Ain Shams University
Faculty of Pharmacy
Pharmacology and Toxicology Department

A Study on the Potential Chemomodulatory Effects of Biochanin-A in Hepatocellular Carcinoma Cells

A thesis submitted for the partial fulfillment of the requirements of M.Sc. in pharmaceutical sciences (Pharmacology and Toxicology).

By:

Mohi el-din Magdy Mahmoud Youssef

B.Sc. Pharmacy (2010), Faculty of Pharmacy, Ain Shams University

T.A. of Pharmacology and Toxicology Department, Faculty of Pharmacy, Egyptian Russian University

Under the Supervision of:

Prof. Dr. Ashraf Bahy Abdel-Naim

Professor of Pharmacology and Toxicology Department
Faculty of Pharmacy
Ain Shams University

Prof. Dr. Amani Emam Khalifa

Professor of Pharmacology and Toxicology Department
Faculty of Pharmacy, Ain Shams University
Seconded as the Strategic Planning Consultant for 57357 Hospital, Cairo, Egypt

Prof. Dr. Suher Kamal Zada

Professor of Immunology
Biology Department
American University in Cairo (AUC)

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Mohi Youssef

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List of Abbreviations:

5-FU	5-fluorouracil
AFB1	Aflatoxin B1
AFP	Alpha-feto protein
AJCC	American Joint Committee Cancer
Bio-A	Biochanin-A
CI	Combination index
CT	Cycle threshold
DAB	3,3'-diaminobenzidine
DCR	Disease control rate
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
DRI	Dose Reduction Index
EGFR	Epidermal growth factor receptor
ELISA	Enzyme-linked immunosorbent assay
Fa	Fraction of cells affected
HBsAG	Hepatitis-B surface antigen
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HFSR	Hand-foot skin reaction
HMEC	Primary human mammary epithelia cells
IACT	Intra-artery chemotherapy
IL-2	Interleukin 2
NASH	Non-alcholic steatohepatitis
OS	Overall survival
PBS	Phosphate-buffered saline
PDGFR	Platelet-derived growth factor receptor
PEI	Percutaneous ethanol injection
PET	Positron emission tomography
PVT	Portal vein thrombosis
qRT-PCR	Quantitative real-time polymerase chain reaction

RECIST	Response evaluation criteria for solid tumours
RNA	Ribonucleic acid
SHARP	Sorafenib hepatocellular carcinoma assessment protocol
SHBG	Sex hormone binding globulin
SOR	Sorafenib
TACE	Trans-arterial chemoembolization
TTP	Time to progression
US	United States
VEGFR	Vascular endothelial growth factor receptor

Abstract

Biochanin-A, a promising isoflavone, is a natural chemopreventive with selective toxicity towards cancer cells only. Sorafenib is considered as the first-line therapy for patients with advanced hepatocellular carcinoma (HCC). The current study was designed to investigate the synergistic cytotoxicity of biochanin-A (Bio-A) combined with sorafenib (SOR) in HepG2 human hepatocellular carcinoma cell line through modulating pro-survival and apoptotic pathways. Bio-A alone showed cytotoxic potentiality against HepG2 cells at relatively lower potency (IC_{50}=22µM) compared to SOR (IC_{50}=3µM) using sulforhodamine-B cytotoxicity assay. Calcusyn analysis indicated that the concurrent treatment of Bio-A and SOR at 10% of IC_{50} was synergistic (CI<1). Cell cycle analysis indicated a significant cellular arrest at pre-G and G_0/G_1 phases and subsequent decreased percentage of HepG2 cells in the other cell cycle phases by SOR/Bio-A co-treatment. Concomitantly, the expression of cyclinD1, a cell cycle regulatory protein, was down-regulated. Further mechanistic studies in HepG2 cells showed that the gene expression pattern of Ki-67, a proliferation marker, was down-regulated upon combination of SOR and Bio-A. Apoptotic pathway signaling investigation for this co-treatment

showed decreased Bcl-2/Bax ratio as well as time-dependant elevation of both, caspases 3, 9 gene expression and caspase 3 activity, compared to either drug alone. Additionaly, this combination exhibited a significant down-regulation of survivin gene which is an inhibitor of apoptosis. In conclusion, Bio-A synergistically enhanced the cytotoxic and apoptotic effects of SOR on HepG2 cells. Our findings suggest that Bio-A may be a potential adjuvant to SOR as a new therapeutic regimen for treating HCC.

Introduction

Hepatocellular Carcinoma

Epidemiology:

Liver cancer is the fifth most common cancer in men worldwide (523,000 cases per year, 7.9% of all cancers) and the seventh in women (226,000 cases per year, 6.5% of all cancers) according to the International Agency for Research on Cancer **(El-Serag, 2012).** An estimated 748,300 new liver cancer cases and 695,900 cancer deaths occurred worldwide in 2008 **(Ferlay *et al.*, 2010)**. In men, it is the second most frequent cause of cancer death. In women, it is the sixth leading cause of cancer death **(Jemal *et al.*, 2011; Globocan, 2012).**

Among primary liver cancers, hepatocellular carcinoma (HCC) represents the major histological subtype accounting for 85%-90% of primary liver cancers **(Kim and Ho-Lim, 2011).** Most cases of HCC (>80%) occur in sub-Saharan Africa and in Eastern Asia, with typical incidence rates of > 20 per 100,000 individuals **(El-Serag, 2012)**, whereas rates are low in South-Central and Western Asia, as well as Northern and Eastern Europe **(Kim and Ho-Lim, 2011)**.

Men have a higher prevalence of HCC than women; the ratio of affected men to affected women varies between 2:1 and 4:1, depending on the geographic region **(El-Serag, 2012).**

The reasons for the disparity between men and women are obscure, but they may include environmental factors such as a higher prevalence of persistent hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, alcohol abuse, smoking in men than in women, genetic and hormonal factors **(Naugler *et al.*, 2007).**

The incidence of HCC increases with age, reaching its highest prevalence among those aged over 65 years **(Motola-Kuba *et al.*, 2006).** Although HCC is rare before the age of 50 years in North America and Western Europe **(Yu *et al.*, 2003)**, a shift in incidence towards younger persons has been noted in the last two decades **(El-Zayadi *et al.*, 2005),** and this may be attributed to emergence of HCV infection **(Montalto *et al.*, 2002)**, as well as to acquisition of both hepatitis B and C virus infection at younger age **(Velazquez *et al.*, 2003).**

Hepatocellular carcinoma in Egypt:

In Egypt, liver cancer constitutes 1.68% of total malignancies. Liver tumors were mostly hepatocellular carcinoma (70.48%) **(Mokhtar *et al.*, 2007).** HCC was reported to account for about 4.7% of chronic liver disease patients **(El-Zayadi *et al.*,**