Cyclooxygenase-2 is induced by p38 MAPK and promotes cell survival

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Abstract. The Na^+ ionophore monensin affects cellular pH and, depending on its concentration, causes the survival or death of tumor cells. In the present study, we elucidated the survival pathway activated in U937 cells, a human lymphoma-derived cell line. These cells treated with monensin at a concentration of 5 μM were growth-arrested in G1, activated p38 mitogen-activated protein kinase (MAPK) and showed an increased expression of cyclooxygenase-2 (COX-2). The latter two molecular events were linked, as pharmacological inhibition of the MAPK did not allow COX-2 increased expression. Furthermore, we showed that p38 and COX-2 keep monensin-stressed U937 cells alive, as pharmacological inhibition of each enzyme caused cell death.

Introduction

Cells sense various forms of injury and insult by biochemical and molecular machinery and execute appropriate programs of response; they undergo cell cycle arrest, to allow adequate time for repair of damage, or they die, if the damage is too severe. In tumor cells surviving cytotoxic/apoptotic stimuli, a network integrating several pathways may involve different survival pathways, such as mitogen-activated protein kinase (MAPK) signaling cascades, the phosphoinositide 3-kinase (PI3K)/Akt pathway, the nuclear factor (NF)-κB signaling system and the heat-shock response (1-5). Among them, of particular relevance are the MAPKs, serine/threonine-protein kinases regulated by dual tyrosine and threonin phosphorylation, which by participating in signaling cascades conserved through evolution regulate important biological activities (6,7). The three major MAPK groups in mammalian cells include extracellular signal-regulated kinases (ERKs), c-Jun NH_2-terminal protein kinase (JNK) and p38 (8-10). MAPK signaling cascades are activated by a variety of different cellular stimuli and mediate diverse responses. ERKs are acutely stimulated by growth and differentiation factors through activated receptor tyrosine kinases, heterotrimeric G protein-coupled receptors or cytokine receptors (8). JNK and p38 MAPKs are activated in response to a variety of stress signals including UV irradiation, chemotherapeutics, osmotic stress, hypoxia/anoxia, hyperthermia, and they are involved in the regulation of apoptosis (8). Stress-activated MAPKs can mediate survival signals, or, on the contrary, death signals (5,11-13).

Monensin plays a role in the exchange of Na^+ and H^+, transporting one molecule of Na^+ into the cells for each molecule of H^+ transported out, resulting in intracellular alkalinization (14). This drug has been reported to cause tumor cell death (15-19).

In the present study, we investigated whether moderate stress, caused by appropriate doses of monensin, can promote survival in U937 cells, derived from a human lymphoma. We thus identified a p38 MAPK/COX-2 dependent mechanism that appears to counter cell death.

Materials and methods

Materials. RPMI-1640, fetal calf serum, L-glutamine, penicillin-streptomycin, phosphate-buffered saline (PBS), monensin, arachidonic acid (AA), NS398, lipopolysaccharide (LPS) and anti-β-actin antibodies were from Sigma-Aldrich (St. Louis, MO, USA). SB203580, SP600125, PD98059 and acetylsalicylic acid (ASA) were from Calbiochem (Inalco, Milan, Italy). Celecoxib was kindly donated by Pharmacia (Uppsala, Sweden). Anti-phospho-p38 and anti-p38 antibodies were from Cell Signaling Technology (Beverly, MA, USA). Anti-COX-1 and anti-COX-2 antibodies were from Cayman Chemical (Cabru, Milan, Italy). Horseradish peroxidase (HRP)-conjugated anti-immunoglobulin antibodies, enhanced chemiluminescence (ECL) reagents and Hyperfilm-ECL film were from Amersham (Arlington Heights, IL, USA). Protein standards for SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and nitrocellulose membranes were from Bio-Rad. The membrane permeant CDCF-DA was from Molecular Probes (SIC, Rome, Italy), and other reagents were of the highest purity and purchased from Bio-Rad or Sigma.

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Cell viability and growth. U937 cells, derived from the pleural effusion of a patient with histiocytic lymphoma (20), were grown in complete medium (RPMI-1640 medium supplemented with 1.0% sodium pyruvate, 5% FCS, 2 mM glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin) at 37˚C, in a fully humidified atmosphere of 95% room air/5% CO_2. Cells were resuspended three times a week in fresh complete medium as $3x10^5$/ml. Cell growth was evaluated by hemocytometry counts of cells excluding Trypan blue (0.04% Trypan blue in PBS, w/v), and viability was assessed by calculating alive (trypan blue excluding) cells as percentage of all cells counted. Cells used in every experiment were ≥94% viable and were obtained from cultures in exponential growth. They were washed once and resuspended in complete medium ($1x10^6$/ml) and transferred to 24-well microplates. They were then treated with inhibitors or vehicles, incubated for 30 min, and subsequently exposed to test agents or, again, to vehicles. At the end of each experiment, the cells were gently mixed and aliquots were obtained for cell counting and cell cycle analysis. The vehicles, even when used in combination, were ≤0.3% (v/v) and did not modify any investigated parameter in comparison with control culture.

Flow cytometric analysis of cell death. Nuclear DNA fragmentation was quantified by flow cytometry of hypodiploid (subG1) DNA after cell fixation and staining with propidium iodide (PI) (21,22). Briefly, cells were washed with PBS, pelleted and fixed in ice cold ethanol/water (70/30, v/v) for 1 h, pelleted again and washed twice with PBS, and finally resuspended in PBS containing RNAse (20 μg/ml) and PI (100 μg/ml). Events in the different cell cycle phases were gated manually using an EPICS XL cytofluorimeter (Beckman Coulter, Hialeah, FL, USA). At least 10,000 events/sample were acquired. Collected data were analyzed using the Multicycle software for DNA content and cell cycle analysis (Phoenix Flow System, San Diego, CA, USA). The subG1 events representative of the apoptotic cells, and the events in the other cell cycle phases, are given as a percentage of the total cell population.

Western blot analysis. Whole cell lysates were prepared as previously described (23,24). Briefly, the cells were kept for 30 min on ice in lysis buffer [NaCl 150 mM, $CaCl_2$ 1 mM, $MgCl_2$ 1 mM, NaN_3 0.1%, NaF 10 mM, Triton X-100 1% (v/v), orthovanadate 1 mM, aprotinin 2 μg/ml, leupeptin 2 μg/ml, iodoacetamide 10 mM, PMSF 2 mM, and pepstatin 20 μM]. The appropriate volumes of 4X SDS-sample buffer and 2-mercaptoethanol 5% (v/v) were then added. Cell lysates were briefly sonicated, warmed at 95˚C for 5 min, and cleared by centrifugation at 14.000 x g in a microfuge for 15 min at 4˚C. Supernatants were collected and proteins were quantified by RC DC protein assay. Equal amounts of proteins were separated from the different samples by SDS-PAGE, and blotted onto nitrocellulose membranes.

Anisomycin-treated U937 cells were used as positive control for p38 MAPK detection. Transfer efficiency was checked with Ponceau staining. The blots were blocked in Tris-buffered saline (TBS), containing BSA 2% (w/v), probed with specific primary antibodies, washed with PBS-Tween-20, and then incubated with a peroxidase-conjugated secondary antibody. Finally, each membrane was probed to detect β-actin. The final dilutions and incubation times suggested by the manufacturer were used for each antibody. Immunodetection was performed using the ECL reagents and Hyperfilm-ECL film. In the case of COX-1 and COX-2 detection, U937 cells were lysed in RIPA buffer and, as positive control for COX-2, the lysates of peripheral blood mononuclear cells were used, separated by Ficoll-Hypaque density-gradient centrifugation, cultured at 37˚C for 16 h in the presence of 10 μg/ml LPS. Proteins were resolved by 10% SDS-PAGE, blotted and processed as indicated above. Immunodetection was performed with monoclonal antibodies anti-COX-2 (1:1,000) or anti-COX-1 (1:1,000) and specific peroxidase-conjugated anti-immunoglobulin antibodies. Proteins were detected by ECL.

Assay of COX-2 and COX-1 activity. To distinguish between the peroxidative role of COX-1 and COX-2, U937 cells resuspended in complete medium ($1x10^6$/ml) were pretreated with either the COXs inhibitors (ASA, celecoxib and NS398) or the vehicle, or were left untreated, and, thereafter, with monensin or, again, with the vehicle for 6 h. After washing with HBSS, incubating for 10 min with CDCF-DA (2 μM) in HBSS and another washing with the same medium, the cells (≥10,000) were analyzed using an EPICS XL cytofluorimeter, with excitation and emission settings at 495 and 525 nm, respectively. Aliquots of CDCF-DA loaded cells were incubated with exogenous AA (50 μM) for 5 min, immediately prior to cytofluorimetry analysis.

CDCF-DA is an oxidation sensitive fluorescent probe, which is first deacetylated inside the cells to the nonfluorescent compound 2',7'-CDCFH and can subsequently be oxidized to the fluorescent compound 2',7'-CDCF by a variety of peroxides. This probe allows to investigate the activity of the COXs, as these enzymes catalyze a) a cyclooxygenase reaction in which AA is converted to PGG2 and b) a peroxidase reaction in which PGG2 is reduced to PGH2 (25). Thus, CDCFH may be oxidized through this peroxidase reaction to CDCF and become fluorescent. Inhibition of COX-2 with either celecoxib or NS398, or inhibition of COX-1 with ASA, reveals the relative contribution of either COX-1 or COX-2 to AA transformation. Fluorescent cells were analyzed by an EPICS XL cytofluorimeter on a log scale (FL2) and recorded as mean fluorescence intensity (MFI) of the whole cell population. A minimum of 10,000 events were examined for each sample.

Statistical analysis. Results are expressed as the means ± standard deviation (SD) of repeated experiments. Statistical differences were evaluated using the paired two-tailed Student's t-test. $P \leq 0.05$ was considered to indicate statistically significant differences.

Results

Monensin and cell survival. The Na^+ ionophore monensin causes cell death in a dose-dependent manner. A 24-h treatment with high concentrations of this drug were cytotoxic for a large proportion of U937 cells, while lower concentrations were less effective, suggesting the activation of a survival pathway (Fig. 1A). In particular, 5 μM monensin caused a slight decrease in trypan blue excluding cells (78±3%) in comparison with untreated cultures (93±2%), in addition to the appearance

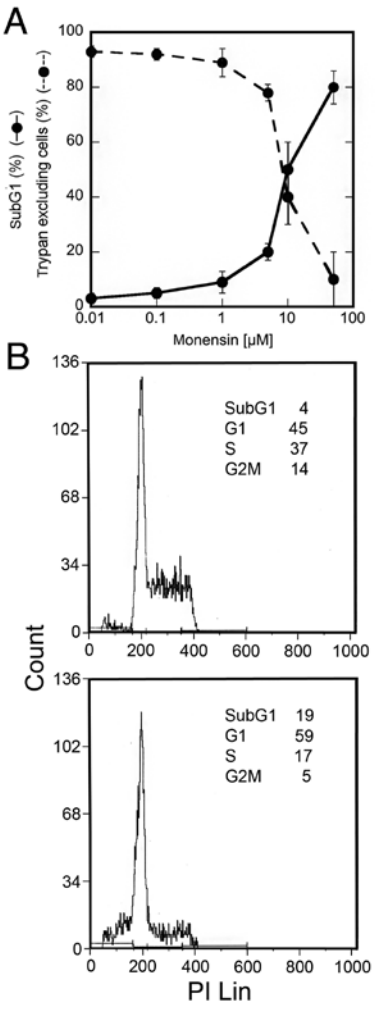

Figure 1. Cell survival depends on the dose of monensin. U937 cells were exposed or not to different concentrations of monensin (A) for 24 h. Cells were counted in a hemocytometer as excluding and not excluding trypan blue. Viability was obtained by calculating live cells (trypan blue excluding) as percentage of all counted cells. A portion of cells were fixed and stained with PI; subG1 events in the cell cycle were evaluated under cytofluorimetry. The reported values represent the means and the error bars the SD of the percentage of live cells (trypan blue excluding) or subG1 events of 12 independent experiments. Assessment of cell survival was investigated and statistically significant differences (P≤0.05) were found between the data obtained using 5 μM monensin and the two highest concentrations of the drug. (B) The cell cycle profiles of one representative experiment out of 12 performed on U937 cells untreated (upper panel) or treated with 5 μM monensin (lower panel) for 24 h. The percentage of events in the different cell cycle phases quantified in the reported experiment are indicated in each panel.

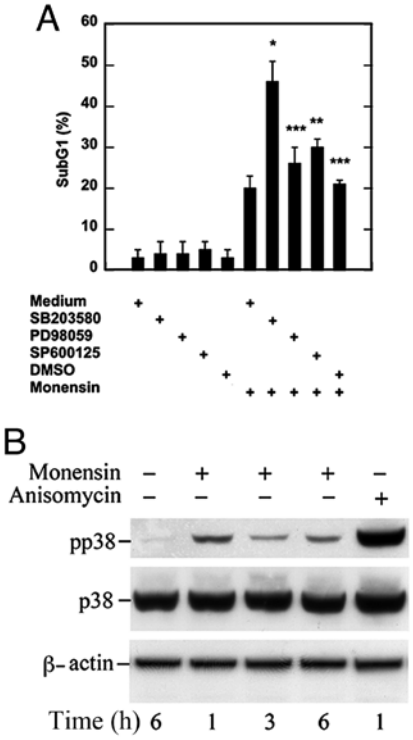

Figure 2. p38 MAPK is activated and promotes survival in U937 cells. (A) U937 cells were pretreated with MAPK inhibitors and then exposed or not to 5 μM monensin for 24 h. The cells were fixed and stained with PI to evaluate subG1 events in the cell cycle under cytofluorimetry. Columns represent means and bars the SD of five replicate experiments. Assessment of cell death showed statistically significant differences between the data obtained in the cultures treated with monensin together with SB203580 (*P=0.009) or with SP600125 (**P=0.04) and not significant with PD98059 (***P>0.05) in comparison with the cultures treated with monensin alone. (B) Western blot analysis of activated p38 in the lysates of U937 cells either exposed or not to monensin for the time indicated. Blotted proteins were probed with anti-phospho-p38 and then with anti-p38 antibodies, each followed by peroxidase-conjugated secondary antibody. Anisomycin-treated cells were used as positive control for the detection of pp38. The level of β-actin is shown at the bottom as a loading control.

of 20±3% of subG1 events. SubG1 events were studied by cytofluorimetry of cell cycle phases of cells fixed and stained with PI; hypodiploid DNA events are easily discernable from the narrow peak of cells with diploid DNA content, and are considered to be indicative of apoptotic nuclei (21,22). Furthermore, analysis of events in the different cell cycle phases showed that monensin (5 μM) caused a decrease in S and G2M phases, while the percentage of G1 events increased (Fig. 1B). Cell counts indicated that at this concentration monensin did not allow cell growth (data not shown). These results suggest that monensin, at this concentration, causes activation of a survival pathway in most U937 cells, increasing the time spent in the G1 cell cycle phase. In order to investigate this survival pathway, the experiments were performed using 5 μM monensin and viability parameters were investigated after 24 h.

p38 MAPK is activated and promotes the survival signal. MAPKs are central mediators of cellular survival and death pathways (11-13). To investigate their involvement in the previously detected survival pathway activated by monensin, we pretreated the cells with pharmacological inhibitors at concentrations affecting specifically one MAPK (26) and then analyzed cell viability.

p38 inhibition with SB203580, but not ERK inhibition with PD98059, caused a significant cell death increase (Fig. 2A). Also SP600125, a JNK inhibitor, caused an increase in cell death (Fig. 2A) even if less significant than that caused by SB203580 (P=0.05 vs. P=0.009). Under the same conditions, neither inhibitor nor DMSO without monensin affected cell viability (Fig. 2A).

To confirm p38 MAPK involvement in the survival pathway activated by monensin, we performed time-kinetic studies in which phosphorylated p38 and then total p38 were analyzed by western blotting with specific antibodies. In the lysate

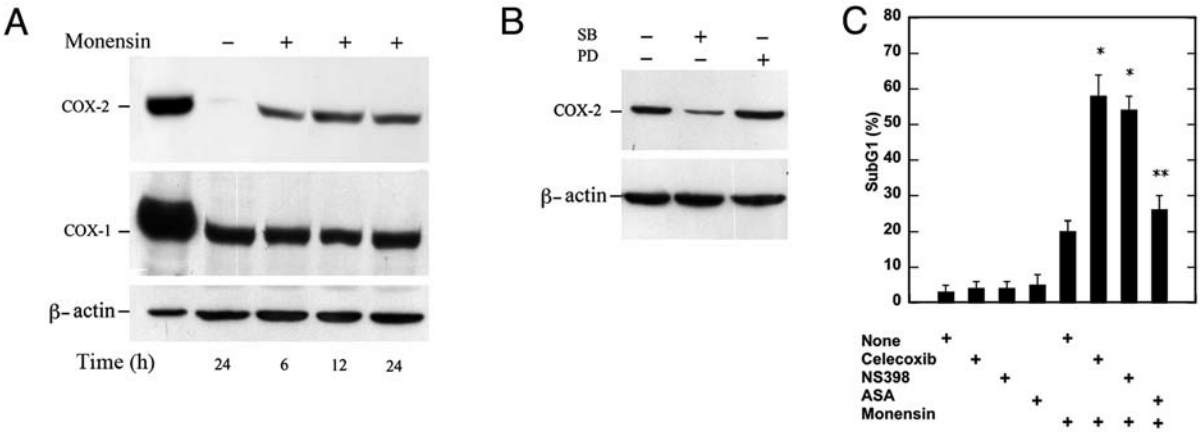

Figure 3. The p38-mediated pro-survival pathway involves COX-2. (A) COX-2 was analyzed by western blot analysis in the lysates of U937 cells exposed to 5 μM monensin or not for the time indicated. Blotted proteins of the same lysates were probed with anti-COX-2 or with anti-COX-1 antibody, each followed by peroxidase-conjugated secondary antibody. In the first lane, the blotted proteins of lysates of human monocytes cultured in the presence of LPS were analyzed and used as positive control for the detection of COX-2. This experiment was performed three times, with comparable results. (B) Western blot analysis of COX-2 in the lysates of U937 cells pretreated or not with SB203580 (10 μM) or PD98059 (10 μM) and then treated with monensin for 6 h. The level of β-actin is shown at the bottom as a loading control. (C) SubG1 events were evaluated in the cell cycle under cytofluorimetry of U937 cells fixed and stained with PI. Assessment of cell death showed statistically significant differences (*P<0.001) between the data obtained in the cultures treated for 24 h with monensin and the COX-2 inhibitors, celecoxib (1 μM) or NS398 (5 μM), in comparison with the cultures treated with monensin alone. Pretreatment of U937 cells with ASA (100 μM) did not have statistically different results (**P>0.05). This type of investigation was performed on ≥10,000 events. The values reported are the means ± SD (n=5).

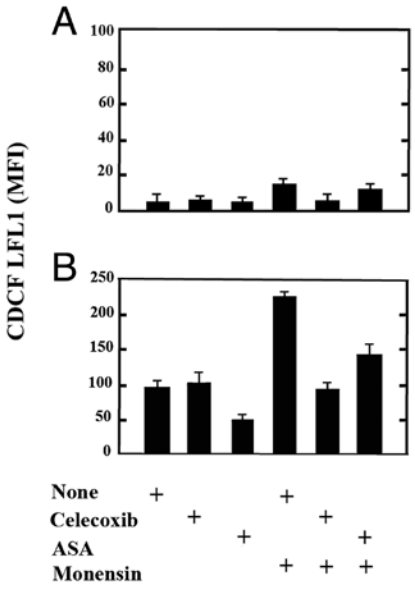

Figure 4. COX-2 is more active than COX-1 to transform AA. (A) U937 cells were pretreated or not with celecoxib (10 μM) or ASA (100 μM) for 30 min and then with or without 5 μM monensin for 6 h. Washed cells were loaded with CDCF-DA and fluorescence was examined under cytofluorimetry. (B) The cells, treated as in (A), were exposed to 50 μM AA for 5 min and examined under cytofluorimetry. The mean fluorescence intensity (MFI) for each cell treatment, detected as Log-fluorescence-1 (LFL1) of ≥10,000 cellular events, is shown. Values are the means ± SD of three independent experiments.

of untreated U937 cells a faint band relative to phospho-p38 was detected, which increased after 1 h and was still present after 6 h of monensin treatment (Fig. 2B). When probed with antibodies against total p38, a 38 kDa band was also present in the lysate of untreated cells and showed no change at the investigated time points (Fig. 2B). Thus, monensin activates p38 MAPK which is involved in a survival pathway in U937 cells.

COX-2 is induced by p38 and contributes to the survival pathway. COX enzymes form prostaglandins (PGs) and thromboxane from AA and, thus far, two COX isoenzymes (COX-1 and COX-2) have been detected. They are differently regulated: COX-1 is constitutively expressed by the majority of cell types, while COX-2 is almost undetectable in normal tissues, is regulated at the transcriptional and at the post-transcriptional levels (27) and its expression can be regulated by MAPK (28,29). Therefore, we examined the expression of this protein by western blotting in the lysates of U937 cells. Untreated cells did not express COX-2 in a considerable amount (30) (Fig. 3A), whereas monensin caused an increase in this protein which became evident after 6 h and was still present after 24 h. The same lysates probed with anti-COX-1 antibodies showed that the expression of this enzyme is constitutive in U937 cells and is apparently not affected by monensin (Fig. 3A). We therefore examined the expression of COX-2 after p38 MAPK inhibition by SB203580 or ERK inhibition by PD98059. p38 MAPK inhibition, but not ERK inhibition, caused a large decrease in COX-2 expression (Fig. 3B).

In comparison to monensin-treated cells, cell death was significantly increased after COX-2 inhibition with celecoxib (1 μM) or NS398 (5 μM) and not after COX-1 inhibition by ASA (100 μM) (Fig. 3C). These results indicate that COX-2 expression is induced by phosphorylated p38 and plays a role in the survival pathway activated by monensin in U937 cells.

COX-2 efficiently transforms AA. The increased expression of COX-2 suggests that COX-1, although constitutively expressed in U937 cells, may function less efficiently than COX-2 and/or that monensin-stressed cells may have an increased need to transform AA or to avoid its accumulation. To examine the function of the COXs in living cells we used CDCF-DA, a probe which becomes fluorescent upon oxidation as a result of the peroxidase activities of either COX-1 or COX-2 (25).

U937 cells untreated or exposed to monensin for 6 h, suspended in a medium containing CDCF-DA, were washed and analyzed. The fluorescence of monensin-treated cells was 3 to 4-fold that of untreated cells and the responsible enzyme was COX-2, as CDCF fluorescence was prevented by celecoxib, but not by ASA (Fig. 4A). By this assay we also analyzed the activity of either COXs towards exogenous AA (50 μM) (Fig. 4B). In comparison with control cells, U937 cells exposed to AA showed an 18 to 22-fold increase in fluorescence, which was due to COX-1 as could be largely prevented by ASA and not by celecoxib (Fig. 4B). Following monensin treatment, the AA exposure caused an increase in fluorescence 45 to 55-fold that of control cells (untreated and not exposed to AA). Such an increase was largely due to COX-2, as celecoxib prevented it by 55-65% and was also, in part, due to COX-1, as ASA inhibited it by 35-40% (Fig. 4B). These results indicate that COX-2 upregulated in monensin-stressed cells is highly active and suggest that, by transforming or avoiding the accumulation of AA, keeps stressed cells alive.

Discussion

In the present investigation we showed that U937 cells treated with subcytotoxic concentrations of monensin were growth-arrested in the G1 cell cycle phase and escaped from death by activation of a survival pathway, in which p38 MAPK and COX-2 were involved.

We investigated the role of cyclooxygenases as it is known that these can be activated by phosphorylated p38 MAPK and can mediate survival signals (31,32). COX-2, an enzyme known to be expressed by several tumor cells, was not apparently expressed in untreated U937 cells, while it was upregulated within 6 h of monensin treatment. Such expression depended on p38, as it could be prevented by SB203580. This result is in agreement with a previously reported finding that p38 causes stabilization of COX-2 transcript (31). The viability assessment after inhibition of COX-2 or COX-1 revealed that the former played a pro-survival role in monensin-treated cells.

It is known that monensin causes an increased release of arachidonic acid (AA) (33) and, therefore, it is plausible that the upregulated COX-2 is needed to decrease free AA. This conclusion is supported by the detection that COX-1 was less active than COX-2 to transform AA. We assayed the activity of COX isoenzymes in alive cells; the specific peroxidative function of COX-1 or COX-2 was explored by loading the cells with the probe CDCF-DA. In this assay, the analysis of cells pretreated with celecoxib or NS398, inhibiting COX-2, or with ASA, inhibiting COX-1, revealed that in monensin-treated cells, COX-2 was more active than the constitutively expressed COX-1. This conclusion was reached considering the activity of the isoenzymes towards endogenous and exogenous AA. It is noteworthy that COX-1, although present, is much less active than COX-2. However, this aspect has already been investigated (34) and some possible explanations are that the Km value of COX-1 for AA is known to be higher than that of COX-2 (10 and 2 μM, respectively); mice deficient in COX-1, the constitutive isoform believed to be responsible for homeostasis, are healthy and live a normal life span, whereas mice lacking COX-2 expression, the inducible isoform, thought to be expressed mainly in disease states, display various developmental problems, female reproductive disorders, and a shortened life span; COX-1 and COX-2 utilize different pools of AA for synthesizing prostanoids: in murine fibroblasts and macrophages, ligand-induced prostaglandin production occurs via expression of COX-2, while COX-1 present in these cells cannot metabolize ligand-released AA.

The COX isoforms have both overlapping as well as distinct physiological and pathological functions; while COX-1 is involved in the homeostasis of various physiological functions, COX-2 is responsible for a number of pathological processes such as inflammation and cancer. COX-2 mRNA levels, as well as COX-2 protein levels, are markedly increased in human colorectal adenocarcinomas relative to normal colonic mucosa and overexpression of COX-2 has been identified as an early central event in colon carcinogenesis (35,36). A previous study showed that constitutive expression of COX-2 in human colon cancer promotes tumor invasion and the metastatic potential of these cells (37). As well as in colon cancer, increased COX-2 expression is also found in other types of cancer, including breast, lung and the mucous membrane of the head and neck (38-40). However, the functions of this enzyme in tumor cells are not completely understood. For example, it is not clear whether COX-2 in tumor cells plays an antiapoptotic role. Furthermore, COX-2 is not expressed in some tumor cells. Thus, in these cases the use of COX-2 inhibitors would be of no benefit. However, in the present study, we detected that the stress caused by monensin in U937 leukemia cells induced COX-2 increased expression upon p38 MAPK activation. This pathway may also be activated by other antineoplastic drugs and should be considered in combination therapy with COX-2 or p38 MAPK inhibitors.

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References

1. Kennedy SG, Wagner AJ, Conzen SD, Jordan J, Bellacosa A, Tsichlis PN and Hay N: The PI 3-kinase/Akt signaling pathway delivers an anti-apoptotic signal. Genes Dev 11: 701-713, 1997.
2. Datta SR, Brunet A and Greenberg ME: Cellular survival: a play in three Akts. Genes Dev 13: 2905-2927, 1999.
3. Finkel T and Holbrook NJ: Oxidants, oxidative stress and the biology of ageing. Nature 408: 239-247, 2000.
4. Martindale JL and Holbrook NJ: Cellular response to oxidative stress: signaling for suicide and survival. J Cell Physiol 192: 1-15, 2002.
5. Wada T and Penninger JM: Mitogen-activated protein kinases in apoptosis regulation. Oncogene 23: 2838-2849, 2004.
6. Widmann C, Gibson S, Jarpe MB and Johnson GL: Mitogen-activated protein kinase: conservation of a three-kinase module from yeast to human. Physiol Rev 79: 143-180, 1999.
7. Kyriakis JM and Avruch J: Mammalian MAPK signal transduction patyhways activated by stress and inflammation: a 10-year update. Physiol Rev 92: 689-737, 2012.
8. Lewis TS, Shapiro PS and Ahn NG: Signal transduction through MAP kinase cascades. Adv Cancer Res 74: 49-139, 1998.
9. Wu J, Suzuki H, Akhand AA, Zhou YW, Hossain K and Nakashima I: Modes of activation of mitogen-activated protein kinases and their roles in cepharanthine-induced apoptosis in human leukemia cells. Cell Signal 14: 509-515, 2002.
10. Platanias LC: MAP kinase signaling pathways and hematologic malignancies. Blood 101: 4667-4679, 2003.

11. Shrode LD, Rubie EA, Woodgett JR and Grinstein S: Cytosolic alkalinization increases stress-activated protein kinase/c-Jun NH_2-terminal kinase (SAPK/JNK) activity and p38 mitogen-activated protein kinase activity by a calcium-independent mechanism. J Biol Chem 272: 13653-13659, 1997.
12. Raciti M, Lotti LV, Valia S, Pulcinelli FM and Di Renzo L: JNK2 is activated during ER stress and promotes cell survival. Cell Death Dis 3: e429, 2012.
13. Okamoto S, Krainc D, Sherman K and Lipton SA: Antiapoptotic role of the p38 mitogen-activated protein kinase-myocyte enhancer factor 2 transcription factor pathway during neuronal differentiation. Proc Natl Acad Sci USA 97: 7561-7566, 2000.
14. Nakazato K and Hatano Y: Monensin-mediated antiport of Na^+ and H^+ across liposome membrane. Biochim Biophys Acta 1064: 103-110, 1991.
15. Zhu WH and Loh TT: Effects of Na^+/H^+ antiport and intracellular pH in the regulation of HL-60 cell apoptosis. Biochim Biophys Acta 1269: 122-128,1995.
16. Park WH, Lee MS, Park K, Kim ES, Kim BK and Lee YY: Monensin-mediated growth inhibition in acute myelogenous leukemia cells via cell cycle arrest and apoptosis. Int J Cancer 101: 235-242, 2002.
17. Park WH, Seol JG, Kim ES, *et al*: Monensin-mediated growth inhibition in human lymphoma cells through cell cycle arrest and apoptosis. Br J Haematol 119: 400-407, 2002.
18. Iljin K, Ketola K, Vainio P, *et al*: High-throughput cell-based screening of 4910 known drugs and drug-like small molecules identifies disulfiram as an inhibitor of prostate cancer cell growth. Clin Cancer Res 15: 6070-6078, 2009.
19. Ketola K, Hilvo M, Hyotylainen T, *et al*: Salinomycin inhibits prostate cancer growth and migration via induction of oxidative stress. Br J Cancer 106: 99-106, 2012.
20. Sundstrom C and Nilsson K: Establishment and characterization of a human histiocytic lymphoma cell line (U-937). Int J Cancer 17: 565-577, 1976.
21. Nicoletti I, Migliorati G, Pagliacci MC, Grignani F and Riccardi C: A rapid and simple method for measuring thymocyte apoptosis by propidium iodide staining and flow cytometry. J Immunol Methods 139: 271-279, 1991.
22. Cirone M, Di Renzo L, Lotti LV, *et al*: Primary effusion lymphoma cell death induced by bortezomib and AG 490 activates dendritic cells through CD91. PLoS One 7: e31732, 2012.
23. Matusali G, Arena G, De Leo A, Di Renzo L and Mattia E: Inhibition of p38 MAP kinase pathway induces apoptosis and prevents Epstein Barr virus reactivation in Raji cells exposed to lytic cycle inducing compounds. Mol Cancer 8: 18, 2009.
24. Marfè G, Morgante E, Di Stefano C, Di Renzo L, *et al*: Sorbitol-induced apoptosis of human leukemia is mediated by caspase activation and cytochrome c release. Arch Toxicol 82: 371-377, 2008.
25. Morita I, Schindler M, Regier MK, Otto JC, Hori T, DeWitt DL and Smith WL: Different intracellular locations for prostaglandin endoperoxide H synthase-1 and -2. J Biol Chem 270: 10902-10908, 1995.
26. Davies SP, Reddy H, Caivano M and Cohen P: Specificity and mechanism of action of some commonly used protein kinase inhibitors. Biochem J 351: 95-105, 2000.
27. Smith WL, DeWitt DL and Garavito RM: Cyclooxygenases: structural, cellular, and molecular biology. Annu Rev Biochem 69: 145-182, 2000.
28. Chen W, Tang Q, Gonzales MS and Bowden GT: Role of p38 MAPK and ERK in mediating ultraviolet-B induced cyclooxygenase-2 gene expression. Oncogene 20: 3921-3926, 2001.
29. Hung JH, Su IJ, Lei HY *et al*: Endoplasmic reticulum stress stimulates the expression of cyclooxygenase-2 through activation of NF-kappaB and pp38 mitogen-activated protein kinase. J Biol Chem 279: 46384-46392, 2004.
30. Rizzo MT, Pudlo N, Farrell L and Leaver A: Specificity of arachidonic acid-induced inhibition of growth and activation of c-jun kinases and p38 mitogen-activated protein kinase in hematopoietic cells. Prostaglandins Leukot Essent Fatty Acids 66: 31-40, 2002.
31. Gauthier ML, Pickering CR, Miller CJ, Fordyce CA, Chew KL, Berman HK and Tlsty TD: p38 regulates cyclooxygenase-2 in human mammary epithelial cells and is activated in premalignant tissue. Cancer Res 65: 1792-1799, 2005.
32. Hendrickx N, Volanti C, Moens U, *et al*: Up-regulation of cyclooxygenase-2 and apoptosis resistance by p38 MAPK in hypericin-mediated photodynamic therapy of human cancer cells. J Biol Chem 278: 52231-52239, 2003.
33. Wang XD, Kiang JG, Scheibel LW and Smallridge RC: Phospholipase C activation by $Na^+/Ca2^+$ exchange is essential for monensin-induced $Ca2^+$ influx and arachidonic acid release in FRTL-5 thyroid cells. J Investig Med 47: 388-396, 1999.
34. Smith WL and Langenbach R: Why there are two cyclooxygenase isozymes. J Clin Invest 107: 1491-1495, 2001.
35. Reddy BS, Rao CV and Seibert K: Evaluation of cyclooxygenase-2 inhibitor for potential chemopreventive properties in colon carcinogenesis. Cancer Res 56: 4566-4569, 1996.
36. Tsujii M, Kawano S and DuBois R: Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential. Proc Natl Acad Sci USA 94: 3336-3340, 1997.
37. Oshima M, Dinchuk JE, Kargman SL, *et al*: Suppression of intestinal polyposis in Apc delta716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). Cell 87: 803-809, 1996.
38. Wolff H, Saukkonen K, Anttila S, Karjalainen A, Vainio H and Ristimaki A: Expression of cyclooxygenase-2 in human lung carcinoma. Cancer Res 58: 4997-5001, 1998.
39. Chan G, Boyle JO, Yang EK, Zhang F, *et al*: Cyclooxygenase-2 expression is up-regulated in squamous cell carcinoma of the head and neck. Cancer Res 59: 991-994,1999.
40. Higashi Y, Kanekura T and Kanzaki T: Enhanced expression of cyclooxygenase (COX)-2 in human skin epidermal cancer cells: evidence for growth suppression by inhibiting COX-2 expression. Int J Cancer 86: 667-671, 2000.