**Non-steroidal anti-inflammatory drugs are safe with respect to the transcriptome of human dermal fibroblasts**

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**ABSTRACT**

Non-steroidal anti-inflammatory drugs (NSAIDs) provide important benefits to millions of patients, but are associated with a number of serious adverse events. These adverse drug reactions are an important clinical issue and a serious public health risk. While most unfortunate responses in human to NSAIDs are mild and may disappear after decreasing the dose or withdrawal of the drug, some of them can produce serious outcomes. Currently, little is known regarding the effects of NSAIDs on global RNA expression in normal, non-transformed cells. Therefore, in this report, the effect of NSAIDs, COX-nonspecific and COX-2-specific inhibitors, indomethacin and nimesulide respectively, commonly used medications worldwide for the reduction of pain, fever, inflammation and stiffness, on transcriptomic signature of human dermal fibroblasts was investigated. A total of 3,803 differentially expressed genes with a fold change greater than or equal to 1.3 and below than or equal to 0.7 for whole genome transcripts, with a P value of < 0.05 were identified in response to all applied conditions. We found that although the total number of deregulated genes was relatively high at such criteria, changes in fibroblast transcriptome profile after treatment at selected experimental conditions were however smallish, as the selected drugs slightly modulate transcriptome with only a few genes with expression altered a bit more than twice. Nevertheless, transcriptomic data has its own limitations and it cannot reflect all post-transcriptional changes, which in turn may cause same risks, especially for a long time of medication.

**Chemical compounds studied in this article**

Indomethacin (PubChem CID: 3715); Nimedulide (PubChem CID: 4495)

**Keywords**

Non-steroidal anti-inflammatory drugs (NSAIDs), Gene expression profiling, Cell cycle, Fibroblasts**1.** **INTRODUCTION**

Non-steroidal anti-inflammatory drugs (NSAIDs) are primarily used as analgesics, antipyretics and anti-inflammatory agents, which are among the most widely used medications worldwide (Conaghan, 2012). It is estimated that everyday about 30 million people consume NSAIDs (Sostres et al., 2013). The main mechanism of action of non-steroidal anti-inflammatory drugs is reduction of prostaglandins’ (PGs) biosynthesis by inhibition the activity of the cyclooxygenase (COX) enzymes. The production of prostaglandins is catalyzed by two COX isoenzymes, commonly referred to as COX-1 and COX-2 (Su and O’Connor, 2013). COX-1 is constitutively expressed in many tissues and its metabolic products are considered to be involved in cellular housekeeping functions. In contrast to COX-1, the expression of COX-2 is highly inducible by various inflammatory mediators and mitogens (Bernardi et al., 2006). Interestingly, COX-2 is significantly up-regulated in a variety of neurological disorders (e.g. Alzheimer’s and Parkinson’s diseases) and numerous types of human tumors, including colorectal, gastric, lung and breast cancers (Ettarh et al., 2010; Gasparini et al., 2004; Tian et al., 2012; Yang et al., 2016). Currently, a growing amount of evidence from numerous *in vitro* and *in vivo* experiments suggests positive effects of NSAIDs, used in combination with other therapies, in achieving additive or synergistic benefits in numerous cancers (Huerta et al., 2015; Liu et al., 2015; Özalp et al., 2012; Stolfi et al., 2013; Trask et al., 2004). However, the exact mechanism underlying cancer preventive and cancer therapeutic actions of NSAIDs is still largely unknown (Hilovska et al., 2014). Moreover, considering NSAIDs with other agents as a potential adjunctive approach for therapy of neuronopathic forms of lysosomal storage disorders (LSDs) appears reasonable (Jeyakumar et al., 2004; Mozolewski et al., 2017; Smith et al., 2009; Williams et al., 2014).

So far, the results from transcriptomic studies reported in the literature concern mostly human cancer cells treated with a high (typically used in preclinical studies) doses of cyclooxygenases inhibitors. Currently, little is known regarding the effects of NSAIDs on global RNA expression in normal, non-transformed cells. Therefore, the purpose of this study was to examine the effect of COX-nonspecific and COX-2-specific inhibitors, indomethacin (2-[1-(4-chlorobenzoyl)-5-methoxy-2-methylindol-3-yl]acetic acid) and nimesulide (N-[4-nitro-2-phenoxyphenyl]-methanesulfonamide) respectively. The concentrations of indomethacin (2 and 10 µmol/l) and nimesulide (5 and 25 µmol/l) used in this work are close to clinically relevant molar doses (Dingle, 1999; Liu et al., 2015; Miura et al., 2004). We used transcriptomic approach to examine global gene expression changes, as well as cell viability and proliferation test to study development of ﬁbroblasts exposed to different concentrations of tested compounds. We also analyzed cell cycle to investigate whether tested drugs can change DNA content in human dermal fibroblasts. Moreover, we tested whether the cell cycle is affected after treatment with selected NSAIDs in cells of patients suffering from mucopolysaccharidoses (MPS), according to recent studies concerning specific disturbances in the cell cycle of MPS type II fibroblasts (Moskot et al., 2016).

**2. MATERIALS AND METHODS**

**2.1 Cell cultures, drugs solutions, supplements**

Human Dermal Fibroblasts, adult (HDFa) (Cascade Biologics, Portland, USA) and MPS fibroblasts type I and II (Children’s Memorial Health Institute, Warsaw, Poland) were cultured in Dulbecco’s modified Eagle’s medium (DMEM, Thermo Fisher Scientific Inc., Paisley, UK) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic/antimycotic solution (Sigma-Aldrich Co. LLC., St. Louis, USA) at 37 °C in a humidified atmosphere of 5% CO2. The non-selective COX inhibitor indomethacin and the selective COX-2 inhibitor nimesulide (Sigma-Aldrich Co. LLC., St. Louis, USA) were dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich Co. LLC., St. Louis, USA) and used at a desired concentration.

**2.2 Cytotoxicity and proliferation assays**

The cell growth and proliferation was assessed using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) (Sigma-Aldrich Co. LLC., St. Louis, USA) which is reduced by viable cells into a purple colored formazan product (Mosmann, 1983). Cells were plated in flat-bottomed 96-well plates in a number of 6 x 103 cells per well (cytotoxicity assay) or 103 cells per well (proliferation assay), and after 24 h they were treated with indicated concentration of indomethacin, nimesulide or 0.05% DMSO (Control) for 24 h, 48 h, and 7 days at 37 °C and 5% CO2. Then, medium was replaced with RPMI (Sigma-Aldrich Co. LLC., St. Louis, USA) supplemented with MTT (1 mg/ml) for another 4 h. The purple formazan crystals (proportional to the number of viable cells) were dissolved in 150 μl of 100% DMSO, and absorbance was determined at 570 nm using Wallac 1420 Multilabel Counter (Perkin Elmer Inc., MA, USA).

**2.3 RNA extraction**

Total RNA was extracted from cells using the High Pure RNA Isolation Kit (Roche Applied Science, IN, USA) and quantified with the Quant-itTM RiboGreen® assay kit (Thermo Fisher Scientific Inc., Paisley, UK) following the manufacturer’s instructions. In addition, the quality of each RNA sample was assessed using the RNA 6000 Nano Assay on the Agilent 2100 Bioanalyzer (Agilent Technologies Inc., CA, USA).

**2.4 Microarray assay performance and statistical analysis**

Whole genome microarray analysis of three biological replicates was performed using Illumina’s Human HT-12v4 Expression BeadChips, targeting more than 25,000 genes with more than 48,000 probes (Illumina Inc., CA, USA), for all tested conditions. BeadChips were scanned using an Illumina BeadArray Reader and the Bead Scan Software (Illumina Inc., CA, USA). The quality of microarray data was controlled by examining raw and adjusted intensity histograms. The assay performance and data extraction was done as described previously (Moskot et al., 2014). All gene expression data have been deposited in the NCBI Gene Expression Omnibus (GEO series accession number GSE63239), according to the MIAME (minimum information about a microarray experiment) standards. An overview of experiment performance was gained by clustering samples using a correlation metric (Illumina® BeadStudio Data Analysis software). The Pearson correlation coefficient method was used to calculate ‘expression distance values’ across experiments and to group samples that have similar expression patterns. The values ranging between 0.98 and 0.99 for biological replicates indicate a high degree of reproducibility and strong correspondences between expression profiles. Genes were considered to be significantly differentially expressed if they obtained a fold change (FC) greater than or equal to 1.3 and below than or equal to 0.7 for whole genome transcripts, respectively, with a P value of < 0.05.

**2.5 Cell cycle analysis**

Human dermal fibroblasts, adult, were seeded into 6-well plate at a density of 1 × 104 cells per well. The next day, the standard DMEM medium was replaced with fresh one containing appropriate amounts of selected drugs or DMSO only used as control. After 24 h cells were harvested by trypsinization and DNA content was analyzed using the MUSE® Cell Analyzer (Merck Millipore, Germany) with a Millipore’s Muse Cell Cycle Assay Kit (Catalog No. MCH100106, Merck Millipore, Germany), according to the manufacturer’s instructions. Briefly, cells samples were centrifuged at 300 x *g* for 5 minutes and after removing the supernatant, 1 ml of PBS per 1 × 106 cells was added to each tube. Then, appropriate volume of ice cold 70% ethanol was added to the re-suspending cell pellet in the residual PBS for the cell fixation. After 3 h, fixed cells were centrifuged again at 300 x *g* for 5 minutes and cell pellet was re-suspended in 200 µl of Muse Cell Cycle Reagent, and incubated for 30 min at room temperature, in the dark. For analysis, samples were transferred into 1.5 ml microcentrifuge tubes. Three independent experiments were conducted, and the mean propidium iodide (PI) fluorescence intensity was obtained from 10,000 cells.

**3. RESULTS**

The cytotoxicity effect of selected drugs was measured after three different periods of time (24 h, 48 h and 7 days), and human dermal fibroblasts were exposed to a range of concentrations of each agent. It is worth noting that the results presented in our recent studies indicated that indomethacin (IN) and nimesulide (NIM) had little cytotoxic effect on human dermal fibroblast, when used at clinically achievable concentrations. However, statistically significant (P < 0.05, ANOVA with Tukey’s HSD post-hoc test) adverse effect on cell growth was observed especially for indomethacin (Mozolewski et al., 2017). In this study estimated concentrations of tested compounds lethal to 25, 50 or 75% (LC25, LC50 or LC75 respectively) of HDFa cells in cultures exposed to selected drugs for 7 days (Tab. a). By definition, the lower concentration of tested drug causing the death of indicated number of cells the more cytotoxic activity is revealed. The lethal concentrations of indomethacin were noticeably lower than those observed for nimesulide, thus confirming that cytotoxic effects occurred more acutely after treatment with indomethacin. Additionally, during cytotoxicity assays, light microscopy observations were conducted in this study. No influence of tested substances on cell morphology was visible (data not shown).

There is a significant knowledge gap regarding the effects of non-steroidal anti-inflammatory drugs on global RNA expression in cells. To test the effect of selected NSAIDs on whole-transcriptome profile studies using microarray technique were performed. In the previous work we found that non-steroidal anti-inflammatory drugs modulate cellular glycosaminoglycan synthesis by affecting epidermal growth factor receptor (EGFR) and phosphoinositide 3-kinase (PI3K) signaling pathways (Mozolewski et al., 2017), while in current work, we put emphasis on global modulation of transcriptome in the context of their safety in normal, untransformed, human cells. Our microarray results were filtered based on desired patterns of expression (FC of gene expression ratios was ≥ 1.3 and ≤ 0.7, P < 0.05) according to our previous work. We found that changes in HDFa transcriptome signature after treatment at selected experimental conditions were smallish between analyzed samples. Although total number of significantly up- and down-regulated genes (i.e. 3,803 genes) is relatively high (Tab. b), it is worth to point out that the level of observed changes is negligible, as the total number of differentially expressed genes have decreased tenfold (i.e. to 377 genes) after analysis with the FC ≥ 1.5 in either direction. It has to be taken into account that this cut off value is also used very often in transcriptomic studies, however implementation of this rate for our studies allowed us to detect much reduced number of genes with changes in their activity. The most altered expression levels of genes under indicated conditions are shown in Tab. c and d. It is worth noticing that, among the most modulated genes more than 20% have uncertain function. The highest number of genes with modulated expression in fibroblasts is observed after 24 h of treatment with 25 µM nimesulide, as is the number of the most up- and down-regulated genes. Interestingly, we found that in all cases selected NSAIDs up-regulate genes expression more effectively rather that down-regulate (Tab. b).

To evaluate effects of selected drugs on cell cycle progression in human dermal fibroblasts, we analyzed this process, using the MUSE® Cell Analyzer. Cell percentages in G0/G1, S and G2/M phases were determined after 24 h of incubation. Moreover, we investigated whether the cell cycle is affected in fibroblasts derived from patients suffering from mucopolysaccharidosis type I and II. We found that, despite observed alterations, the HDFa treatment did not show any statistically significant changes (P < 0.05, *t*-Student test) between fractions of cells in G0/G1, S, and G2/M phases compared to the control (Fig. 1A). Similar tendency was observed in MPS I and MPS II fibroblasts (Fig. 1B and 1C), however, we noted statistically significant changes in MPS I fibroblasts (Tab. e).

**4. DISCUSSION**

In this study we have evaluated the effects of two selected non-steroidal anti-inflammatory drugs, indomethacin and nimesulide, in human dermal fibroblasts model. So far, mounting evidence suggested that NSAIDs are able to inhibit cell growth and interfere with numerous cellular pathways, for instance, cell cycle and apoptosis (Bernardi et al., 2006; Brooks et al., 2003; De Luna-Bertos et al., 2014; Valle et al., 2013). However, most of studies on human cells were performed with cancer-derived cell lines, and to our knowledge, there are only few reports concerning non-transformed cells (Brooks et al., 2003; Chang et al., 2009, 2007; Nguyen and Lee, 1992). Moreover, in vast majority of works, high or very high concentrations of NSAIDs were used. Our group previously reported that therapeutic doses of indomethacin and nimesulide after 24 h of treatment can significantly modulate expression of genes involved in crucial metabolic pathways (Mozolewski et al., 2017). To our knowledge, there are no reports about effects of indomethacin and nimesulide on global gene expression profile of human dermal fibroblasts. These data might however be important from the point of view of the safety of medicines, including the long-term treatments. Thus, in this study we put emphasis on quantitative changes in HDFa transcriptome and show that selected drugs slightly modulate transcriptome with only a few genes with expression altered more than twice (Tab. b, c and d). Moreover, our recent studies have revealed modulation of expression of genes involved in basic metabolism which, as demonstrated recently, may contribute to cell cycle progression (Konieczna et al., 2015). Some previous studies have shown that indomethacin and nimesulide can affect cell growth by increasing G0/G1 cells population in a range of cell types, mostly tumor (Brooks et al., 2003; De Luna-Bertos et al., 2014; Díaz-Rodríguez et al., 2012; Ricchi et al., 2002; Valle et al., 2013). Since our transcriptomic analysis has revealed that some of the most up- and down-regulated genes are involved in regulation of cell cycle we have analyzed the proportion of cells in the G0/G1, S, and G2/M phase after 24 h treatment with 10 µM indomethacin or 25 µM nimesulide. In the previous study the most significant inhibition for both drugs was observed after 24 h (Mozolewski et al., 2017), thus, this time of exposure was implemented in the study of cell cycle progression. Interestingly, we did not find any significant changes in the normal HDFa cell distribution after drugs treatment compared to control (Tab. e).

Our previously reported results have shown that indomethacin and nimesulide, especially in the combination with isoflavone genistein, can be considered as a method for improvement of efficiency of therapy for mucopolysaccharidoses (Mozolewski et al., 2017). Thus, in this study we decided to investigate the influence of these two drugs on cell cycle in fibroblasts from patients suffering from mucopolysaccharidosis type I and II. We detected a slight, but statistically significant effects of tested compounds only in MPS I fibroblasts (Tab. e). Interestingly, we noticed an increased (P < 0.05) G2/M fraction of MPS I and MPS II cells in comparison to HDFa cells (Fig. 1). These data are partially consistent with recently published results, which showed that after 24 h of incubation G2/M fraction of MPS II fibroblasts increased relative to normal fibroblasts (Moskot et al., 2016).

So far, the main focus has been put on investigating the role of NSAIDs in variety of tumors cell lines and very little attention has been given to the effect of these drugs on untransformed cells. The advantage of this study, in relation to previous works is that the results presented here were obtained from normal, human dermal fibroblasts. It is worth to mention, that there are some contradictory results concerning effects of NSAIDs on cell cycle, probably dependent on chemical classes, doses and duration of treatment (Bernardi et al., 2006; Brooks et al., 2003; De Luna-Bertos et al., 2014). Therefore, better comparative data from *in vitro* studies, using clinically realistic doses of NSAIDs, are required. As there is growing evidence in the literature that NSAIDs can affect crucial cellular pathways leading to numerous changes (Gasparini et al., 2004; Hilovska et al., 2014; Özalp et al., 2012), it is important to expand our knowledge concerning non-steroidal anti-inflammatory drugs.

**COMPETING INTERESTS**

The authors declare that they have no conflict of interest.

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**REFERENCES**

Bernardi, A., Jacques-Silva, M.C., Delgado-Cañedo, A., Lenz, G., Battastini, A.M.O., 2006. Nonsteroidal anti-inflammatory drugs inhibit the growth of C6 and U138-MG glioma cell lines. Eur. J. Pharmacol. 532, 214–222. doi:10.1016/j.ejphar.2006.01.008

Brooks, G., Yu, X.-M., Wang, Y., Crabbe, M.J.C., Shattock, M.J., Harper, J. V., 2003. Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit vascular smooth muscle cell proliferation via differential effects on the cell cycle. J. Pharm. Pharmacol. 55, 519–526. doi:10.1211/002235702775

Chang, J.-K., Li, C.-J., Liao, H.-J., Wang, C.-K., Wang, G.-J., Ho, M.-L., 2009. Anti-inflammatory drugs suppress proliferation and induce apoptosis through altering expressions of cell cycle regulators and pro-apoptotic factors in cultured human osteoblasts. Toxicology 258, 148–56. doi:10.1016/j.tox.2009.01.016

Chang, J.-K., Li, C.-J., Wu, S.-C., Yeh, C.-H., Chen, C.-H., Fu, Y.-C., Wang, G.-J., Ho, M.-L., 2007. Effects of anti-inflammatory drugs on proliferation, cytotoxicity and osteogenesis in bone marrow mesenchymal stem cells. Biochem. Pharmacol. 74, 1371–82. doi:10.1016/j.bcp.2007.06.047

Conaghan, P.G., 2012. A turbulent decade for NSAIDs: update on current concepts of classification, epidemiology, comparative efficacy, and toxicity. Rheumatol. Int. 32, 1491–502. doi:10.1007/s00296-011-2263-6

De Luna-Bertos, E., Ramos-Torrecillas, J., Manzano-Moreno, F.J., García-Martínez, O., Ruiz, C., 2014. Effects on Growth of Human Osteoblast-Like Cells of Three Nonsteroidal Anti-Inflammatory Drugs Metamizole, Dexketoprofen, and Ketorolac. Biol. Res. Nurs. 17, 1099800414527155. doi:10.1177/1099800414527155

Díaz-Rodríguez, L.., García-Martínez, O.., Arroyo-Morales, M.., Rodríguez-Pérez, L.., Rubio-Ruiz, B.., Ruiz, C.. d, 2012. Effects of indomethacin, nimesulide, and diclofenac on human mg-63 osteosarcoma cell line. Biol. Res. Nurs. 14, 98–107. doi:10.1177/1099800411398933

Dingle, J.T., 1999. The effects of NSAID on the matrix of human articular cartilages. Z. Rheumatol. 58, 125–129. doi:10.1007/s003930050161

Ettarh, R., Cullen, A., Calamai, A., 2010. NSAIDs and cell proliferation in colorectal cancer. Pharmaceuticals 3, 2007–2021. doi:10.3390/ph3072007

Gasparini, L., Ongini, E., Wenk, G., 2004. Non-steroidal anti-inflammatory drugs (NSAIDs) in Alzheimer’s disease: Old and new mechanisms of action. J. Neurochem. 91, 521–536. doi:10.1111/j.1471-4159.2004.02743.x

Hilovska, L., Jendzelovsky, R., Fedorocko, P., 2014. Potency of non-steroidal anti-inflammatory drugs in chemotherapy (Review). Mol. Clin. Oncol. 3–12. doi:10.3892/mco.2014.446

Huerta, C., Aberturas, M. del R., Molpeceres, J., 2015. Nimesulide-loaded nanoparticles for the potential coadjuvant treatment of prostate cancer. Int. J. Pharm. 493, 152–160. doi:10.1016/j.ijpharm.2015.07.027

Jeyakumar, M., Smith, D.A., Williams, I.M., Borja, M.C., Neville, D.C.A., Butters, T.D., Dwek, R.A., Platt, F.M., 2004. NSAIDs increase survival in the Sandhoff disease mouse: Synergy withN-butyldeoxynojirimycin. Ann. Neurol. 56, 642–649. doi:10.1002/ana.20242

Konieczna, A., Szczepańska, A., Sawiuk, K., Węgrzyn, G., Łyżeń, R., 2015. Effects of partial silencing of genes coding for enzymes involved in glycolysis and tricarboxylic acid cycle on the enterance of human fibroblasts to the S phase. BMC Cell Biol. 16, 16. doi:10.1186/s12860-015-0062-8

Liu, J.-R., Wu, W.-J., Liu, S.-X., Zuo, L.-F., Wang, Y., Yang, J.-Z., Nan, Y.-M., 2015. Nimesulide inhibits the growth of human esophageal carcinoma cells by inactivating the JAK2/STAT3 pathway. Pathol. Res. Pract. 211, 426–434. doi:10.1016/j.prp.2015.01.007

Miura, S., Tatsuguchi, A., Wada, K., Takeyama, H., Shinji, Y., Hiratsuka, T., Futagami, S., Miyake, K., Gudis, K., Mizokami, Y., Matsuoka, T., Sakamoto, C., 2004. Cyclooxygenase-2-regulated vascular endothelial growth factor release in gastric fibroblasts. AJP Gastrointest. Liver Physiol. 287, G444–G451. doi:10.1152/ajpgi.00537.2003

Moskot, M., Gabig-Cimińska, M., Jakóbkiewicz-Banecka, J., Wesięrska, M., Bocheńska, K., Węgrzyn, G., 2016. Cell cycle is disturbed in mucopolysaccharidosis type II fibroblasts, and can be improved by genistein. Gene 585, 100–103. doi:10.1016/j.gene.2016.03.029

Moskot, M., Montefusco, S., Jakóbkiewicz-Banecka, J., Mozolewski, P., Węgrzyn, A., Di Bernardo, D., Węgrzyn, G., Medina, D.L., Ballabio, A., Gabig-Cimińska, M., 2014. The phytoestrogen genistein modulates lysosomal metabolism and transcription factor EB (TFEB) activation. J. Biol. Chem. 289, 17054–69. doi:10.1074/jbc.M114.555300

Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods 65, 55–63.

Mozolewski, P., Moskot, M., Jakóbkiewicz-Banecka, J., Węgrzyn, G., Bocheńska, K., Banecki, B., Gabig-Cimińska, M., 2017. Nonsteroidal anti-inflammatory drugs modulate cellular glycosaminoglycan synthesis by affecting EGFR and PI3K signaling pathways. Sci. Rep. 7, 43154. doi:10.1038/srep43154

Nguyen, K.D., Lee, D. a, 1992. Effect of steroids and nonsteroidal antiinflammatory agents on human ocular fibroblast. Invest. Ophthalmol. Vis. Sci. 33, 2693–2701.

Özalp, S.S., Eren, C.Y., Bostancioglu, R.B., Koparal, A.T., 2012. Induction of apoptosis and inhibition of cell proliferation by the cyclooxgenase enzyme blocker nimesulide in the Ishikawa endometrial cancer cell line. Eur. J. Obstet. Gynecol. Reprod. Biol. 164, 79–84. doi:10.1016/j.ejogrb.2012.05.018

Ricchi, P., Di Matola, T., Ruggiero, G., Zanzi, D., Apicella, a, di Palma, a, Pensabene, M., Pignata, S., Zarrilli, R., Acquaviva, a M., 2002. Effect of non-steroidal anti-inflammatory drugs on colon carcinoma Caco-2 cell responsiveness to topoisomerase inhibitor drugs. Br. J. Cancer 86, 1501–1509. doi:10.1038/sj.bjc.6600289

Smith, D., Wallom, K.-L., Williams, I.M., Jeyakumar, M., Platt, F.M., 2009. Beneficial effects of anti-inflammatory therapy in a mouse model of Niemann-Pick disease type C1. Neurobiol. Dis. 36, 242–251. doi:10.1016/j.nbd.2009.07.010

Sostres, C., Gargallo, C.J., Lanas, A., 2013. Nonsteroidal anti-inflammatory drugs and upper and lower gastrointestinal mucosal damage. Arthritis Res. Ther. 15, 1–8. doi:10.1186/ar4175

Stolfi, C., De Simone, V., Pallone, F., Monteleone, G., 2013. Mechanisms of action of non-steroidal anti-inflammatory drugs (NSAIDs) and mesalazine in the chemoprevention of colorectal cancer. Int. J. Mol. Sci. 14, 17972–17985. doi:10.3390/ijms140917972

Su, B., O’Connor, J.P., 2013. NSAID therapy effects on healing of bone, tendon, and the enthesis. J. Appl. Physiol. 115, 892–9. doi:10.1152/japplphysiol.00053.2013

Tian, J.J., Lu, S.M., Yu, L., Ma, J.K., Mu, Y.K., Wang, H.B., Xu, W., 2012. Nimesulide inhibited the growth of hypopharyngeal carcinoma cells via suppressing Survivin expression. Head Neck Oncol. 4, 2–9. doi:10.1186/1758-3284-4-7

Trask, D., Bock, J., Goswami, P., Menon, S., 2004. Celecoxib and sulindac sulfide enhance the cytotoxic effect of standard chemotherapeutic agents and possess anti-tumor activity mediated by p21(cipl/waf1) expression. J. Clin. Oncol. 22, 3186.

Valle, B.L., D’Souza, T., Becker, K.G., Wood, W.H., Zhang, Y., Wersto, R.P., Morin, P.J., 2013. Non-Steroidal Anti-inflammatory Drugs Decrease E2F1 Expression and Inhibit Cell Growth in Ovarian Cancer Cells. PLoS One 8. doi:10.1371/journal.pone.0061836

Williams, I.M., Wallom, K.-L., Smith, D.A., Al Eisa, N., Smith, C., Platt, F.M., 2014. Improved neuroprotection using miglustat, curcumin and ibuprofen as a triple combination therapy in Niemann–Pick disease type C1 mice. Neurobiol. Dis. 67, 9–17. doi:10.1016/j.nbd.2014.03.001

Yang, H., Yin, P., Shi, Z., Ma, Y., Zhao, C., Zheng, J., Chen, T., 2016. Sinomenine, a COX-2 inhibitor, induces cell cycle arrest and inhibits growth of human colon carcinoma cells in vitro and in vivo. Oncol. Lett. 11, 411–418. doi:10.3892/ol.2015.3838

**FIGURE LEGENDS**

Fig. 1. Cell percentages in G0/G1, S and G2/M phases in HDFa (a), MPS I (b) and MPS II (c) fibroblasts after 24 h treatment with 0.05% DMSO (Control), 10 µM indomethacin (IN) or 25 µM nimesulide (NIM). Experiments were in triplicate and the mean was obtained from 10,000 cells for each condition.

Tab. a Concentration of tested compounds that is lethal (LC) to 25, 50 or 75% of HDFa cells in a culture exposed to selected drugs for 7 days.

|  |  |  |  |
| --- | --- | --- | --- |
| **7-days of exposure** | **LC25 (µM)** | **LC50 (µM)** | **LC75 (µM)** |
| Indomethacin | 80 | 250 | 430 |
| Nimesulide | 260 | 550 | 850 |

Tab. b Number of genes whose expression was altered as a function of the treatment type identified in the microarray analysis among whole genome sequences and transcripts of HDFa cells (significantly differentially expressed genes had a fold change as described in the table, respectively, for whole genome, with a P value of < 0.05, *n* ≥ 3 experiments).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Fold change range for genes with altered expression** | **Concentration [µM]** | | | | | | | |
| **Indomethacin** | | | | **Nimesulide** | | | |
| **2** | | **10** | | **5** | | **25** | |
| **Time of exposure [h]** | | | | | | | |
| **24** | **48** | **24** | **48** | **24** | **48** | **24** | **48** |
| 1.5 ≤ FC < 3.0  1.3 ≤ FC < 1.5 | 13 | 17 | 64 | 51 | 25 | 38 | 295 | 0 |
| 68 | 92 | 236 | 211 | 170 | 159 | 435 | 511 |
| 0.5 < FC ≤ 0.7  0.3 ≤ FC ≤ 0.5 | 40 | 26 | 249 | 160 | 43 | 124 | 503 | 236 |
| 0 | 1 | 5 | 1 | 1 | 4 | 19 | 6 |

Tab. c Selected genes with the most changed expression profile (3 most up- and down-regulated) after 24- and 48 h treatment with indomethacin. The symbols of genes that were up-regulated are bolded and genes present in more than one conditions are underlined.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Conditions** | | **Gene/Locus** | **Description** | **Fold change**  **± S.D.** |
| **2 µM** | **24 h** | ***ZNF346*** | **Zinc Finger Protein 346** | **1.76 ± 0.47** |
| ***LOC729806*** | **uncertain function** | **1.69 ± 0.20** |
| ***MIR1974*** | **microRNA 1974** | **1.69 ± 0.25** |
| *PROSAPIP1* | Proline Rich Synapse Associated Protein Interacting Protein 1 | 0.61 **±** 0.10 |
| *LOC651075* | uncertain function | 0.60 ± 0.06 |
| *PIGL* | Phosphatidylinositol-Glycan Biosynthesis Class L Protein | 0.57 ± 0.13 |
| **48 h** | ***LOC654116*** | **uncertain function** | **1.78 ± 0.45** |
| ***SPHAR*** | **S-Phase Response Protein** | **1.75 ± 0.40** |
| ***SLC12A8*** | **Solute Carrier Family 12 Member 8** | **1.74 ± 0.11** |
| *PRUNE* | Prune Exopolyphosphatase | 0.66 ± 0.14 |
| *YKT6* | YKT6 V-SNARE Homolog (S. Cerevisiae) | 0.56 ± 0.08 |
| *LOC653629* | uncertain function | 0.51 ± 0.09 |
| **10 µM** | **24 h** | ***NEK7*** | **NIMA Related Kinase 7** | **2.32 ± 0.76** |
| ***SOCS6*** | **Suppressor Of Cytokine Signaling 6** | **2.15 ± 0.55** |
| ***UBXN7*** | **UBX Domain Protein 7** | **1.92 ± 0.56** |
| *SYMPK* | Symplekin | 0.54 ± 0.06 |
| *LOC651881* | uncertain function | 0.53 ± 0.03 |
| *ZNF579* | Zinc Finger Protein 579 | 0.52 ± 0.00 |
| **48 h** | ***PLEKHF2*** | **Pleckstrin Homology And FYVE Domain Containing 2** | **2.30 ± 0.67** |
| ***LOC730167*** | **uncertain function** | **2.15 ± 0.12** |
| ***LOC653803*** | **uncertain function** | **1.92 ± 0.22** |
| *METT10D* | Methyltransferase 10 Domain-Containing Protein | 0.56 ± 0.03 |
| *SYMPK* | Symplekin | 0.56 ± 0.10 |
| *TMEM63B* | Transmembrane Protein 63B | 0.48 ± 0.07 |

Tab. d Selected genes with the most changed expression profile (3 most up- and down-regulated) after 24- and 48 h treatment with nimesulide. The symbols of genes that were up-regulated are bolded and genes present in more than one conditions are underlined.

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| **Conditions** | | **Gene/Locus** | **Description** | **Fold change**  **± S.D.** |
| **5 µM** | **24 h** | ***SACS*** | **Sacsin Molecular Chaperone** | **2.05 ± 0.13** |
| ***PTGER4*** | **Prostaglandin E Receptor 4** | **1.94 ± 0.10** |
| ***SOCS6*** | **Suppressor Of Cytokine Signaling 6** | **1.76 ± 0.13** |
| *SNORD25* | Small Nucleolar RNA, C/D Box 25 | 0.57 ± 0.05 |
| *TNRC6B* | Trinucleotide Repeat Containing 6B | 0.56 ± 0.26 |
| *ASAP1IT1* | ASAP1 Intronic Transcript 1 | 0.54 ± 0.27 |
| **48 h** | ***PLEKHF2*** | **Pleckstrin Homology And FYVE Domain Containing 2** | **2.07 ± 0.25** |
| ***SPHAR*** | **S-Phase Response Protein** | **1.91 ± 0.27** |
| ***NKAP*** | **NFKB Activating Protein** | **1.73 ± 0.20** |
| *LOC650840* | uncertain function | 0.54 ± 0.02 |
| *PHACS* | ACC Synthase-Like Protein 1 | 0.52 ± 0.05 |
| *MFHAS1* | Malignant Fibrous Histiocytoma Amplified Sequence 1 | 0.51 ± 0.10 |
| **25 µM** | **24 h** | ***TXNL1*** | **Thioredoxin Like 1** | **2.65 ± 0.47** |
| ***LOC653631*** | **uncertain function** | **2.51 ± 0.77** |
| ***SBDS*** | **SBDS Ribosome Assembly Guanine Nucleotide Exchange Factor** | **2.50 ± 0.47** |
| *MKI67* | Marker Of Proliferation Ki-67 | 0.45 ± 0.04 |
| *RECQL4* | RecQ Like Helicase 4 | 0.44 ± 0.02 |
| *WDR62* | WD Repeat Domain 62 | 0.42 ± 0.02 |
| **48 h** | ***LOC730083*** | **uncertain function** | **2.36 ± 0.14** |
| ***TUBE1*** | **Tubulin Epsilon 1** | **2.26 ± 0.22** |
| ***LOC730167*** | **uncertain function** | **2.10 ± 0.25** |
| *XIST* | X Inactive Specific Transcript (Non-Protein Coding) | 0.48 ± 0.36 |
| *TAF1* | TATA-Box Binding Protein Associated Factor 1 | 0.45 ± 0.10 |
| *MFHAS1* | Malignant Fibrous Histiocytoma Amplified Sequence 1 | 0.42 ± 0.08 |

Tab. eNumerical data for cell cycle fluorescence profile of HDFa, MPS I and MPS II fibroblasts in culture treated for 24 h with indomethacin (IN) dose of 10 µM or nimesulide (NIM) dose of 25 µM. The indicated data represent mean values ± S.D. from 3 independent experiments. Asterisks indicate P < 0.05 in *t*-Student test if the difference untreated (Control) and treated cells is statistically significant.

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| **Cultured cells** | **Conditions** | **G0/G1** | **S** | **G2/M** |
| **Mean ± S.D.** | **Mean ± S.D.** | **Mean ± S.D.** |
| HDFa | Control | 46.8 ± 6.95 | 25.0 ± 2.37 | 25.2 ± 7.65 |
| 10 µM IN | 56.4 ± 0.84 | 24.8 ± 0.21 | 17.5 ± 0.81 |
| 25 µM NIM | 49.7 ± 8.56 | 24.7 ± 1.13 | 22.9 ± 5.44 |
| MPS I fibroblasts | Control | 57.7 ± 1.10 | 28.1 ± 1.62 | 12.8 ± 0.50 |
| 10 µM IN | 60.7 ± 0.70  **(*p* = 0.016)\*** | 27.9 ± 0.74 | 10.2 ± 0.56  **(*p* = 0.003)\*** |
| 25 µM NIM | 60.1 ± 0.35  **(*p* = 0.021)\*** | 26.3 ± 2.19 | 12.5 ± 2.04 |
| MPS II fibroblasts | Control | 66.1 ± 1.80 | 21.8 ± 0.95 | 10.7 ± 1.81 |
| 10 µM IN | 67.4 ± 1.27 | 20.2 ± 3.72 | 10.9 ± 4.89 |
| 25 µM NIM | 66.5 ± 0.21 | 21.5 ± 3.15 | 10.0 ± 3.60 |