Cellular and Molecular Biology

Atari -Hajipirloo *et al. Cell. Mol. Biol.*2016, 62 (2): 68-74
ISSN: 1165-158X
doi: 10.14715/cmb/2016.62.2.12

Original Research

The effect of celecoxib and its combination with imatinib on human HT-29 colorectal cancer cells: Involvement of COX-2, Caspase-3, VEGF and NF-κB genes expression

S. Atari-Hajipirloo[1], S. Nikanfar[1], A. Heydari[2], F. Noori[3], F. Kheradmand[4*]

[1] Department of Biochemistry, Urmia University of Medical Sciences, Urmia, Iran
[2] Department of Pharmacology, Faculty of Pharmacy and Center for Cellular and Molecular Research, Urmia University of Medical Sciences, Urmia, Iran
[3] Urmia Lake Research Institute, Urmia University, Urmia, Iran
[4] Department of Biochemistry, Faculty of Medicine and Center for Cellular and Molecular Research, Urmia University of Medical Sciences, Urmia, Iran

Abstract: It has been shown that combination of imatinib (IM) with other agents may have some advantages in avoiding toxicity and resistance caused by this drug. The selective cyclooxygenase-2 inhibitor, celecoxib (CX), has been known to have antitumor and chemo-sensitizing effect in the treatment of colorectal cancer. In this study, we investigated the effectiveness of CX and its combination with anticancer agent IM on human colorectal cancer HT-29 cell and their probable molecular targets. Cultured HT-29 cells were exposed to IC50 dose of CX, IM, and their combination (half dose of IC50) for 24 hours to assess their effect on proliferation inhibition by MTT assay. The caspase-3 activity was estimated in HT-29 cells with colorimetric kit. COX-2, Caspase-3, VEGF and NF-κB genes expression was also investigated using real-time PCR method. Combined treatment with IM and CX, resulted in a significant ($P<0.05$) decrease in cell viability and increased caspase-3 enzyme activity. Decreased COX-2 gene expression has been found in CX and combined treated group. Significant increase in Caspase-3 gene expression has been shown in IM and combined treated cells. In conclusion, the present in vitro study with colon cancer cell line demonstrated that CX and its combination with IM improved the anticancer activity of each component. Caspase-3 and COX-2 dependent molecular targets seem to be involved in mediating the anti-proliferative effects of IM and CX combination. Of course, the other molecular pathways are also likely to play the role and should be explored in future studies.

Key words: Imatinib, celecoxib, colorectal cancer, COX-2, Caspase-3, VEGF, NF-κB.

Introduction

Colorectal cancer is one of the most prevalent human malignancies worldwide (1). Imatinib mesylate (Glivec) is a selective tyrosine kinase inhibitor, inhibiting kinases of BCR-Abl, c-kit, and platelet-derived growth factor receptor (PDGFR) (2). This drug has FDA approval for the treatment of chronic myeloid leukemia and gastrointestinal stromal tumors (GIST) with positive c-kit expression (3). Several studies have shown that using various combinations of chemotherapeutic agents exert greater efficacy than a single agent (4-6) and may be useful in decreasing chemotherapy associated side effects.

The use of non-steroidal anti-inflammatory drugs (NSAIDs) in colon cancer treatment and prevention has attracted attention due to their anti-proliferative and apoptosis-promoting properties (7). According to *in vitro* and *in vivo* studies, cyclooxygenase2 (COX-2) inhibitor celecoxib (CX), can reduce the risk of colorectal cancer (8). In addition, this drug reduces the count of colorectal polyps in patients with familial adenomatous polyposis (9) and can enhance the antitumor efficacy of chemotherapeutic agents (10).

Chemo-preventive effects of CX on HT-29 colorectal cancer cells may be mediated by COX-2 dependent and independent mechanisms. However, the precise mechanisms are not yet known (11). Apoptosis and Caspase-3 induction has been known to be a target for cancer chemoprevention by some chemotherapeutic agents (12, 13). Besides, nuclear factor κB (NF-κB) which is important in inflammatory reaction and cell cycle control has been shown to regulate the expression of sets of genes involved in tumorigenesis (14-17). Also vascular endothelial growth factor (VEGF) is most strongly associated with tumor growth and metastasis (18). According to previous studies, CX down- or up-regulates the expression of VEGF and NF-κB in other tumoral cells (19-23). The activity of NF-κB in colon cancer cell lines is abnormally high (24) therefore, inhibition of NF-κB signaling pathway by COX-2 inhibitors including CX may improve the response of colon cancer cells to chemotherapy. For this purpose, we assessed the effects of CX alone and its combination with IM on cell viability and COX-2, Caspase-3, VEGF and NF-κB genes expression in colorectal cancer HT-29 cell line.

Materials and Methods

Cell culture and drug treatment

The HT-29 human colorectal cancer cell line was ob-

Received November 25, 2015; **Accepted** February 26, 2016; **Published** February 29, 2016

*** Corresponding author:** Fatemeh Kheradmand, Department of Biochemistry, Faculty of Medicine and Center for Cellular and Molecular Research, Urmia University of Medical Sciences, Iran. Email: fkheradmand@yahoo.com

tained from Iranian Biological Resource Center (IBRC). Cells were grown in DMEM medium with stable glutamine (pAA) supplemented with 10% FBS (pAA) and 100 units/mL of penicillin.

IM and CX were purchased from Cayman Chemical Co. (Ann Arbor, MI, USA) and Sigma-Aldrich Co. (St. Louis, MO, USA), respectively. Stock solutions of the drugs (20 mM) were prepared in dimethyl sulfoxide (DMSO) and diluted in DMEM before use. The final concentrations of drugs was selected on the basis of their IC50 values obtained in our previous work which were 7 µM for IM and 30 µM for celecoxib (26). HT-29 cells were treated with IC50 dose of IM (7 µM) and CX (30 µM) or their combination (half dose of IC50) for 24 hr. DMSO was used as the vehicle to deliver drugs and its concentration in control wells was equal to test wells.

Cell viability

Cell proliferation was analyzed by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide [MTT, Cayman Chemical Co. (Ann Arbor, MI, USA)] in triplicate. Briefly, HT29 cells (5000 cells/well) were seeded in 96-well plates. At 24 hr after seeding, cells were treated with mentioned concentrations of drugs. Then 10 µl of MTT reagent was added per well 24 hr following treatments and the plates incubated at 37˚C for another 3 hr. The reaction was stopped by removal of MTT-containing media. Thereafter, 100 µl of crystal dissolving solution was added to solubilize formazan crystals. Absorbance at 570 nm was recorded using an ELISA micro-plate reader (Stat-Fax 2100, Awareness Technology Inc.). The percent of cell viability was calculated as the absorbance ratio of treated samples compared with the untreated control.

Measurement of caspase-3 enzyme activity

The caspase-3 colorimetric assay kit (Abnova, Taiwan) was used to determine the caspase-3 enzyme activity. Briefly, HT-29 cells were seeded in 24-well tissue culture plates. After 24 hr the medium was changed and cells were treated with mentioned concentrations of drugs for 24 hr. The medium was removed and the cells were collected by centrifugation at 14,000 rpm for 5 min. Then, 50 µL of cell lysis buffer (10mM Tris–HCl pH 7.6, 150 mMNaCl, 5 mM EDTA, 1% Triton X-100) was added to the cells to resuspend them. After the lysation, cells were kept on ice for 10 min. Then, they were centrifuged at 10,000 rcf for 1 min. Protein concentration was assayed with a Bradford Protein Assay kit. Into each well of a 24-well plate, 50 µg protein was diluted by adding Cell Lysis Buffer to make a volume of 100 µl. 2X Reaction Buffer 50 µl (containing 10 mM dithiothreitol) was added to each well. 5 µL of the 4 mM DEVD-*p*NA substrate (200 µM final concentration) was added and the plates were incubated at 37°C for 2 h. The sample plate was read at 400 or 405 nm with a microtiter plate reader.

RNA extraction & cDNA synthesis

Total RNA was isolated from treated cells using the RNX-plus™ kit (CinnaGen Inc, Iran) according to the protocol provided by the manufacturer. Briefly, 1 ml of RNX-plus was added to a tube containing 10^6 homogenized cells, and the mixture was incubated at room temperature for 5 min. Chloroform was added to the solution and centrifuged at 12000 rpm for 15 min. The upper aqueous phase was then transferred to new tube and an equal volume of isopropanol was added. The mixture was then centrifuged at 12000 rpm for 15 min and the resulting pellet was then washed in 70% ethanol and dissolved in DEPC-treated water. The purity of the extracted RNA was confirmed by measuring the ratio of optical density at 260 nm to that at 280 nm and its integrity was examined by electrophoresis on agarose gel.

Total RNA was used to generate single-stranded cDNA with 2-step RT-PCR kit (Vivantis, Malaysia) by the following reaction: Purified total RNA was mixed with 1 µl of random Hexamers primer (50ng/µl), 1 µl dNTPs mix (10mM) and up to 10 µl of water. This mixture was added to 10 µl of a cDNA synthesis mix that included 2 µl of buffer M-MuLV (10X) and 100 unit M-MuLV reverse transcriptase. The mixture was incubated at 42°C for 60 min, and then for 5 min at 85°C. The synthesized cDNA was directly used as template for real time RT-PCR.

Real time RT-PCR

Primer design was done with regard to primer dimer formation, self-priming formation, and primer melting temperature, and was checked with Gene runner software. Blast search in the published sequence database Gene Bank (http://blast. ncbi. nlm. nih. gov/ Blast. cgi) revealed the primers to be gene specific (Table 1). Human beta actin gene was used as a housekeeping gene.

Real time RT-PCR was performed using the AccuPower® 2X Greenstar qPCR Master mix (Bioneer, Korea) in a total volume of 25 µl according to the manufacturer's instruction. The expression of mentioned genes

Table 1. Sequences of primers used to evaluate expression of β-actin, Caspase-3, VEGF, NF-κB and COX-2 genes in HT-29 cell line.

Target gene		Primer sequence	Product Size (bp)
β-actin	Forward	5′- CTGGAACGGTGAAGGTGACA-3′	161
	Reverse	5′-TGGGGTGGCTTTTAGGATGG-3′	
Caspase-3	Forward	5′- AGAACTGGACTGTGGCATTGAG -3′	191
	Reverse	5′-GCTTGTCGGCATACTGTTTCAG-3′	
VEGF	Forward	5'- AGGAGGAGGGCAGAATCATC-3'	144
	Reverse	5'-GGCACACAGGATGGCTTGAA-3'	
NF-κB	Forward	5'-GGAGATCGGGAAAAAGAGC-3'	315
	Reverse	5'- GACTCCACCATTTTCTTCCTC-3'	
COX-2	Forward	5'-GGAACACAACAGAGTATGCG-3'	250
	Reverse	5'-AAGGGGATGCCAGTGATAGA-3'	

was analyzed using an iQ5 Real-Time PCR detection system (Bio-Rad, CA, USA). In addition, a no template control was used to test the potential contamination and primer dimer formation. The reactions were prepared in a 96-well optical plate for 10 min at 95˚C followed by 40 cycles of 20 sec at 95˚C and 45 sec at 59˚C. A melting curve analysis was conducted to confirm the specificity of the amplification reactions. Each sample was replicated three times. The relative expression of each mRNA was calculated using the $2^{-\Delta\Delta Ct}$ method, where Ct is the threshold cycle (27). Relative expression levels of mRNA were normalized to β-actin and analyzed for statistical significance.

Statistical analysis

All data were presented as mean ± standard error for at least three separate experiments for each treatment. Statistical significance of differences between mean values was analyzed by one way ANOVA followed by Tukey's HSD post-hoc test using SPSS 16 statistical analysis software (SPSS Inc. Chicago, IL). The level of significant difference was set at $P<0.05$. The fold differences of gene expression normalized to control was presented graphically in the form of histograms, using Microsoft Excel computer program.

Results

Interactions between IM and CX in the inhibition of HT-29 cell viability

The percentage of viable cells detected after treatment with CX (15 μM) in combination with at half dose of IC50, 15 μM) was reduced to 40% compared to the control ($P<0.05$), whereas, lower effects were produced by treatments with higher concentrations of CX (30 μM) or IM (7 μM) alone (Figure 1).

IM in combination with celecoxib induced caspase-3 activity in HT-29 cells

Caspase-3 activity studies reported an increase in the activity levels in HT-29 cells treated with CX (15 μM) in combination with IM (at half dose of IC50, 3.5 μM) approximately 60% compared to the control ($P<0.001$) (Figure 2).

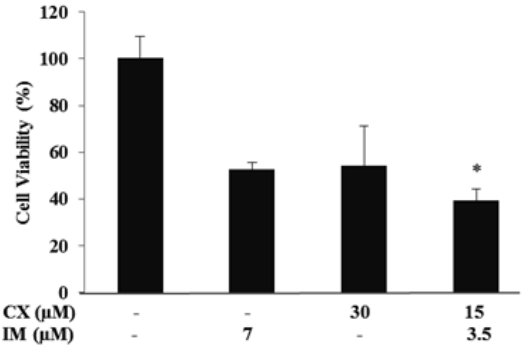

Figure 1. Effects of imatinib (IM), celecoxib (CX) and their half dose combination on HT-29 cell viability. Viable cell number was determined using the MTT colorimetric assay. Vertical bars indicate the mean cell viability ± SEM in each treatment group. * $P<0.05$ as compared to the vehicle-treated control group.

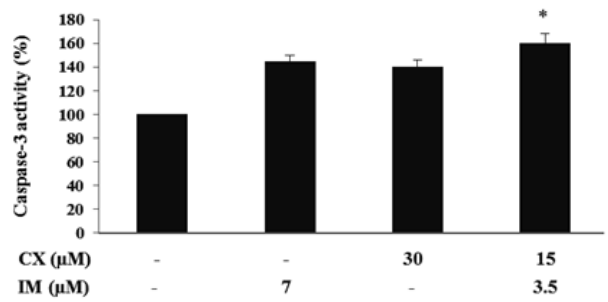

Figure 2. Effects of imatinib (IM), celecoxib (CX) and their half dose combination on HT-29 cell apoptosis. The HT-29 colorectal cancer cells were treated for 24 hr. Apoptosis induction was assessed by caspase-3 assay. IM in combination with CX induced apoptosis significantly compared to control cells. Data are presented as the mean ± SEM and the mean number of untreated control cell was set at 100%. Asterisks indicate sample that is significantly different compared to other samples using analysis of variance. (* $P<0.05$).

The expression level of Caspase-3, COX-2, NF-κB and VEGF after treatment with IM, CX and their combination in HT-29 colorectal cancer cells

As shown in Figure 3A, there was an approximately 3-fold increasing of Caspase-3 mRNA as a result of treatment with IC50 dose of IM (7 μM, $P=0.04$) and its combination with CX (at half dose of IC50, $P=0.04$) compared to untreated HT-29 cells. The levels of Caspase-3 mRNA were increased insignificantly and approximately 1-fold in treatment with CX alone (30 μM, $P>0.05$). Taken together, these results indicate that half dose IM combination with CX increased expression of Caspase-3 mRNA almost the same as IM alone and this effect may mostly related to IM.

According to Figure 3B, CX (30 μM, $P<0.001$) had significantly down-regulated the expression of COX-2 mRNA in comparison to control (DMSO), whereas IM (7 μM, $P=0.9$) had no effect on the expression of COX-2 mRNA. There was an approximately 2-fold reduction of COX-2 mRNA as a result of combination treatment of IM with CX ($P=0.04$) compared to IM alone. Quantitative real-time PCR analyses demonstrated that the combined treatment was significantly effective at inhibiting COX-2 expression.

According to qRT-PCR results, treatments with IM (7 μM) increased but CX (30 μM) decreased VEGF mRNA level insignificantly. Combined treatment with 3.5 μM IM and 15 μM CX, resulted in more slight decrease in VEGF mRNA level but these differences were not found to be significant (Figure 3C). In addition, treatments with IM, CX and their combination showed only a minimal and not significant reduction of NF-κB expression in HT-29 cells (Figure 3D).

Discussion

Abounding evidence for functional participation of CX in tumor suppression has been reported in several studies including breast cancer (28, 29), esophageal cancer (30, 31), prostate cancer (32), cervical cancer (33, 34) and colorectal cancer (35, 36). Besides multiple lines of evidence indicate that CX exerts its effect in

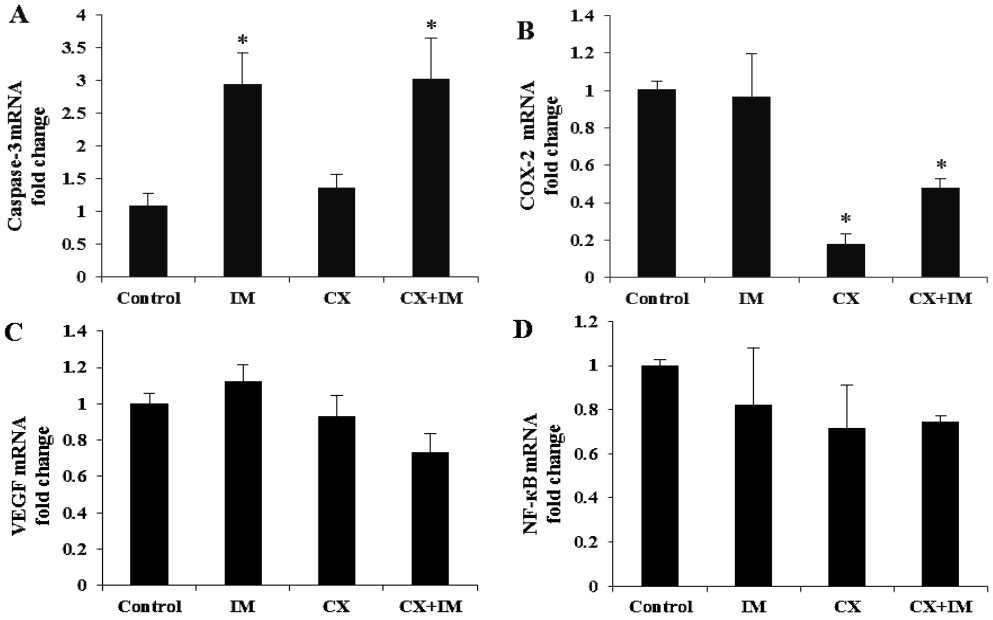

Figure 3. Real-time quantitative RT-PCR analysis to determine the effects of imatinib (IM), celecoxib (CX) and their combination on Caspase-3 (A), COX-2 (B), VEGF (C) and NF-κB (D) mRNA levels with b-actin as the internal control. Vertical bars indicate the mean fold changes ± SEM for three independent experiments in each treatment group. *: P<0.05 versus control.

combination with other drugs including curcumin (37), 5-fluorouracil (38, 39), atorvastatin (40) in colon cancer cells, cisplatin (31) in human esophageal squamous cell carcinoma cells and IM in K562 leukemia cells (41). There was no study about combination of IM and CX on HT-29 colorectal cancer cells.

In this study, we demonstrated that CX, IM and their combination decreased cell viability which was significantly lower in the case of combination treatment compared to control group in HT-29 colorectal cancer cells. We also observed that IM in combination with CX significantly increased caspase-3 activity in these cells. Anti-proliferative effects of IM in combination with CX were associated with decrease in COX-2 and increase in Caspase-3 mRNA levels. Alteration in VEGF and NF-κB genes expression was not statistically significant.

In accordance to our results, Kusunoki et al. showed that CX and its derivative inhibits proliferation and COX-2 expression in HT-29 colorectal cancer cells (42). Ninomiya et al. also demonstrated that CX reduces the growth and metastatic potential of HT-29 colorectal carcinoma in mice through COX-2 inhibition and proposed that CX has a high potential to use as a clinical agent for inhibition of hematological and lymphatic metastases of colorectal cancer (43). Besides, Zhang et al. reported that higher doses of CX (80–160 μM) significantly reduce viability and down regulate the expression of COX-2 mRNA/protein in K562 cells (41). Combination of CX and IM have also been shown to have synergistic effects in terms of anti-proliferation on K562 cells (44). Over-expression or down-regulation of COX-2 in K562 cells treated with combination of CX (10 μM) and IM (10 μM) or CX (10 μM) alone, respectively have also been demonstrated (6). In addition it has been shown that IM increased COX-2 expression in squamous cell carcinoma (45) and over-expression of COX-2 in K562 cells involved in the resistance to IM (46). Then decrease in COX-2 expression by CX and combination treatment and not by IM in our study indicates the usefulness of CX especially in combination with IM.

There are some lines of evidence suggesting that COX-2 inhibitors exert part of their anti-cancer properties without affecting COX-2 activity (47). In accordance to our results, some studies point to apoptosis as a mechanism of the effect of CX (48-50). In the study by Ninomiya et al. reduced growth and metastatic potential of colorectal carcinoma in mice, suppressed VEGF protein expression and induced apoptosis have been demonstrated by CX through COX-2 inhibition (43). On the other hand, Du et al. reported up-regulation of VEGF in HCT116 colorectal cancer cells (19). Also increased VEGF gene expression in chronic myelogenous leukemia patients (51) and decreased VEGF levels in some patients with gastrointestinal stromal tumors have been shown following IM treatment (52). In our study, IM increased but CX and its combination with IM decreased VEGF mRNA level insignificantly. If so, decreasing VEGF level by CX and its combination with IM might inhibit angiogenic process in HT-29 colorectal cancer cells.

In addition we observed that treatment with IM, CX and their combination showed a minimal (not significant) reduction of NF-κB expression in HT-29 cells. Besides, NF-κB level was lower in the cells treated with CX and its combination with IM. Consistent literature data on the effect of CX on NF-κB are available (14-16). Accordingly it has been shown that CX might prevent colorectal cancer in the early stages by down regulating NF-κB in experimentally induced colorectal cancer (53). However, because of statistically insignificant data in our study, CX induced decrease in VEGF and NF-κB levels seems to be less important and should be evaluated in the future.

As stated, observed changes in cell viability and gene expression were stronger in combined drug treatments. Since drug resistance and side effects related to IM treatment may significantly limit the effectiveness of this drug, using suitable combinations of IM along with other preventive agents like CX can be advantageous in lowering the required dose of anticancer drugs and,

consequently, minimizing undesirable side effects (54).

Stahtea et al. used combination of IM with 5- fluorouracil and showed significant growth inhibition of the highly tumourigenic HT-29 cells (55). Also Zhang et al. reported that CX combined with 5- fluorouracil could inhibit the growth of HT-29 induced xenograft carcinoma by inducing apoptosis and decreasing COX-2 expression (39). Consistent with the results, of mentioned studies we showed effectiveness of IM-CX combination in HT-29 colorectal cancer cells treatment. COX-2 inhibition and Caspase-3 activation seems to be the most important pathways which were involved in anti-proliferative effect of combined drugs in this cell line. Of course, more studies are necessary to clarify combined drugs usefulness for clinical usage and finding the best dosage of the drugs. Yamaguchi et al. used the combination of gefitinib and CX in a pilot study and showed that this combination was generally well tolerated in patients with advanced GI cancer (56).

In conclusion, the present in vitro study on colon cancer cell lines demonstrated that IM in combination with CX significantly improved the anticancer activity of each component. In this regard, our study presents Caspase-3 and COX-2 dependent molecular targets as probable mechanisms mediating the anti-proliferative effects of IM and CX combination. Of course studying other molecular targets and cell lines, could be helpful to learn more in-depth mechanism by using combined treatment with IM and CX.

Acknowledgments

This study was conducted with financial support that was provided by the Urmia University of Medical Sciences. We also thank Dr Mohammad Reza Vardast (Department of Medicinal Chemistry, Faculty of Pharmacy, Urmia University of Medical Sciences, Urmia, Iran) for providing us technical supports.

References

1. Weitz J, Koch M, Debus J, Hohler T, Galle PR, Buchler MW. Colorectal cancer. Lancet. 2005;365(9454):153-65. doi:10.1016/S0140-6736(05)17706-X.

2. Buchdunger E, O'Reilly T, Wood J. Pharmacology of imatinib (STI571). European journal of cancer. 2002;38 Suppl 5:S28-36. doi:10.1016/S0959-8049(02)80600-1.

3. Joensuu H, Roberts PJ, Sarlomo-Rikala M, Andersson LC, Tervahartiala P, Tuveson D, et al. Effect of the tyrosine kinase inhibitor STI571 in a patient with a metastatic gastrointestinal stromal tumor. The New England journal of medicine. 2001 Apr 5;344(14):1052-6. doi:10.1056/NEJM200104053441404.

4. Altun A, Turgut NH, Kaya TT. Anticancer effect of COX-2 inhibitor DuP-697 alone and in combination with tyrosine kinase inhibitor (E7080) on colon cancer cell lines. Asian Pacific journal of cancer prevention : APJCP. 2014;15(7):3113-21. doi:10.7314/APJCP.2014.15.7.3113.

5. Swamy MV, Cooma I, Patlolla JM, Simi B, Reddy BS, Rao CV. Modulation of cyclooxygenase-2 activities by the combined action of celecoxib and decosahexaenoic acid: novel strategies for colon cancer prevention and treatment. Molecular cancer therapeutics. 2004;3(2):215-21.

6. Dharmapuri G, Doneti R, Philip GH, Kalle AM. Celecoxib sensitizes imatinib-resistant K562 cells to imatinib by inhibiting MRP1-5, ABCA2 and ABCG2 transporters via Wnt and Ras signaling pathways. Leukemia research. 2015;39(7):696-701. doi:10.1016/j.leukres.2015.02.013.

7. Ruder EH, Laiyemo AO, Graubard BI, Hollenbeck AR, Schatzkin A, Cross AJ. Non-steroidal anti-inflammatory drugs and colorectal cancer risk in a large, prospective cohort. The American journal of gastroenterology. 2011;106(7):1340-50. doi:10.1038/ajg.2011.38.

8. Rahme E, Barkun AN, Toubouti Y, Bardou M. The cyclooxygenase-2-selective inhibitors rofecoxib and celecoxib prevent colorectal neoplasia occurrence and recurrence. Gastroenterology. 2003 Aug;125(2):404-12. doi:10.1016/S0016-5085(03)00880-1.

9. Steinbach G, Lynch PM, Phillips RK, Wallace MH, Hawk E, Gordon GB, et al. The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. The New England journal of medicine. 2000;342(26):1946-52. doi:10.1056/NEJM200006293422603.

10. Trifan OC, Durham WF, Salazar VS, Horton J, Levine BD, Zweifel BS, et al. Cyclooxygenase-2 inhibition with celecoxib enhances antitumor efficacy and reduces diarrhea side effect of CPT-11. Cancer research. 2002;62(20):5778-84.

11. Cervello M, Bachvarov D, Cusimano A, Sardina F, Azzolina A, Lampiasi N, et al. COX-2-dependent and COX-2-independent mode of action of celecoxib in human liver cancer cells. Omics : a journal of integrative biology. 2011;15(6):383-92. doi:10.1089/omi.2010.0092.

12. Schwartz LM, Smith SW, Jones ME, Osborne BA. Do all programmed cell deaths occur via apoptosis? Proceedings of the National Academy of Sciences of the United States of America. 1993;90(3):980-4. doi:10.1073/pnas.90.3.980.

13. Hu W, Lee SK, Jung MJ, Heo SI, Hur JH, Wang MH. Induction of cell cycle arrest and apoptosis by the ethyl acetate fraction of Kalopanax pictus leaves in human colon cancer cells. Bioresource technology. 2010;101(23):9366-72. doi:10.1016/j.biortech.2010.06.091.

14. Funakoshi-Tago M, Shimizu T, Tago K, Nakamura M, Itoh H, Sonoda Y, et al. Celecoxib potently inhibits TNFalpha-induced nuclear translocation and activation of NF-kappaB. Biochemical pharmacology. 2008;76(5):662-71. doi:10.1016/j.bcp.2008.06.015.

15. Li Y, Ahmed F, Ali S, Philip PA, Kucuk O, Sarkar FH. Inactivation of nuclear factor kappaB by soy isoflavone genistein contributes to increased apoptosis induced by chemotherapeutic agents in human cancer cells. Cancer research. 2005;65(15):6934-42. doi:10.1158/0008-5472.CAN-04-4604.

16. Vaish V, Rana C, Piplani H, Vaiphei K, Sanyal SN. Sulindac and Celecoxib regulate cell cycle progression by p53/p21 up regulation to induce apoptosis during initial stages of experimental colorectal cancer. Cell biochemistry and biophysics. 2014;68(2):301-19. doi:10.1007/s12013-013-9711-8.

17. Garg A, Aggarwal BB. Nuclear transcription factor-kappaB as a target for cancer drug development. Leukemia. 2002;16(6):1053-68. doi:10.1038/sj.leu.2402482.

18. Carmeliet P. Blood vessels and nerves: common signals, pathways and diseases. Nature reviews Genetics. 2003;4(9):710-20. doi:10.1038/nrg1158.

19. Du H, Li W, Wang Y, Chen S, Zhang Y. Celecoxib induces cell apoptosis coupled with up-regulation of the expression of VEGF by a mechanism involving ER stress in human colorectal cancer cells. Oncology reports. 2011;26(2):495-502. doi:10.3892/or.2011.1297.

20. Zhang YF, Ruan LH, Zhao XQ. [Effects of COX-2 inhibitor celecoxib on expressions of VEGF, b-FGF and TGF-beta mRNA in acute leukemia cells]. Zhongguo shi yan xue ye xue za zhi / Zhongguo bing li sheng li xue hui = Journal of experimental hematology / Chinese Association of Pathophysiology. 2012;20(5):1086-9.

21. Li WZ, Wang XY, Ding YQ. [Effects of celecoxib on PGE2 synthesis and COX-2 and VEGF-C mRNA expression in Tca8113 cell lines]. Nan fang yi ke da xue xue bao = Journal of Southern

Medical University. 2009;29(3):466-8.
22. Subhashini J, Mahipal SV, Reddanna P. Anti-proliferative and apoptotic effects of celecoxib on human chronic myeloid leukemia in vitro. Cancer letters. 2005;224(1):31-43. doi:10.1016/j.canlet.2004.11.002.
23. Kim SH, Song SH, Kim SG, Chun KS, Lim SY, Na HK, et al. Celecoxib induces apoptosis in cervical cancer cells independent of cyclooxygenase using NF-kappaB as a possible target. Journal of cancer research and clinical oncology. 2004;130(9):551-60. doi:10.1007/s00432-004-0567-6.
24. Grivennikov SI, Karin M. Dangerous liaisons: STAT3 and NF-kappaB collaboration and crosstalk in cancer. Cytokine & growth factor reviews. 2010;21(1):11-9. doi:10.1016/j.cytogfr.2009.11.005.
25. Thanigaimani S, Kichenadasse G, Mangoni AA. The emerging role of vascular endothelial growth factor (VEGF) in vascular homeostasis: lessons from recent trials with anti-VEGF drugs. Current vascular pharmacology. 2011;9(3):358-80. doi:10.2174/157016111795495503
26. Nikanfar S, Attari-hajipirloo S, Heydari A, Kheradmand F, editors. Poster No. 68. 5th International Congress on Cell Membranes and Oxidative Stress: Focus on Calcium Signaling and TRP Channels; 2014.
27. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001;25(4):402-8. doi:10.1006/meth.2001.1262.
28. Harris RE, Namboodiri KK, Farrar WB. Nonsteroidal antiinflammatory drugs and breast cancer. Epidemiology. 1996;7(2):203-5.
29. Bocca C, Bozzo F, Bassignana A, Miglietta A. Antiproliferative effects of COX-2 inhibitor celecoxib on human breast cancer cell lines. Molecular and cellular biochemistry. 2011;350(1-2):59-70. doi:10.1007/s11010-010-0682-4.
30. Funkhouser EM, Sharp GB. Aspirin and reduced risk of esophageal carcinoma. Cancer. 1995;76(7):1116-9. doi:10.1002/1097-0142(19951001)76:7<1116::AID-CNCR2820760703>3.0.CO;2-I.
31. Yu L, Chen M, Li Z, Wen J, Fu J, Guo D, et al. Celecoxib antagonizes the cytotoxicity of cisplatin in human esophageal squamous cell carcinoma cells by reducing intracellular cisplatin accumulation. Molecular pharmacology. 2011;79(3):608-17. doi:10.1124/mol.110.069393.
32. Hsu AL, Ching TT, Wang DS, Song X, Rangnekar VM, Chen CS. The cyclooxygenase-2 inhibitor celecoxib induces apoptosis by blocking Akt activation in human prostate cancer cells independently of Bcl-2. The Journal of biological chemistry. 2000;275(15):11397-403. doi:10.1074/jbc.275.15.11397.
33. Thun MJ, Namboodiri MM, Heath Jr CW. Aspirin use and reduced risk of fatal colon cancer. New England Journal of Medicine. 1991;325(23):1593-6. doi:10.1056/NEJM199205073261912.
34. Kim SH, Hwang CI, Juhnn YS, Lee JH, Park WY, Song YS. GADD153 mediates celecoxib-induced apoptosis in cervical cancer cells. Carcinogenesis. 2007;28(1):223-31. doi:10.1093/carcin/bgl227.
35. Gupta RA, Dubois RN. Colorectal cancer prevention and treatment by inhibition of cyclooxygenase-2. Nature reviews Cancer. 2001;1(1):11-21. doi:10.1038/35094017.
36. Arber N, DuBois RN. Nonsteroidal anti-inflammatory drugs and prevention of colorectal cancer. Current gastroenterology reports. 1999;1(5):441-8. doi:10.1007/s11894-999-0027-1
37. Lev-Ari S, Strier L, Kazanov D, Madar-Shapiro L, Dvory-Sobol H, Pinchuk I, et al. Celecoxib and curcumin synergistically inhibit the growth of colorectal cancer cells. Clinical cancer research : an official journal of the American Association for Cancer Research. 2005;11(18):6738-44. doi:10.1158/1078-0432.CCR-05-0171.
38. Lim YJ, Rhee JC, Bae YM, Chun WJ. Celecoxib attenuates 5-fluorouracil-induced apoptosis in HCT-15 and HT-29 human colon cancer cells. World journal of gastroenterology. 2007;13(13):1947-52. doi:10.3748/wjg.v13.i13.1947.
39. Zhang DQ, Guo Q, Zhu JH, Chen WC. Increase of cyclooxygenase-2 inhibition with celecoxib combined with 5-FU enhances tumor cell apoptosis and antitumor efficacy in a subcutaneous implantation tumor model of human colon cancer. World journal of surgical oncology. 2013;11:16. doi:10.1186/1477-7819-11-16.
40. Xiao H, Zhang Q, Lin Y, Reddy BS, Yang CS. Combination of atorvastatin and celecoxib synergistically induces cell cycle arrest and apoptosis in colon cancer cells. International journal of cancer Journal international du cancer. 2008;122(9):2115-24. doi:10.1002/ijc.23315.
41. Zhang GS, Liu DS, Dai CW, Li RJ. Antitumor effects of celecoxib on K562 leukemia cells are mediated by cell-cycle arrest, caspase-3 activation, and downregulation of Cox-2 expression and are synergistic with hydroxyurea or imatinib. American journal of hematology. 2006;81(4):242-55. doi:10.1002/ajh.20542.
42. Kusunoki N, Ito T, Sakurai N, Handa H, Kawai S. A celecoxib derivative potently inhibits proliferation of colon adenocarcinoma cells by induction of apoptosis. Anticancer research. 2006;26(5A):3229-36.
43. Ninomiya I, Nagai N, Oyama K, Hayashi H, Tajima H, Kitagawa H, et al. Antitumor and anti-metastatic effects of cyclooxygenase-2 inhibition by celecoxib on human colorectal carcinoma xenografts in nude mouse rectum. Oncology reports. 2012;28(3):777-84. doi:10.3892/or.2012.1885.
44. Li RJ, Gong FJ, Zhang GS. [Cytotoxic activities of Celecoxib on leukemic cells and the synergistic effects of Celecoxib with Imatinib thereupon]. Zhonghua yi xue za zhi. 2006;86(20):1417-20.
45. Johnson FM, Yang P, Newman RA, Donato NJ. Cyclooxygenase-2 induction and prostaglandin E2 accumulation in squamous cell carcinoma as a consequence of epidermal growth factor receptor activation by imatinib mesylate. Journal of experimental therapeutics & oncology. 2004;4(4):317-25.
46. Arunasree KM, Roy KR, Anilkumar K, Aparna A, Reddy GV, Reddanna P. Imatinib-resistant K562 cells are more sensitive to celecoxib, a selective COX-2 inhibitor: role of COX-2 and MDR-1. Leukemia research. 2008;32(6):855-64. doi:10.1016/j.leukres.2007.11.007.
47. Liu H, Huang P, Xu X, Liu J, Guo C. Anticancer effect of celecoxib via COX-2 dependent and independent mechanisms in human gastric cancers cells. Digestive diseases and sciences. 2009;54(7):1418-24. doi:10.1007/s10620-008-0510-9.
48. Vaish V, Sanyal SN. Role of Sulindac and Celecoxib in chemoprevention of colorectal cancer via intrinsic pathway of apoptosis: exploring NHE-1, intracellular calcium homeostasis and Calpain 9. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie. 2012;66(2):116-30. doi:10.1016/j.biopha.2011.11.019
49. Liu HF, Hsiao PW, Chao JI. Celecoxib induces p53-PUMA pathway for apoptosis in human colorectal cancer cells. Chemico-biological interactions. 2008;176(1):48-57. doi:10.1016/j.cbi.2008.07.012.
50. Peng J, Zhang GY, Xiao ZQ. [Effects of celecoxib on the proliferation and apoptosis of human colorectal cancer cell line HT-29]. Zhong nan da xue xue bao Yi xue ban = Journal of Central South University Medical sciences. 2004;29(3):261-5.
51. Legros L, Bourcier C, Jacquel A, Mahon FX, Cassuto JP, Auberger P, et al. Imatinib mesylate (STI571) decreases the vascular endothelial growth factor plasma concentration in patients with chronic myeloid leukemia. Blood. 2004;104(2):495-501. doi:10.1182/blood-2003-08-2695.
52. McAuliffe JC, Lazar AJ, Yang D, Steinert DM, Qiao W, Thall PF, et al. Association of intratumoral vascular endothelial growth

factor expression and clinical outcome for patients with gastrointestinal stromal tumors treated with imatinib mesylate. Clinical cancer research : an official journal of the American Association for Cancer Research. 2007;13(22 Pt 1):6727-34. doi:10.1158/1078-0432.CCR-07-0895.

53. Vaish V, Tanwar L, Sanyal SN. The role of NF-kappaB and PPARgamma in experimentally induced colorectal cancer and chemoprevention by cyclooxygenase-2 inhibitors. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2010;31(5):427-36. doi:10.1007/s13277-010-0051-7.

54. RADUJKOVIC A, TOPALY J, FRUEHAUF S, ZELLER WJ. Combination treatment of imatinib-sensitive and-resistant BCR-ABL-positive CML cells with imatinib and farnesyltransferase inhibitors. Anticancer research. 2006;26(3A):2169-77.

55. Stahtea XN, Roussidis AE, Kanakis I, Tzanakakis GN, Chalkiadakis G, Mavroudis D, et al. Imatinib inhibits colorectal cancer cell growth and suppresses stromal-induced growth stimulation, MT1-MMP expression and pro-MMP2 activation. International journal of cancer Journal international du cancer. 2007;121(12):2808-14. doi:10.1002/ijc.23029.

56. Yamaguchi NH, Mayer IA, Malzyner A, de Andrade CJ, Murad AM, Del Giglio A, et al. Gefitinib and celecoxib in advanced metastatic gastrointestinal tumors: a pilot feasibility study. Journal of gastrointestinal oncology. 2014 Feb;5(1):57-66. doi:10.3978/j.issn.2078-6891.2013.056.