PUFAs are collectively named oxylipins (OXLs). The initial formation of hydroperoxides may occur either by chemical oxidation or by the action of enzymes such as lipoxygenase and α-dioxygenase [1]. Subsequent transformations of these hydroperoxides give rise to a great variety of oxylipin classes, which have many different biological functions and can act as inter-kingdom signaling molecules [2].

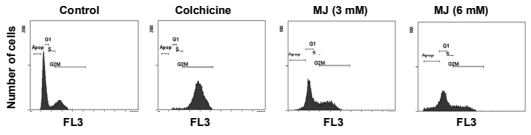
Microalgae are a valuable source of fatty acids of the ω3 and ω6 families. PUFAs, such as α-linolenic acid (ALA, C18:3n-3), arachidonic acid (ARA, C20:4n-6), eicosapentaenoic acid (EPA, C20:5n-3), and docosahexanoic acid (DHA, C22:6n-3), are known to be beneficial in the prevention of neurodegenerative, age-related disorders, and cancer [3,4]. We have recently described the isolation, chemical characterization, and *in vitro* anti-inflammatory activity of several oxylipins isolated from cultures of the freshwater microalga *Chlamydomonas debaryana* and of the seawater species *Nannochloropsis gaditana* [5]. The oxylipins were hydroxy acids derived from C16, C18, and C20 PUFAs. In addition, the oxylipin-containing biomass of *C. debaryana* and its major oxylipin constituent (9Z,11E,13S,15Z)-13-hydroxyoctadeca-9,11,15-trienoic acid (13-HOTE) showed a preventive effect in acute trinitrobenzenesulfonic acid (TNBS)-induced colitis in rats [6].

Plant oxylipins are mostly produced from linoleic acid (LA, 18:2n-6) and α-linolenic acid (ALA, 18:3n-3). Oxylipins formed in flowering plants include the plant hormones 12-oxo phytodienoic acid (OPDA) and jasmonic acid (JA) [7]. Upon developmental or environmental stimuli, jasmonates are synthesized and play key roles in plant development and defense responses in multiple biotic and abiotic stresses [8]. From the biomedical point of view, JA, methyl jasmonate (MJ) and some of their synthetic derivatives, have been shown to exhibit anti-cancer activity, *in vivo* and *in vitro*, against cancer cells of various histological origins [9-12]. Further, MJ has recently attracted attention because of its specific pro-apoptotic properties in a wide range of malignancies [13,14].

This study was aimed to investigate the cytotoxic activity of microalgal-derived OXLs, taking MJ as a model. Although plant jasmonates had shown cytotoxicity against various types of cancer cells [9-15], no data concerning the effects of MJ on HT-29 human colon carcinoma cells were available. Thus, the first objective of this study was to evaluate the cytotoxicity of MJ against HT-29; the melanoma skin cancer (UACC-62) cells were used for comparison, because MJ has anti-cancer effects against experimental melanoma [16]. The anticancer effects of MJ are related to its capacity to deplete adenosine 5'-triphosphate (ATP) in several experimental models [17-19]. Herein, the relevance of this mechanism of action was investigated in the two cancer cell lines. In a second step, a similar study was performed with the OXLs isolated from the microalgae. In addition, a possible synergism with 5-fluorouracil (5-FU), which is one of the most common chemotherapeutic agents against colon cancer, was evaluated.

The effect of MJ on cell cycle in human colon cancer HT-29 cells was analyzed by flow cytometry (Figure 1). Colchicine, which inhibits microtubule polymerization [25], induced cell cycle arrest at M, thus decreasing cell number in $G_0/G_1$ and increasing it at $G_2/M$. MJ caused a decrease in cell number at $G_0/G_1$ and an increase in S and $G_2/M$, which is consistent with cell cycle arrest at $G_2/M$. Interestingly, a concentration-dependent accumulation of cells in apoptotic sub-$G_1$ phase was also observed, with a significantly increased cell number from 0.98 ± 0.18 to 8.56 ± 0.51 at 3 mM MJ, and to 20.33 ± 0.43 at 6 mM MJ.

The cytotoxic effect of MJ on human colorectal adenocarcinoma (HT-29) and melanoma skin cancer (UACC-62) cells was evaluated after 24 h of treatment using the SRB assay (Figure 2). MJ




| % | Control | Colchicine (0.5 µM) | MJ (3 mM) | MJ (6 mM) |
|---|---|---|---|---|
| Apoptotic sub-$G_1$ | 1.0 ± 0.2 | 1.1 ± 0.1 | 8.6 ± 0.5*** | 20.3 ± 0.4*** |
| $G_0/G_1$ | 64.2 ± 1.5 | 4.6 ± 1.6*** | 41.3 ± 0.1 | 37.1 ± 0.2 |
| S | 7.6 ± 0.5 | 3.5 ± 0.6 | 13.0 ± 0.2** | 12.2 ± 0.1** |
| $G_2$/M | 27.0 ± 0.8 | 91.1 ± 0.8*** | 37.0 ± 0.4** | 30.4 ± 0.3 |

**Figure 1:** Effect of MJ on cell cycle distribution of HT-29 cells. Cells were treated with 3 and 6 mM MJ for 24 h. Colchicine (0.5 µM) was used as positive control. Data are means ± SE of three experiments. ***P*<0.01, ****P*<0.001 compared with the solvent control in each respective cell cycle phase.

concentration-dependently reduced the viability of HT-29 and UACC62 cells. The $IC_{50}$ values were, respectively, 5.69 ± 0.17 mM and 1.41 ± 0.01 mM. The results showed that MJ had the highest cytotoxic activity towards UACC-62 cells.

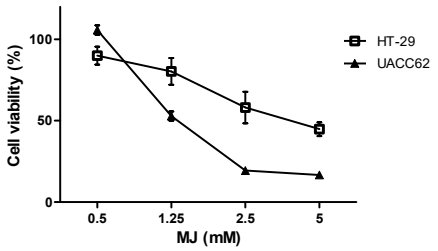



**Figure 2:** Cytotoxic effect of MJ on HT-29 and UACC-62 cells. Cell viability was evaluated by the SRB assay after 24 h of treatment. Data are means ± SE of three experiments.

The major OXL constituent of *Chlamydomonas debaryana* biomass (13-HOTE) and 15-HEPE from *Nannochloropsis gaditana* were assayed on HT-29 and UACC-62 cells (Table 1). These two OXLs displayed higher cytotoxicity against UACC-62 cells than against HT-29 cells, both at 48 h and at 72 h of treatment. The $IC_{50}$ values at 72 h were 71.9 ± 3.6 µM for 13-HOTE and 53.9 ± 6.4 µM for 15-HEPE in UACC-62 cells, and above 100 µM in HT-29 cells.

Table 1 shows the cytotoxic activity of MJ and OXLs measured with the SRB, MTT reduction, and LDH leakage assays. Comparable results were obtained with the three assays. In all cases, the cytotoxicity of both MJ and OXLs was higher against UACC-62 cells than against HT-29 cells. Taken together, these results show the cytotoxic activity of MJ in the mM range, the same as that reported in other studies [19, 26], and of OXLs in the µM range, the same at which biological activity for these compounds had been reported [5].

**Table 1:** Cytotoxic effect of OXLs on HT-29 and UACC-62 cells.

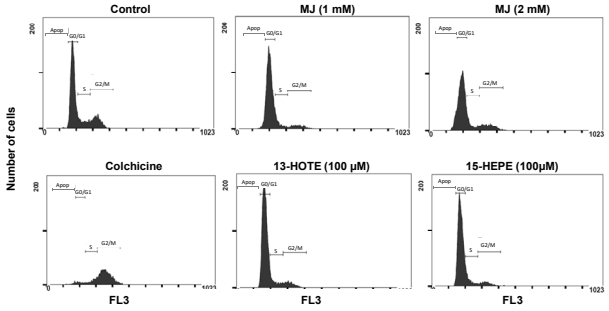
| Compounds | Cell line | SRB ($IC_{50}$) | | MTT ($IC_{50}$) | | LDH ($IC_{50}$) |
|---|---|---|---|---|---|---|
| | | 48 h | 72 h | 48 h | 72 h | 48 h |
| **MJ** (mM) | HT-29 | 2.98 ± 0.25 | 0.52 ± 0.09 | 1.95 ± 0.16 | 0.78 ± 0.20 | 1.86 ± 0.06 |
| | UACC-62 | 1.38 ± 0.11 | 0.43 ± 0.15 | 0.84 ± 0.12 | 0.62 ± 0.02 | 1.45 ± 0.11 |
| **13-HOTE** (µM) | HT-29 | >100 | >100 | >100 | >100 | >100 |
| | UACC-62 | >100 | 71.9 ± 3.6 | >100 | 68.2 ± 0.2 | 99.0 ± 25.1 |
| **15-HEPE** (µM) | HT-29 | >100 | >100 | >100 | >100 | >100 |
| | UACC-62 | 72.3 ± 3.5 | 53.9 ± 6.4 | >100 | 78.8 ± 4.6 | 93.0 ± 14.0 |

[a]Cell viability was evaluated by the SRB, MTT and LDH assays after 48 and 72 h of treatment. [b]Data are means ± SE of three experiments.

The effect of MJ and OXLs on cell cycle in UACC-62 cells was analyzed by flow cytometry (Figure 3). Colchicine was used as control; it induced cell cycle arrest at $G_2$/M, and likewise in HT-29 cells. MJ caused a concentration-dependent accumulation of cells in the apoptotic sub-$G_1$ phase. On the other hand, both 13-HOTE and 15-HEPE at µM concentration induced about 10% cell cycle arrest in $G_0/G_1$, increased the number of apoptotic cells, and decreased cell number in the S and $G_2$/M phases.

MJ has been reported to target the mitochondria and cause ATP depletion [18]. In addition, the combination of MJ with the glycolysis inhibitor 2-deoxyglucose (2-DG) was accounted to result in synergistic effects [26]. 2-DG was used expecting a cooperative effect of the two agents (MJ and 2-DG) in short-time treatments that would cause assessable ATP depletion without killing the cells.

The effect of MJ on cellular ATP level was evaluated in HT-29 and UACC-62 cells with and without addition of 2-DG (Figure 4). A 30 min treatment with increasing concentrations of MJ caused a dose-dependent decrease of ATP levels in HT-29 (Figure 4A) and UACC-62 cells (Figure 4B), which were further lowered by MJ in combination with 2-DG. The effect was of statistical significance for 3 and 4 mM MJ in both cell lines. It is worth noting that ATP levels were higher in control UACC62 cells (4.10 ± 0.30 µM) relative to control HT-29 cells (0.70 ± 0.03 µM). For this reason, the effect of MJ, 2-DG, and the combination of both compounds, had a higher impact on UACC-62 cells. For example, 4 mM MJ combined with 5 mM 2-DG decreased ATP level by 19-fold in UACC-62 and by 10-fold in HT-29.




| % | Control | Colchicine (0.5 µM) | MJ (1 mM) | MJ (2 mM) | 13-HOTE (100 µM) | 15-HEPE (100 µM) |
|---|---|---|---|---|---|---|
| Apoptotic sub-$G_1$ | 1.8 0.5 | 8.9 0.9* | 6.3 0.1 | 9.3 0.2* | 3.3 2.4 | 2.8 0.7 |
| $G_0/G_1$ | 63.1 0.2 | 8.4 0.1** | 73.8 1.0 | 58.9 1.5 | 78.3 1.4 | 72.8 3.6 |
| S | 14.9 0.1 | 14.9 2.5 | 10.8 0.1* | 12.6 0.2 | 9.5 0.7 | 12.5 0.4 |
| $G_2$/M | 20.1 0.8 | 67.8 1.6* | 9.1 0.4** | 18.7 1.6 | 9.0 0.4* | 12.0 2.6 |

**Figure 3:** Effect of MJ, 13-HOTE and 15-HEPE on cell cycle distribution of UACC-62 cells. Cells were treated with 1 and 2 mM MJ, 100 µM 13-HOTE and 100 µM 15-HEPE for 24 h. Colchicine (0.5 µM) was used as positive control. Data are means ± SE of three experiments. **P*<0.05, ***P*<0.01 compared with the solvent control in each respective cell cycle phase.

OXLs treatment did not induce significant changes in ATP levels in HT-29 cells (Figure 5). On the contrary, they significantly lowered ATP levels of UACC-62 cells in a dose-dependent manner, with 13-HOTE and 15-HEPE among the most active. These effects were magnified when OXLs were combined with 2-DG.

Jasmonates are known to display cooperative cytotoxicity with several anticancer drugs [26]. The cytotoxic activity of MJ (Figure 6A, 6B), 13-HOTE (Figure 6C) and 15-HEPE (Figure 6D) in combination with the anticancer drug 5-FU was tested in HT-29 cells at 48 h (MJ) and 72 h (OXLs). The combination index (CI)-isobologram equation allows quantitative determination of drug interactions, where CI <1, =1, and >1 indicate synergism, additive effect, and antagonism, respectively. The figures on the left show % of cell viability estimated with the SRB assay, and figures on the right show the fraction-affected (Fa)/CI plots constructed by computer analysis with the CompuSyn software.

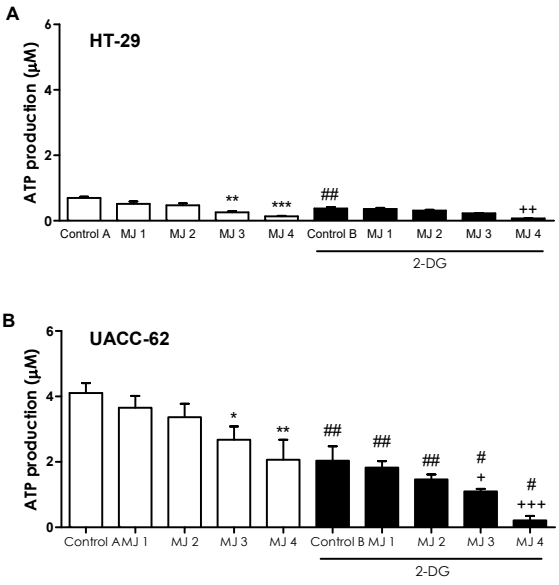



**Figure 4:** Effect of MJ and 2-DG on ATP level. (**A**) HT-29 cells. (**B**) UACC-62 cells. Cells were treated when indicated with 5 mM 2-DG for 3 h, and/or increasing concentrations of MJ (1, 2, 3 and 4 mM) for 30 min. Data are means ± SE of six experiments. **P*<0.05, ***P*<0.01, ****P*<0.001 *vs* solvent control (control A) for the treatments without 2-DG. $^{+}P$<0.05, $^{++}P$<0.01, $^{+++}P$<0.001 *vs* control with 2-DG (control B) for the treatments with 2-DG. $^{\#}P$<0.05, $^{\#\#}P$<0.01 for treatments with 2-DG and MJ compared with the corresponding treatment with MJ alone.

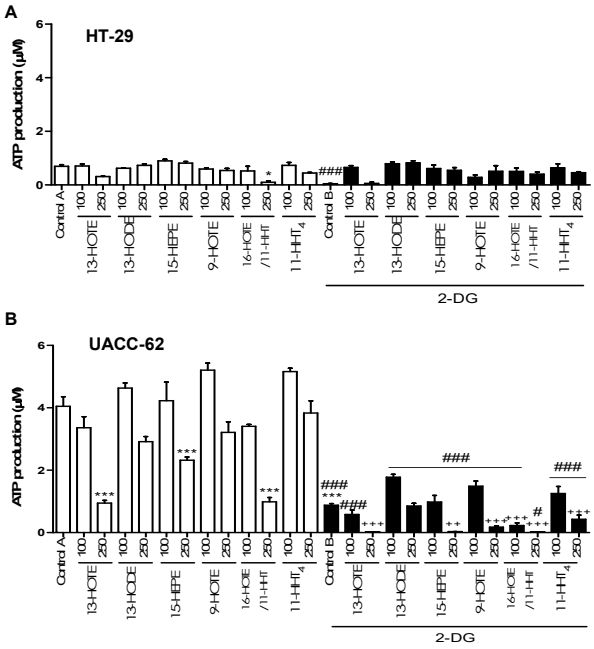



**Figure 5:** Effect of OXLs and 2-DG on ATP level. (**A**) HT-29 cells. (**B**) UACC-62 cells. Cells were treated when indicated with 5 mM 2-DG for 3 h, and/or 100 and 250 µM OXLs for 30 min. Data are means ± SE of four experiments. **P*<0.05, ****P*<0.001 *vs* solvent control (control A) for the treatments without 2-DG. $^{++}P$<0.01, $^{+++}P$<0.001 *vs* control with 2-DG (control B) for the treatments with 2-DG. $^{\#}P$<0.05, $^{\#\#\#}P$<0.001 for treatments with 2-DG and OXLs compared with the corresponding treatment with OXLs alone.

Plant jasmonates, such as JA, MJ, and several synthetic derivatives, have been shown to display anti-cancer activity in both *in vitro* and *in vivo* models [9-15]. MJ has been demonstrated to reduce the ATP intracellular level in a range of cancer cell lines, preceding apoptotic cell death induction [18]. These observations are consistent with the results herein described, which show that MJ induced both apoptosis and ATP-depletion in HT-29 cells. The combination of MJ with the glycolysis inhibitor 2-DG, which has been reported to enhance the anticancer effects of MJ [26-27], potentiated the effect of MJ on ATP level. In addition, it could be observed that the higher cytotoxic activity of MJ against UACC-62 cells, in comparison with HT-29 cells, was accompanied by a higher effect on ATP level in melanoma cells. The OXLs from microalgae (13-HOTE, 13-HODE, 11-HHT, 9-HOTE, 16-HOTE, 11-$HHT_4$ and 15-HEPE) had also a higher reductive effect on ATP level and higher cytotoxic activity in UACC-62 than in HT-29 cells, either alone or combined with 2-DG. These results suggest that these OXLs have an interesting therapeutic potential against cancer cells with high growth rate and elevated metabolic requirements.

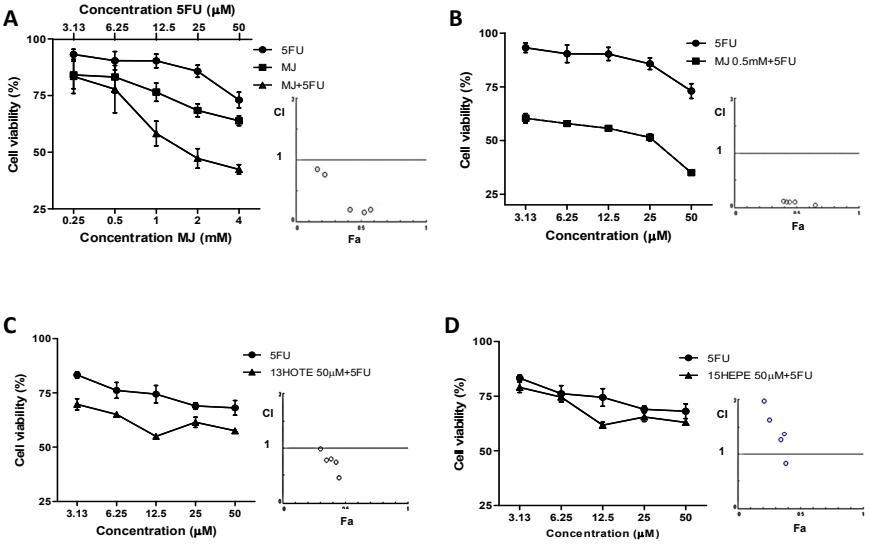



**Figure 6:** Cytotoxic activity of MJ or OXLs in combination with 5-FU. (**A**) HT-29 cells were exposed for 48 h to different concentrations of MJ, 5-FU, or MJ in combination with 5-FU. (**B**) HT-29 cells were exposed for 48 h to 0.5 mM MJ in combination with different concentrations of 5-FU. (**C**) HT-29 cells were exposed for 72 h to 50 µM 13-HOTE in combination with different concentrations of 5-FU. (**D**) HT-29 cells were exposed for 72 h to 50 µM 15-HEPE in combination with different concentrations of 5-FU. The percentage of cell viability (mean ± SE) was determined with the SRB assay, and the parameter Combination Index (CI) was calculated with the computer software CompuSyn. Each experiment was performed in triplicate and repeated three times.

One of the key points of the anticancer activity of jasmonates is their capacity to cooperate with other anti-cancer agents causing synergistic effects. Cooperative effects for MJ and several clinical anticancer drugs (cisplatin, paclitaxel, doxorubicin, carmustine) have been demonstrated in many cancer cell lines [26-27]. With respect to colorectal cancer, MJ has been shown to sensitize cancer cells toward apoptosis induced by TNF-related apoptosis-inducing ligand (TRAIL) [28]. In the same line, MJ combined with algal extracts was shown to predispose prostate cancer cells to undergo apoptosis [29]. The use of multi-agent drug combinations may allow reduction of the administered dose, decrease unwanted side effects, and reduce the likelihood that the tumor will display resistance to the combined therapy. Thus, the synergism between MJ and 5-FU against HT-29 cells described in this paper may be interesting in the treatment of colorectal cancer. The clinical use of MJ is limited for the need of high doses of the compound, in the millimolar range. The future development of more active synthetic analogs may overcome this restriction. In this respect, the OXLs displaying structures of hydroxylated fatty acids studied in this work show similar activities (cytotoxicity, ATP-depletion) at much lower concentrations, in the micromolar range.

Several lines of evidence associate chronic intestinal inflammation to greater risk of developing colorectal cancer [30]. We have shown that 13-HOTE, the major oxylipin constituent of the biomass of the microalga *Chlamydomonas debaryana*, could effectively ameliorate TNBS-induced inflammation in rat colon tissue [6]. This work demonstrated that 13-HOTE had a synergistic effect on 5-FU cytotoxicity against HT-29 cells, thus expanding its potential for preventing inflammatory bowel disease and derived colorectal cancer.

In conclusion, this study shows that MJ and microalgal-derived OXLs can impact on the energetic metabolism of cancer cell lines, causing ATP depletion. The effect on ATP level was closely linked to the cytotoxic activity of these compounds, which was higher against human melanoma cancer cell line UACC-29 (high basal ATP levels) than against human colon adenocarcinoma cell line HT-29 (low basal ATP level). The present study further reveals the synergistic cooperation between the chemotherapeutic drug 5-FU and MJ or 13-HOTE against HT-29. The anti-inflammatory activity of the OXLs previously described by our group increases their significance in prevention of inflammatory bowel disease and derived colorectal cancer.

## Experimental

***Compounds:*** MJ and 5-FU were obtained from Sigma-Aldrich (Saint Louis, MO, USA). The OXLs used in this study were obtained as previously described [5]. The compounds (9*Z*,11*E*,13*S*,15*Z*)-13-hydroxyoctadeca-9,11,15-trienoic acid (13-HOTE), (9*Z*,11*E*,13*S*)-13-hydroxyoctadeca-9,11-dienoic acid (13-HODE), (7*Z*,9*E*,11*S*,13*Z*)-11-hydroxyhexadeca-7,9,13-trienoic acid (11-HHT), (10*E*,12*Z*,15*Z*)-9-hydroxyoctadeca-10,12,15-trienoic acid (9-HOTE), (9*Z*,12*Z*,14*E*)-16-hydroxyoctadeca-9,12,14-trienoic acid (16-HOTE), and (4*Z*,7*Z*,9*E*,11*S*,13*Z*)-11-hydroxyhexadeca-4,7,9,13-tetraenoic acid (11-HHT4) were isolated from *Chlamydomonas debaryana*. The compound (5*Z*,8*Z*,11*Z*,13*E*,15*S*,17*Z*)-15-hydroxyeicosa-5,8,11,13,17-pentaenoic acid (15-HEPE) was isolated from *Nannochloropsis gaditana*. MJ, 5-FU and OXLs stocks were prepared in DMSO at 0.5 M, 0.5 M, and 10 mM, respectively, and diluted to the desired concentration with culture medium. Controls were incubated with the corresponding quantity of DMSO, which was always below 1% and did not affect cell viability.

***Cell lines:*** The human colonic adenocarcinoma cell line HT-29 was obtained from the European Collection of Cell Cultures (ECACC) and cultured in McCoy's 5A medium (PAA Laboratories, Pasching, Austria). The human melanoma cell line UACC-62, a kind gift of Dr Soengas (Centro Nacional de Investigaciones Oncológicas, Madrid, Spain), was cultured in RPMI medium supplemented with 2 mM L-Gln and 25 mM HEPES (Gibco, USA). The culture media were supplemented with 10% heat-inactivated fetal bovine serum, 100 U/mL penicillin and 100 mg/mL streptomycin (PAA Laboratories, Pasching, Austria). Cultures were incubated at 37°C in a 5% $CO_2$ atmosphere.

***Flow cytometry and cell cycle determination:*** HT-29 and UACC-62 cells were seeded at 8 x $10^5$ cells/well in 6-wells NUNC plates and were treated with either MJ or OXLs for 24 h. Cells were treated with trypsin and collected by centrifugation (375 *g*, 5 min, 25°C), and fixed in 70% ethanol ($10^6$ cells $ml^{-1}$) for 24 h. Ethanol was eliminated and cells were resuspended in PBS containing 5 mg $mL^{-1}$ pancreatic A ribonuclease (Sigma-Aldrich) and kept at 4°C for 48 h under mild agitation. Then, propidium iodide was added at 5 µg $mL^{-1}$, and cells were incubated at 4°C for 1 h. DNA content was determined on a Beckman Coulter Cytomics FC 500 MPL (Beckman Coulter Inc, CA; USA) with MXP Software examining $10^4$ cells. Percentages of cells in apoptotic-sub $G_1$, $G_0/G_1$, S and $G_2/M$ were calculated using CXP software.

***Cell proliferation assay:*** The cytotoxic activity of MJ and OXLs was measured with the sulforhodamine B (SRB) assay [20], the (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium (MTT) reduction assay [21], and the lactate deshydrogenase (LDH) leakage assay [22], as described. The half maximal inhibitory concentration ($IC_{50}$) is the concentration causing 50% of cell death at a fixed time.

***ATP quantification:*** Cells in growth medium were seeded into 24-well plates at 3 x $10^5$ cells/well and kept at 37°C in a 5% $CO_2$ atmosphere for 24 h. When indicated, cells were supplied with 5 mM 2-deoxyglucose (2-DG) for 3 h, and then, with different concentrations of either MJ or OXLs for 30 min. To secure that the effect of the compounds was the cause, and not the consequence, of cell death, the viability of the cells was confirmed by trypan blue staining of a subset of cells after the treatments. Cells were washed with cold PBS and adenylates were extracted with 0.5 mL of 0.6 M $HClO_4$. The acid suspension was rapidly neutralized with 75 µL of 2 M $K_2CO_3$ and the residue was removed by centrifugation. Supernatants were stored at -35°C. The assay of ATP was performed with a luciferin-luciferase Adenosine 5'-triphosphate (ATP) Bioluminescent Assay kit (Sigma-Aldrich, St. Louis. Mo., USA), using a Luminoskan TL (LabSystems Oy, Helsinki, Finland) luminometer. ATP is consumed and light is emitted when firefly luciferase catalyzes the oxidation of D-luciferin [23]. ATP concentration of samples was calculated by comparison with a standard curve prepared with serial dilutions of the ATP standard supplied with the kit.

***Drug interaction analysis:*** The interaction between MJ, 13-HOTE or 15-HEPE and 5-FU was evaluated by median-effect plot analyses and the combination index (CI) method [24]. Compounds were combined at concentrations below their $IC_{50}$ values. Data analysis was performed using the CompuSyn software (Combosyn, Inc., NJ, USA).

***Statistical analysis:*** Data are expressed as mean values ± Standard Error (SE) from at least 3 independent experiments conducted at

least in duplicate. The data were analyzed in one-way analysis of variance (ANOVA) followed by Bonferroni´s test, using GraphPad Prism 5.0. Differences were considered statistically significant at $P<0.05$.

**Acknowledgments** - This work was supported by a grant from MINECO (Spain) and FEDER (research project IPT-2011-1370-060000). We thank A. de la Jara (Instituto Tecnológico de Canarias, Spain) for providing lyophilized microalgal samples and Centro de Investigación, Tecnología e Innovación (CITIUS) of the University of Seville for providing technical assistance.

## References

[1] Mosblech A, Feussner I, Heilmann I. (**2009**) Oxylipins: Structurally diverse metabolites from fatty acid oxidation. *Plant Physiology and Biochemistry*, ***47***, 511-517.
[2] Pohl CH, Kock JL. (**2014**) Oxidized fatty acids as inter-kingdom signaling molecules. *Molecules*, ***19***, 1273-1285
[3] Morin C, Rousseau E, Fortin S. (**2013**) Anti-proliferative effects of a new docosapentaenoic acid monoacylglyceride in colorectal carcinoma cells. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, ***89***, 203-213.
[4] Penny MK, William SH, Lawrence JA. (**2003**) Omega-3 fatty acids and cardiovascular disease. New recommendations from the American Heart Association. *Arteriosclerosis, Thrombosis and Vascular Biology*, ***23***, 151-152.
[5] de los Reyes C, Ávila-Román J, Ortega MJ, de la Jara A, García-Mauriño S, Motilva V, Zubía E. (**2014**) Oxylipins from the microalgae *Chlamydomonas debaryana* and *Nannochlorosis gaditana* and their activity as TNF-α inhibitors. *Phytochemistry*, ***102***, 152-161.
[6] Ávila-Román J, Talero E, Alcaide A, de los Reyes C, Zubía E, García-Mauriño S, Motilva, V. (**2014**) Preventive effect of the microalga *Chlamydomonas debaryana* in the acute phase of experimental colitis in rats. *British Journal of Nutrition*, ***112***, 1055-1064.
[7] Vellosillo T, Martínez M, López MA, Vicente J, Cascón T, Dolan L, Hamberg M, Castresana C. (**2007**) Oxylipins produced by the 9-lipoxygenase pathway in Arabidopsis regulate lateral root development and defense responses through a specific signaling cascade. *The Plant Cell*, ***19***, 831-846.
[8] Santino A, Taurino M, De Domenico S, Bonsegna S, Poltronieri P, Pastor V, Flors V. (**2013**) Jasmonate signaling in plant development and defense response to multiple (a)biotic stresses. *Plant Cell Reports*, ***32***, 1085-1098.
[9] Flescher E. (**2005**) Jasmonates-a new family of anti-cancer agents. *Anticancer Drugs*, ***16***, 911-916.
[10] Flescher E. (**2007**) Jasmonates in cancer therapy. *Cancer Letters*, ***245***, 1-10.
[11] Raviv Z, Cohen S, Reischer-Pelech D. (**2013**) The anti-cancer activities of jasmonates. *Cancer Chemotherapy and Pharmacology*, ***71***, 275-285.
[12] Ghasemi Pirbalouti A, Sajjadi SE, Parang K. (**2014**) A review (research and patents) on jasmonic acid and its derivatives. *Archiv der Pharmazie (Weinheim)*, ***347***, 229-239.
[13] Cohen S, Flescher E. (**2009**) Methyl jasmonate: a plant stress hormone as an anti-cancer drug. *Phytochemistry*, ***70***, 1600-1609.
[14] Klippel S, Jakubikova J, Delmore J, Ooi M, McMillin D, Kastritis E, Laubach J, Richardson PG, Anderson KC, Mitsiades CS. (**2012**) Methyljasmonate displays *in vitro* and *in vivo* activity against multiple myeloma cells. *British Journal of Haematology*, ***159***, 340-351.
[15] Wasternack C. (**2007**) Jasmonates: an update on biosynthesis, signal transduction and action in plant stress response, growth and development. *Annals of Botany*, ***100***, 681-697.
[16] Reischer D, Heyfets A, Shimony S, Nordenberg J, Kashman Y, Flescher E. (**2007**) Effects of natural and novel synthetic jasmonates in experimental metastatic melanoma. *British Journal of Pharmacology*, ***150***, 738-749.
[17] Rotem R, Heyfets A, Fingrut O, Blickstein D, Shaklai M, Flescher E. (**2005**) Jasmonates: novel anticancer agents acting directly and selectively on human cancer cell mitochondria. *Cancer Research*, ***65***, 1984-1993.
[18] Elia U, Flescher E. (**2013**) Combined chemotherapy or biotherapy with jasmonates: targeting energy metabolism for cancer treatment. *Current Pharmaceutical Biotechnology*, ***14***, 331-341.
[19] Cesari IM, Carvalho E, Figueiredo Rodrigues M, Mendonça Bdos S, Amôedo ND, Rumjanek FD. (**2014**) Methyl jasmonate: putative mechanisms of action on cancer cells cycle, metabolism, and apoptosis. *International Journal of Cell Biology*, **2014** Article ID 572097. doi: 10.1155/2014/572097.
[20] Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesh H, Kenney S, Boyd MR. (**1990**) New colorimetric cytotoxicity assay for anticancer-drug screening. *Journal of the National Cancer Institute*, ***82***, 1107-1112.
[21] Mosmann T. (**1983**) Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, ***65***, 55-63.
[22] Decker T, Lohmann-Matthes M. (**1988**) A quick and simple method for the quantitation of lactate dehydrogenase release in measurements of cellular cytotoxicity and tumor necrosis factor (TNF) activity. *Journal of Immunological Methods*, ***15***, 61-69.
[23] Leach FR. (**1981**) ATP determination with firefly luciferase. *Journal of Applied Biochemistry*, ***3***, 473-517.
[24] Chou TC. (**2006**) Theoretical basis, experimental design, and computerized simulation of synergism and antagonism in drug combination studies, *Pharmacological Reviews*, ***58***, 621-681.
[25] Taylor EW. (**1965**) The mechanism of colchicine inhibition of mitosis: I. Kinetics of inhibition and the binding of H3-colchicine. *The Journal of Cell Biology*, ***25***, 145-160.
[26] Heyfets A, Flescher E. (**2007**) Cooperative cytotoxicity of methyl jasmonate with anti-cancer drugs and 2-deoxy-d-glucose. *Cancer Letters*, ***250***, 300-310.
[27] Fingrut O, Reischer D, Rotem R, Goldin N, Altboum I, Zan-Bar I, Flescher E. (**2005**) Jasmonates induce nonapoptotic death in high-resistance mutant p53-expressing B-lymphoma cells. *British Journal of Pharmacology*, ***146***, 800-808.
[28] Raviv Z, Zilberberg A, Cohen S, Reischer-Pelech D, Horrix C, Berger MR, Rosin-Arbesfeld R, Flescher E. (**2011**) Methyl jasmonate down-regulates survivin expression and sensitizes colon carcinoma cells towards TRAIL-induced cytotoxicity. *British Journal of Pharmacology*, ***164***, 1433-1444.
[29] Farooqi AA, Butt G, Razzaq Z. (**2012**) Algae extracts and methyl jasmonate anti-cancer activities in prostate cancer: choreographers of 'the dance macabre'. *Cancer Cell International*, DOI: 10.1186/1475-2867-12-50.
[30] Dyson JK, Rutter MD. (**2012**) Colorectal cancer in inflammatory bowel disease: What is the real magnitude of the risk? *World Journal of Gastroenterology*, ***18***, 3839-3848.