Genes & Genomics
DOI 10.1007/s13258-011-0168-0

RESEARCH ARTICLE

Cyclooxygenase-2 inhibition by novel Bisaryl imidazolyl imidazole derivatives increases *Bax/Bcl-2* ratio and upregulates *Caspase-3* gene expression in Caco-2 colorectal cancer cell line

Reza Entezari Heravi · Farzin Hadizadeh · Mojtaba Sankian · Jalil Tavakol Afshari · Javad Behravan

Received: 21 August 2011 / Accepted: 07 December 2011 / Published online: 09 April 2012

Abstract

Cyclooxygenase-2 (COX-2) inhibitors including celecoxib inhibit cell growth and induce apoptosis in cancer cells. In this study, the relation of *Bax* (an apoptosis promoter) to *Bcl-2* (an apoptosis inhibitor) ratio with the apoptosis co-ordination enzyme, caspase-3 was investigated in correlation with the treatment of 4,5-bisaryl imidazolyl imidazoles as novel selective COX-2 inhibitors in Caco-2 colorectal cancer cells . Recently, the organic reactions under microwave irradiation attracted attention of scientists due to their high reaction rate, mild reaction conditions and the formation of clean products. Therefore, a microwave-assisted method was used to synthesize our compounds. The effects of these COX-2 inhibitors on the proliferation of Caco-2 cells were evaluated by MTT assay. cDNA microarray and clustering analysis were used to evaluate effects of our synthetic compounds on gene expression pattern of 112 genes involved in apoptosis pathways. *Bax*, *Bcl-2* and *caspase-3* mRNA expression and their relationship were analyzed by quantitative real-time PCR. Results indicated that proliferation of Caco-2 cells after treatment with 4,5-bisaryl imidazolyl imidazoles on Caco-2 cells were time and dose dependent. We conclude that increase in *Bax*/*Bcl-2* ratio leads to an up-regulation in *caspase-3* mRNA expression.

Keywords *Bax/Bcl-2*; *caspase-3*; Apoptosis; Selective COX-2 inhibitors

R. E. Heravi · F. Hadizadeh (✉) · J. Behravan (✉)
Biotechnology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
e-mail: Hadizadehf@mums.ac.ir
e-mail: behravanj@mums.ac.ir

M. Sankian · J. T. Afshari
Immunology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

R. E. Heravi · J. Behravan
Department of Pharmaceutical Biotechnology, Mashhad University of Medical Sciences, Mashhad, Iran

F. Hadizadeh
Department of Medicinal Chemistry, Mashhad University of Medical Sciences, Mashhad, Iran

Introduction

Apoptosis maintains homeostasis in living organisms by depleting cells in response to various stimuli. On the other hand, apoptosis is regulated via the action of several oncogenes and subsequently oncoproteins that display inhibiting or promoting actions. The susceptibility of normal and cancer cells to induction of apoptosis depends on the balance between pro-apoptotic and anti-apoptotic genes. *Bcl-2*, a gene located on chromosome 18q21, encodes a 26-kD protein that blocks programmed cell death without affecting cellular proliferation (Hockenbery et al., 1990). DNA microarrays can be used to study expression of thousands of genes simultaneously. They are important tools for advance biological discovery (Kudoh et al., 2000). In combination with cluster analysis, one can identify underlying gene expression patterns in complex data collected from multiple microarray experiments (Eisen et al., 1998). Herein, we explored the utility of this method to compare effects of our synthetic compounds with celecoxib on gene expression pattern of apoptosis related genes. *Bax* gene is a member of bcl-2 family that promotes apoptosis. The ratio of *Bax* to *Bcl-2* determines the susceptibility of a cell to apoptosis (Yang et al., 2002). It is well known that a family of cysteinyl proteases, caspases, is involved in the apoptotic cell death. Caspase-3 is known to act downstream of *Bax/Bcl-2* control and play a key role in the execution of apoptosis. Cyclooxygenase (COX) is the rate-limiting enzyme in the synthesis of prostaglandins from arachidonic acids. The cyclooxygenase enzyme includes two isoforms, COX-1 and COX-2.