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Exploring the Antitumor Effects of Dasatinib Alone or in Combination with Celecoxib in HCT-116 Colorectal Carcinoma Cell Line

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Abstract

Colorectal cancer is a heterogeneous disease driven by genetic and epigenetic changes that allow cells to overgrow and evade programmed cell death. Many of these changes map to overlapping signal transduction cascades that govern cell proliferation/invasion/metastasis. Novel approaches for better disease management aimed at co-targeting more than one of these cascades. Src and cyclooxygenase-2 are plausible targets involved in the pathogenesis of colorectal cancer. In this context, the present work was carried out to unravel the anticarcinogenic effects resulting from co-targeting Src and cyclooxygenase-2 pathways using dasatinib as a Src inhibitor, and celecoxib as a selective cyclooxygenase-2 inhibitor in HCT-116 colorectal carcinoma cell line. MTT cell proliferation assay was used to determine the GI50 of both drugs. HCT-116 cells were treated with dasatinib, celecoxib, and their combination at their GI50s along with a control group for 72 h. Then, cells were collected and pellets were stored at -80°C. After that, cells were prepared for ELISA to determine c-Src, NF-κB, TNF-Aα, p-AKT, p-STAT-3, MMP-9, cyclin D1, caspase-3, and VEGF protein levels. Expression of c-Src gene was assessed by quantitative real-time PCR. On one hand, Dasatinib, Celecoxib, and their combination reduced c-Src, NF-κB, TNF-Aα, p-AKT, STAT-3, VEGF, MMP-9, and Cyclin D1 protein levels compared with the control group. On the other hand, there was no statistically significant difference in caspase-3 protein level upon Dasatinib and Celecoxib treatments, but Dasatinib/Celecoxib combination exerted a pronounced effect on caspase-3 protein level. c-Src gene was up-regulated by 1.3, and 1.7-fold by each of Dasatinib and Celecoxib, respectively; however, the combination didn't influence c-Src gene expression level. To sum up, the combination has a beneficial role that results from the use of Celecoxib as an add-on therapy to Dasatinib. This needs to be explored in other types of cancer and in cancer patients as Celecoxib can be used as adjuvant therapy for the reduction of inflammation and proliferation in addition to apoptosis stimulation.

Keywords: Colorectal cancer; Src signaling cascade; COX-2 signaling cascade; Dasatinib; Celecoxib

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Introduction

According to Globocan 2018, Colorectal Cancer (CRC) is the second most common cancer in females after breast cancer, and the third after lung and prostate cancers in males, with approximately 1.2 million newly diagnosed cases every year, and the third cause of cancer-related mortality, with around 600,000 deaths yearly [1]. CRC occurs due to both genetic and epigenetic changes that promote the uncontrolled proliferation of colonocytes [2]. Genetic deregulation is evident in CRC and comprises a multitude of deregulated signaling cascades. The advent of small-molecule inhibitors targeting these cascades provides a basis for exploiting them as probable targets in CRC treatment [3].

One plausible target involved in CRC pathogenesis is Src. Average Src expression levels are 2.4-fold higher in CRC patients than those in the normal mucosa. The highly metastatic CRC cells showed significantly up-regulated Src expression compared to the poorly metastatic CRC cells [4]. Src activation promotes proliferation, invasiveness, and metastasis of CRC cells *via* activation of various downstream targets including: Phosphatidylinositol 3-Kinase (PI3K)/AKT, Growth Factor Receptor-Bound Protein 2 (GRB2)/Rat Sarcoma Viral Oncogene (RAS)/V-raf Murine Sarcoma

Viral Oncogene (RAF)/Mitogen-Activated Protein Kinase (MAPK), and Signal Transducers and Activators of Transcription protein (STATs) pathways [4].

Dasatinib, Src/Abl family kinase inhibitor, is approved by the FDA for imatinib-resistant chronic myelogenous and Philadelphia chromosome-positive acute lymphoblastic leukemia's [5,6]. Dasatinib can suppress tumor progression of a multitude of solid tumors via inhibition of cell migration/invasion/metastasis, and induction of cell cycle G1 arrest and apoptotic cell death [7].

Cyclooxygenase-2 (COX-2) is another target overexpressed in breast, ovarian, thyroid, lung, and colorectal cancers [8]. COX-2 up-regulation was evidenced in precancerous as well as cancerous lesions in the colon [9]. COX-2 was overexpressed in colorectal adenomas and sporadic colon carcinomas by about 50% and 85%, respectively. COX-2 seems to have a role in polyp formation. COX-2 up-regulation is correlated with poor survival in CRC [10]. COX-2 inhibitors can suppress polyp growth, enhance apoptotic cell death, and reduce the expression of proangiogenic factors. Additionally, COX-2 inhibitors down-regulate PI3K signaling cascade, which plays a pivotal role in carcinogenesis [10].

Celecoxib has antitumor activities in a multitude of human cancers, such as breast, lung, and colorectal cancers [11] and has been suggested as an agent that can target various signal transduction cascades associated with COX-2 expression [12].

To sum up, this work aimed at unraveling the antitumor effects of Dasatinib as a Src inhibitor, Celecoxib as a selective COX-2 inhibitor as well as their combination in HCT-116 colorectal carcinoma cell line.

Materials and Methods

Drugs under study

Dasatinib and Celecoxib (Selleckchem, TX, USA) were dissolved in dimethyl sulphoxide and stored at -20°C.

Cell lines

HCT-116, an epithelial CRC cell line, is purchased from the American Type Culture Collection (ATCC® CCL-247™).

Cell cultures

HCT-116 cells were kept as a monolayer culture in T-25 flasks at 37°C and 5% CO2 in Dulbecco's Modified Eagle's Media (Lonza Biowhitaker™, B-4800 Verviers, Belgium) augmented with fetal bovine serum (10%) (Sigma-Aldrich Co., Germany). Penicillin/Streptomycin (Lonza Biowhitaker™, B-4800 Verviers, Belgium) were used at the concentrations of 100 units/ml and 100 µg/ml, respectively. Cells were passaged when they were 80% confluent.

Growth inhibition assay

Cell viability was determined by MTT assay [13]. HCT-116 cells were seeded in 96-well plates, treated with various concentrations of the selected drugs. The concentrations tested for Dasatinib were 3.75 µM, 7.5 µM, 15 µM, 30 µM, 45 µM, 90 µM, and those for Celecoxib were 3.75 µM, 7.5 µM, 15 µM, 30 µM, 60 µM, and 120 µM. MTT (10 µl) was added after 72 h, then incubation was carried out at 37°C for 4 h, then absorbance was measured at 570 nm. The Growth Inhibition 50 (GI50) was assessed using the Compu Syn 3.0.1 software.

Experimental design

Three replicas of HCT-116 cells received either dimethyl sulphoxide as a vehicle, dasatinib (16.83 µM), celecoxib (22.30 µM), or dasatinib (16.83 µM)/Celecoxib (22.30 µM) combination. All experiments were conducted as per the regulatory aspects regarding the use of cell lines.

Biochemical analyses

Protein levels of c-Src, Nuclear Factor kappa B (p-NF-κB), Tumor Necrosis Factor Aα (TNF-Aα), p-AKT, p-STAT-3, Matrix Metaloproteases 9 (MMP-9), cyclin D1, and VEGF were assessed using the following ELISA kits: Human c-Src ELISA kit (Ela science Biotechnology Co., Ltd) (Cat#: E-EL-H0017), human p-NF-κB ELISA kit (Ray Biotech Ray Bio®) (Cat#: PEL-NFKBP65-S536), human TNF-Aα ELISA kit (Ela science Biotechnology Co., Ltd) (Cat#: E-EL-H0109), human p-AKT ELISA kit (Ray Biotech, USA) (Cat#: PEL-AKT-S473-T), human p-STAT-3 ELISA kit (Ela science Biotechnology Co., Ltd) (Cat#: E-EL-H2132), human MMP-9 ELISA kit (Ela science Biotechnology Co., Ltd) (Cat#: E-EL-H1451), human Cyclin D1 ELISA kit (Ela science Biotechnology Co., Ltd) (Cat#: E-EL-H2092), human Vascular Endothelial Growth Factor (VEGF) based ELISA assay kit (Ela science Biotechnology Co., Ltd) (Cat#: E-EL-H0111), respectively according to the manufacturer's instructions.

Active caspase-3 determination

Caspase 3 activity was assessed using caspase-3 colorimetric kit (Sigma Aldrich, USA) (Cat#: CASP-3-C) according to the manufacturer's instructions.

Analysis of Src gene expression using quantitative real-time polymerase chain reaction

Expression of c-Src gene was determined using step one real-time Polymerase Chain Reaction (PCR) (Applied Biosystem, USA) where total RNA was isolated using Easy-REDTM total RNA extraction kit (Intron Biotechnology, South Korea) (Cat#: 17063) according to the manufacturer's instructions. Total RNA quantification and purity checking were done using Nanodrop 2000 spectrophotometer (Thermo Fischer Scientific, USA). Real-time PCR reactions were done using the SensiFast™ SYBR® No-ROX one-step kit (Bioline Co., USA) (Cat#: BIO-72001). Then, the relative expression of c-Src gene was assessed against *β-actin* as a housekeeping gene. The sequences of the forward and reverse primers for c-Src gene are: Forward: 5' GGACAGTGGCGGATTCTACATC-3', and reverse: 5' AGCTGCTGCAGGCTGTTGA-3' whereas those for GAPDH gene were: forward: 5'-TGCACCACCAACTGCTTAGC-3' and reverse: 5'-GGCATGGACTGTGGTCATGAG-3' [14,15]. The sequences of the primers were blasted against NCBI/primer blast to confirm the expected unique amplification of c-Src and *GAPDH* genes. The relative expression of c-Src gene against *GAPDH* relied on the ΔΔ comparative threshold method. The analyses were performed as triplicates.

Statistical analysis of the data

Data were presented as mean ± standard error of the mean. One-way analysis of variance followed by Tukey post-hoc test was used for data analysis. The statistical analyses were executed by GraphPad Prism Software (version 3.0). The level of significance was fixed at $p<0.05$.

Results

Determination of the GI50 for dasatinib and celecoxib in HCT-116 cells

Data depicted in (Figure 1) revealed that the GI50 for Dasatinib

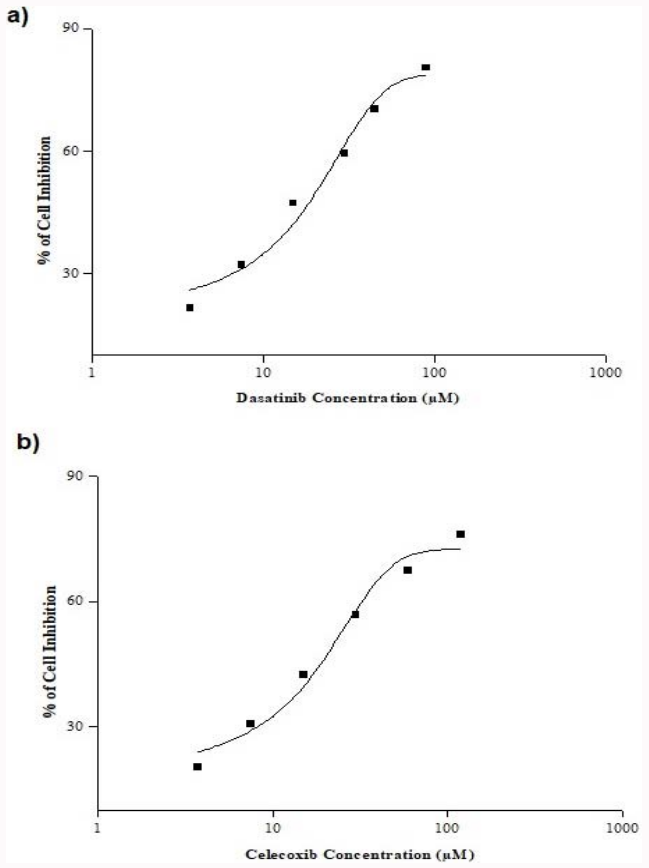

Figure 1: Cell inhibition (%) of HCT-116 cells upon treatment with (a) Dasatinib and (b) Celecoxib. MTT was conducted to determine the cell viability. Data points represent mean ± standard error of the mean, each performed in triplicate.

was 16.83 µM while that for Celecoxib was 22.30 µM.

Effect of Dasatinib (16.83 µM), Celecoxib (22.30 µM), and Dasatinib (16.83 µM)/Celecoxib (22.30 µM) combination on c-Src gene expression and protein levels in HCT-116 cell lysates after 72 h of treatment.

As illustrated in (Figure 2), c-Src gene was up-regulated by 1.3 and 1.7-fold by each of Dasatinib and Celecoxib, respectively in HCT-116 cell line compared with the control group (p=0.005). However, Dasatinib/Celecoxib combination didn't influence c-Src gene expression.

The data herein (Figure 3a) inferred that Dasatinib, Celecoxib, and their combination reduced c-Src protein level significantly by about 34%, 42%, and 87% compared with the control group (p=0.005).

Effects of Dasatinib (16.83 µM), Celecoxib (22.30 µM), and Dasatinib (16.83 µM)/Celecoxib (22.30 µM) combination on NF-κB protein level (µg/mg protein) in HCT-116 cell lysates after 72 h of treatment.

As presented in (Figure 3b), Dasatinib, Celecoxib, and their combination reduced p-NF-κB protein level by approximately 66%, 54%, and 83% relative to the control group (p=0.006, p=0.012, p=0.003, respectively).

Effects of Dasatinib (16.83 µM), Celecoxib (22.30 µM), and Dasatinib (16.83 µM)/Celecoxib (22.30 µM) combination on TNF-

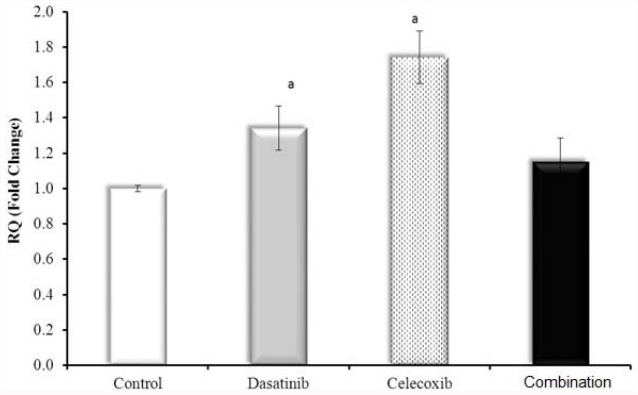

Figure 2: Effect of Dasatinib (16.83 µM), Celecoxib (22.30 µM) and their combination on *c-Src* gene expression level.

Aα protein level (Pg/mg protein) in HCT-116 cell lysates after 72 h of treatment.

Data depicted in (Figure 3c) revealed that Dasatinib, Celecoxib, and their combination decreased TNF-Aα protein level by around 50%, 44%, and 77% compared to the control group (p=0.001).

Effects of Dasatinib (16.83 µM), Celecoxib (22.30 µM), and Dasatinib (16.83 µM)/Celecoxib (22.30 µM) combination on p-AKT protein level (µM/mg protein) in HCT-116 cell lysates after 72 h of treatment.

The results presented in (Figure 4a) revealed that Dasatinib, Celecoxib, and their combination reduced p-AKT protein level by 55%, 49%, and 82% relative to the control group (p=0.001).

Effects of Dasatinib (16.83 µM), Celecoxib (22.30 µM), and Dasatinib (16.83 µM)/Celecoxib (22.30 µM) combination on p-STAT-3 protein level (ng/mg protein) in HCT-116 cell lysates after 72 h of treatment.

As illustrated in (Figure 4b), Dasatinib, Celecoxib, and their combination decreased p-STAT-3 level by about 52%, 46%, and 78% relative to the control group.

Effects of Dasatinib (16.83 µM), Celecoxib (22.30 µM), and Dasatinib (16.83 µM)/Celecoxib (22.30 µM) combination on MMP-9 protein level (ng/mg protein) in HCT-116 cell lysates after 72 h of treatment.

As presented in (Figure 4c), MMP-9 protein level was reduced upon treatment with Dasatinib, Celecoxib, and their combination by 55%, 40%, and 81% compared to the control group (p=0.003, p=0.011, and p=0.001, respectively).

Effects of Dasatinib (16.83 µM), Celecoxib (22.30 µM), and Dasatinib (16.83 µM)/Celecoxib (22.30 µM) combination on cyclin D1 protein level (ng/mg protein) in HCT-116 cell lysates after 72 h of treatment.

Data presented in (Figure 5a) revealed that cyclin D1 protein level was decreased by about 52%, 45%, and 78% relative to the control group in Dasatinib-treated, Celecoxib-treated, and combination-treated cells, respectively (p<0.001).

Effects of Dasatinib (16.83 µM), Celecoxib (22.30 µM), and Dasatinib (16.83 µM)/Celecoxib (22.30 µM) combination on caspase-3 protein level (ng/mg protein) in HCT-116 cell lysates

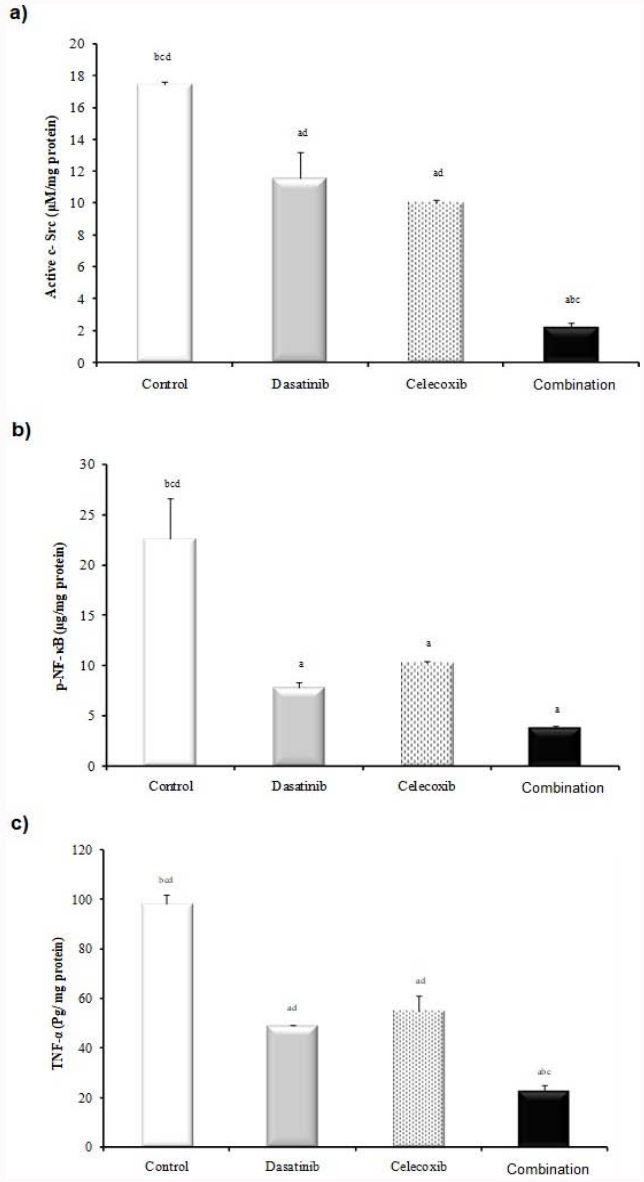

Figure 3. Effect of Dasatinib (16.83 Î¼M), Celecoxib (22.30 Î¼M) and their combination on (a) c-Src (ÂµM/mg protein), (b) NF-Î°B (Âµg/mg protein), and (c) TNF-Î± (Pg/mg protein) protein levels in HCT-116 cell lysates after 72 hours of treatment.

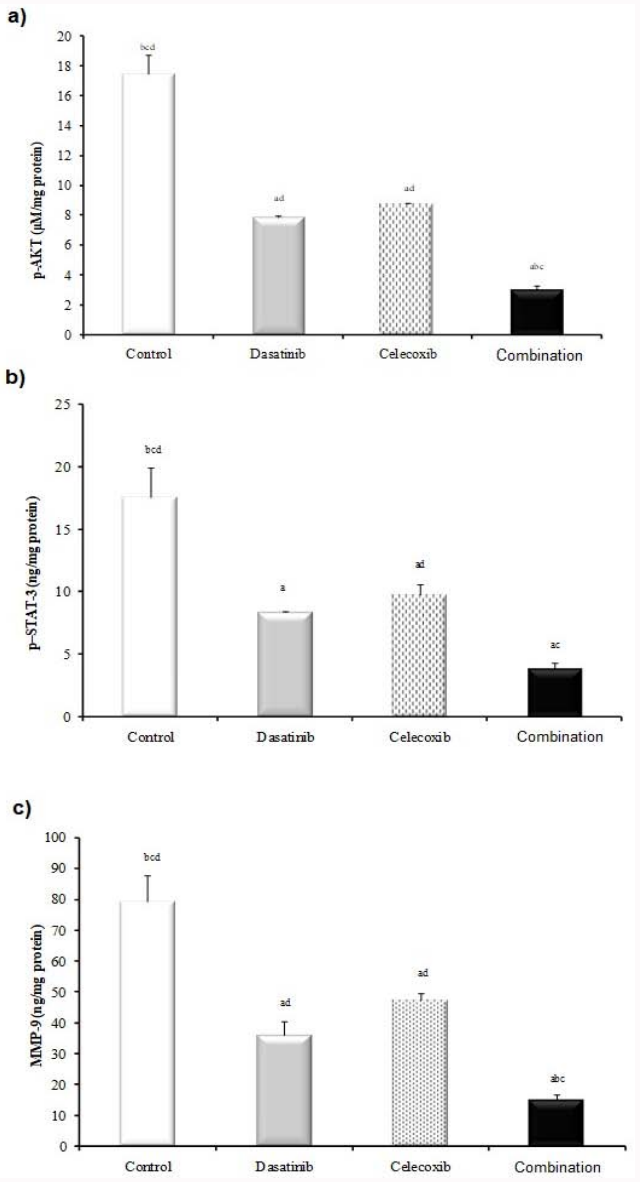

Figure 4: Effect of Dasatinib (16.83 µM), Celecoxib (22.30 µM) and their combination on (a) p-p-AKT (µM/mg protein), (b) p-STAT-3 (ng/mg protein), and (c) MMP-9 (ng/mg protein) protein levels in HCT-116 cell lysates after 72 hours of treatment.

after 72 h of treatment.

Data herein (Figure 5b) showed that both drugs didn't influence caspase-3 protein level relative to the control group (p=0.066, and p=0.082, respectively). On the contrary, the combination increased caspase-3 protein level significantly compared with the control group (260%, p=0.001).

Effects of Dasatinib (16.83 µM), Celecoxib (22.30µM), and Dasatinib (16.83 µM)/Celecoxib (22.30 µM) combination on VEGF protein level (Pg/mg protein) in HCT-116 cell lysates after 72 h of treatment.

Our results presented in (Figure 5c) revealed that VEGF protein level was reduced by about 59%, 46%, and 82% relative to the control group in Dasatinib-treated, Celecoxib-treated, and combination-treated cells, respectively (p<0.001).

Discussion

Up to our knowledge, this study is the first to unravel the potential antitumor effects resulting from co-targeting Src and COX-2-mediated pathways using Dasatinib and Celecoxib in HCT-116 CRC cell line. Inhibition of c-Src is the main point of interaction between Dasatinib and Celecoxib, and that was reflected on the downstream signaling pathways as represented by the inhibition of NF-κB, TNF-α, STAT-3, VEGF, MMP-9, and cyclin D1 as determined by ELISA and quantitative real-time PCR techniques.

The upregulatory effect of Dasatinib on c-Src gene expression level in our study could be ascribed to the presence of a positive feedback loop to recover for Src inhibition. That is in line with what was reported in various tumor cell lines upon treatment with other Src inhibitors [16-18]. Dasatinib significantly reduced Src protein level

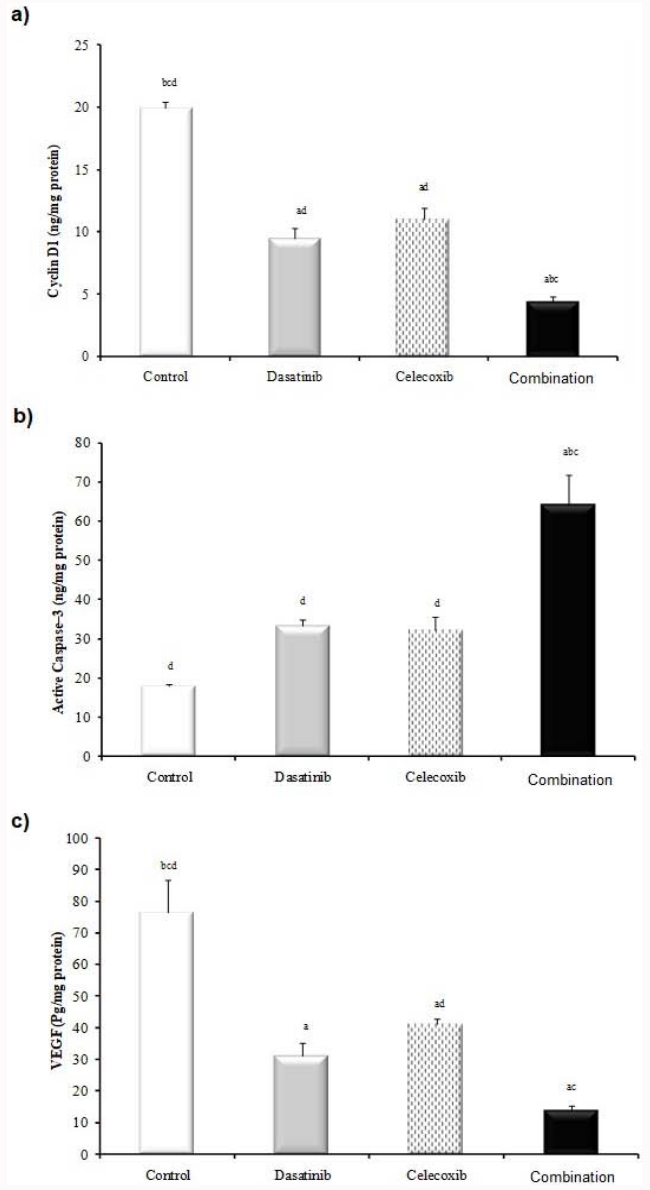

Figure 5: 5a: Effect of Dasatinib (16.83 Î¼M), Celecoxib (22.30 Î¼M) and their combination on (a) cyclin D1 (ng/mg protein), (b) caspase-3 (ng/mg protein), and (c) VEGF protein levels in HCT-116 cell lysates after 72 hours of treatment.

making our results consistent with previous studies where Dasatinib inhibited Src kinase activity in MG-63 bone sarcoma cell line [16] and human melanoma cell lines [19], and blocked phosphorylation of Src at Tyr 416 in breast cancer cell lines [20] and lung inflammation and fibrosis in acute experimental silicosis [21]. Dasatinib reduced both Src activation and p27 phosphorylation in Burkitt's esophageal cells [22], and suppressed phosphorylation of c-Src and its downstream effectors in MDA-MB-468 breast cancer cells [23].

Dasatinib significantly reduced NF-κB making our data in line with a previous study reporting that Dasatinib might influence the activation of NF-κB signaling cascade, as NF-κB activation is dependent on Src tyrosine kinase [24]. Moreover, Dasatinib decreased NF-κB in chronic myeloid leukemia cell lines; however, the mechanism is not well understood [25]. Various stimuli such as Tumor Necrosis Factor Aα (TNF-Aα) activate NF-κB cascade, and this leads to the phosphorylation of IκB kinase (IKK) and the degradation of IkBa by the proteasome, highlighting the presence of crosstalk between TNF-Aα, NF-κB, and c-Src [26].

Our results herein inferred that Dasatinib decreased TNF-Aα protein level significantly making our results concordant with previous studies [21,27]. Moreover, Dasatinib significantly inhibited p-AKT and since PI3K/AKT is downstream of Src, the antiproliferative effect of Dasatinib and the modulation of PI3K/AKT pathway activity were linked [7,28-30].

It was reported that Src activates different transduction cascades that activate STAT-3 pathway [31]. Several lines of evidence inferred that Dasatinib reduced STAT-3 phosphorylation, hence suppress cell proliferation/metastasis and promote apoptotic cell death [32-35]. Dasatinib markedly inhibited STAT-3 phosphorylation in PC-3 prostate cancer cells [36] and acute lymphocytic leukemia cells [37].

MMP-9 protein level was reduced herein upon treatment with Dasatinib making our results consistent with a study examining 12 sarcoma cell lines in which Dasatinib inhibited migration and invasion in 11 out of the 12 examined cell lines. Dasatinib also inhibited cellular adhesion/migration/invasion in prostate cancer cells [38], glioblastoma cells [39], colon cancer cells [40-42], Head and Neck Squamous Cell Carcinoma Cells (HNSCC), and non-small cell lung cancer cells *in-vitro* [43]. These studies emphasized that Src is involved in the migration and attachment of epithelial cells as it is an integral part of the focal adhesion complex that links integrins to the cytoskeleton [43].

Dasatinib significantly inhibited cyclin D1, making our findings agreeing with multiple studies reporting that Dasatinib inhibited proliferation in various cancer cell types [23,29,30,35,43-51]. Our results agreed with a previous study reporting that Dasatinib didn't induce apoptosis in HCT-116 CRC cell line [52] but disagreed with studies reporting that Dasatinib induced caspase-3 in various cancer types [23,30,44,53-56]. As for VEGF, our results agree with a previous study reporting that Dasatinib decreased VEGF in advanced non-small-cell lung carcinoma patients [57].

Regarding Celecoxib, the present finding demonstrated that Src gene was up-regulated by Celecoxib while Src protein was inhibited making our results in agreement with a previous study in which aspirin, as well as other Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), activated c-Src gene in SW480 CRC cells [58]. It was reported that NSAIDs activated c-terminal src kinase (csk), a c-Src negative regulator, to suppress c-Src activity in HT29 CRC cells which might be due to dose, type of the NSAID, and time of exposure [58].

Our data demonstrated that Celecoxib caused a significant reduction in NF-κB protein level, as supported by a previous study [59]. Celecoxib suppressed NF-κB *via* inhibition of IKK, IκB phosphorylation and degradation, p65 phosphorylation, and nuclear translocation. Accordingly, Celecoxib inhibited NF-κB-regulated gene products such as COX-2, MMP-9, and Cyclin D1 [60].

The current study demonstrated significant inhibition in TNF-Aα protein level upon Celecoxib treatment. In parallel with our results, Celecoxib suppressed TNF-induced activation of AKT, ERK, c-Jun N-terminal Kinases (JNKs), and p38 MAPK [59]. Celecoxib suppressed TNFAα-induced transcriptional and DNA binding activities of NF-kB; however, Celecoxib didn't influence TNFAα-induced activation of IKK and degradation of IkBa and

IkBb. Intriguingly, Celecoxib abolished TNFAα-induced nuclear accumulation of the NF-kB p65 subunit. Accordingly, Celecoxib inhibited TNF-Aα induced expression of inflammatory cytokines. Celecoxib didn't influence TNF-Aα induced nuclear translocation of c-jun and activation of ERK, JNK, p38, and AKT [61].

Celecoxib significantly decreased p-AKT protein level in our study. A previous study reported a novel mechanism by which it selectively inhibits AKT activation, thereby attenuating a main anti-apoptotic pathway activity. The action of Celecoxib may require AKT regulators downstream of PI3-kinase or involve PI3-kinase-independent pathways [62]. Celecoxib distinctly down-regulated p-AKT in SGC-7901 gastric cancer cells, which leads to the overexpression of caspase-8 and -9 and activation of procaspase-8 and -9, thus promoting apoptosis via the death receptor and mitochondrial pathways [63].

The present study showed a decrease in p-STAT-3 protein level upon Celecoxib treatment. Consistent with the present finding, a previous study demonstrated that Celecoxib down-regulated p-STAT-3 and other STAT-3-related genes in medulloblastoma–double-positive cells [64]. Moreover, Celecoxib suppressed STAT-3 phosphorylation in nasopharyngeal carcinoma cell lines [HNE1, and CNE1-LMP1] [65].

In agreement with our results, MMP-9 was significantly decreased by Celecoxib in MG-63 osteosarcoma cells [66]. Celecoxib exerted its antitumor effect *via* the down-regulation of the membrane-anchored glycoprotein, RECK, which inhibited MMP-9 on a post-transcriptional level to block blood vessel formation and metastasis [66]. Celecoxib also suppressed MMP-9 activity in lung adenocarcinoma cells cultured in conditioned media [67,68].

Supporting our finding, Celecoxib markedly inhibited proliferation in animal models of skin, breast, lung, bladder, and colorectal cancers [69]. Furthermore, Celecoxib inhibited growth and promoted cell-cycle arrest at the G0/G1 phase in various cancer cell types *in-vitro* [65,70-75].

The present data inferred that Celecoxib didn't influence caspase-3 protein level; however, apoptosis was promoted by Celecoxib in various cancer cell types [76-79] *via* both COX-dependent and COX-independent mechanisms [69].

Several studies inferred that COX-2 enhances angiogenesis via the up-regulation of VEGF [80-84]. Supporting our findings, Celecoxib decreased VEGF gene expression in ovarian SKOV-3 carcinoma xenografts [85] and reduced microvessel density in human breast cancer [86]. In a rat cornea model, the antiangiogenic effect of Celecoxib involved COX-2 inhibition [87,88]. PGs binding to its receptor promote VEGF expression *via* hypoxia-inducible factor 1 alpha explaining in part the correlation between VEGF and COX-2/PGs [89].

It was reported that COX-2 independent mechanisms contribute to the antiangiogenic effect of Celecoxib. In rat hepatoma cells, Celecoxib suppressed the activation of the early growth response factor (Egr-1) [90] that plays a pivotal role in the transcriptional regulation of the fibroblast growth factor [91,92]. Therefore, the down-regulation of Egr-1 gene suppresses angiogenesis. Celecoxib down-regulated VEGF in human pancreatic cancer cells via inhibiting Sp1 transcriptional factor [93]. The Sp1-binding site in the promoter region of VEGF appears to be essential for VEGF expression [94]. Therefore, inhibition of Sp1 might be another COX-2 independent mechanism for Celecoxib [67].

Taken all together, they obtained results refer to they potential points of crosstalk between the two signaling pathways c-Src and COX-2 with their downstream molecular targets that are involved in colorectal carcinogenesis. Furthermore, up to our knowledge, this is the first study that offers supporting evidence of the beneficial antitumor effects of combining Dasatinib and Celecoxib in colorectal cancer cell line model. In this context, adding Celecoxib to Dasatinib succeeded to enhance the reduction induced by Dasatinib alone on active c-Src protein level and Src gene expression as well. This determinant effect further reflected on all downstream molecular targets including inhibition of p-AKT, NF-κB, TNF-Aα, STAT-3, VEGF, MMP-9, Cyclin D1, and induction of Caspase-3. Further preclinical and clinical investigational studies are highly recommended to explore the proposed favourable antitumor effects of combining Dasatinib and Celecoxib, not only on colorectal cancer, but also other different types of cancer.

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