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