ANTI-COLON CARCINOGENESIS OF CURCUMIN ANALOGS COMBINATION WITH 5-FLUOROURACIL

KHANITTA SRIMUANGWONG

A Thesis Submitted to the Graduate School of Naresuan University
in Partial Fulfillment of the Requirements
for the Doctor of Philosophy Degree in Anatomy
April 2012

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The thesis entitled "Anti-colon carcinogenesis of curcumin analogs combination with 5-fluorouracil" submitted by Khanitta Srimuangwong in partial fulfillment of the requirements for the Doctor of Philosophy degree in Anatomy is hereby approved.

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ABSTRACT

5-Fluorouracil (5-FU) is a widely used chemotherapeutic drug in colorectalcancer, but its chemo-resistance and toxicity limit the success to chemotherapy. A combination of 5-FU with natural substances is often used to enhance the efficacy and reduce the toxicity of this drug. Curcumin (CUR), a major pigment in turmeric, is widely used to prevent and treat a variety of cancers. CUR specifically inhibited mRNA and protein expression of cyclooxygenase-2 (COX-2), which highly expressed in a variety of human cancers, but did not suppressed the cyclooxygenase-1 (COX-1) that maintains body homeostasis. However, CURwas poor solubility and poor absorption which unsuitable for drug design. This study was performed to study the combining effects of 5-FU and hexahydrocurcumin (HHC), a major CUR metaboliteon HT-29 colon cancer cell line and DMH-induced colon carcinogenesis in rats.The present study demonstrates that, in HT-29 cells, HHC significantly decreases cell viability and down-regulates the COX-2 mRNA expression compared to the control but does not alter the COX-1 mRNA. These results suggested that HHC may exert its anti-carcinogenesis by suppressing COX-2 mRNA expression. Furthermore, the combined effect of HHC with a low dose of 5-FU for 24 and 48 h of treatment were showed a quantitative synergistic inhibitory effect on growth of HT-29 cells. Moreover, this combined treatment significantly reduced the expression of COX-2 mRNA and protein and induced the apoptosis to a greater degree than 5-FU or HHC monotherapy. In theDMH- induced colorectal carcinogenesis of rats, HHC treatment alonemarkedly

inhibited the formation of aberrant crypt foci (ACF), preneoplastic lesions in colorectal carcinogenesis as compared to a control group. It is reasonable to assume that HHC exerts growth inhibitory effect of colorectal carcinogenesis. Moreover, HHC in combination with 5-FU showed significantly reduced the ACF formation when compared either HHC or 5-FU treated alone. This combined treatment could decrease the level of COX-2 protein and induced apoptosis when compared to a vehicle group but dose not difference to 5-FU or HHC monotherapy. In addition, this combined treatment dose not alters the level of COX-1 protein. Therefore, the combination effects of these agents in inhibit the ACF formation may be exhibit through other mode of actions. The results from *in vitro* and *in vivo* studies illustrate that HHC is a specific COX-2 inhibitor that lack of toxicity and side effects to normal cells by did not alter the expression of COX-1 and no adverse effects on growth rate of colorectal cancer rat model and encouraging for further study the other mechanism and investigation in pre-clinical trial.

LIST OF CONTENTS

LIST OF CONTENTS (CONT.)

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (CONT.)

LIST OF FIGURES (CONT.)

LIST OF FIGURES (CONT.)

Figure **Page**

LIST OF FIGURES (CONT.)

LIST OF FIGURES (CONT.)

ABBREVIATIONS

%	=	Percent
5-FU	=	5-Fluorouracil
ACF	=	Aberrant crypt foci
ANOVA	=	Analysis of variance
APC	=	Adenomatous polyposis coli
BCl-2	=	B-cell/lymphoma 2
BW	=	Body weight
°C	=	Degree Celsius
CC	=	Crohn's colitis
COX-1	=	Cyclooxygenase-1
COX-2	=	Cyclooxygenase-2
CRC	=	Colorectal cancer
DCA	=	Deoxycholic acid
DMH	=	1,2-dimethyhydrazine
DMSO	=	Dimethyl sulfoxide
DPD	=	dihydropyrimidine dehydrogenase
DSS	=	Dextran sulfate sodium
ECL	=	Enhanced Chemiluminescence
ECM	=	Extracellular matrix
EDTA	=	Ethylenediaminetetraacitic acid
FAP	=	Familial adenomatous polyposis
FdUMP	=	Fluorodeoxyuridine monophosphate
FUTP	=	Fluorouridine triphosphate
HHC	=	Hexahydrocurcumin
HNPCC	=	Hereditary Non-Polyposis Colorectal Cancer
LCA	=	Lithocholic acid
IC50	=	Inhibitory concentration 50%
mg	=	Miligram
Min	=	Minutes

ABBREVIATIONS (CONT.)

ml	=	Mililiter
mM	=	Milimolar
MMP	=	Matrix metalloproteinase
MMR	=	Mismatch repair
MTT	=	3,(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide
mRNA	=	messenger Ribonucleic acid
MVD	=	Microvessel density
NO	=	Nitric oxide
NSAIDs	=	Non-steroidal anti-inflammatory drugs
OPRT	=	Orotatephosphoribosyltransferase
PBS	=	Phosphate buffer saline
PGE2	=	Phorbol ester-induced prostaglandin E2
PGF	=	Phosphatidylinositol-3-kinase
PVDF	=	Polyvinulidene fluoride
RONS	=	Reactive oxygen and nitrogen species
SEM	=	Standard error of mean
TCC	=	Transitional cell carcinoma
THC	=	Tetrahydrocurcumin
TS	=	Thymidylate synthase
UC	=	Ulcerative colitis
μM	=	Micromolar
VEGF	=	Vascular epithelial growth factor