

ห้องสมุดงานวิจัย สำนักงานคณะกรรมการวิจัยแห่งชาติ



E47341

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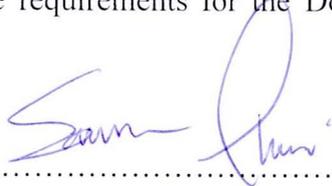
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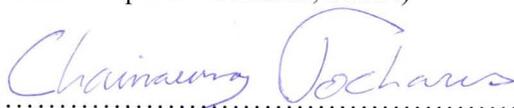
The thesis entitled "Anti-colon carcinogenesis of curcumin analogs combination with 5-fluorouracil" submitted by Khanitta Srimuangwong in partial fulfillment of the requirements for the Doctor of Philosophy degree in Anatomy is hereby approved.



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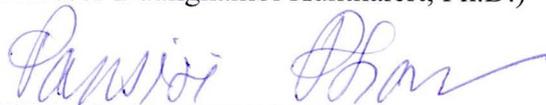
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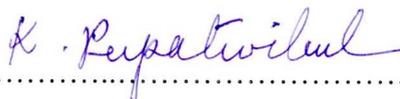


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Title ANTI-COLON CARCINOGENESIS OF CURCUMIN
ANALOGS COMBINATION WITH 5-FLUOROURACIL

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ABSTRACT

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5-Fluorouracil (5-FU) is a widely used chemotherapeutic drug in colorectal cancer, 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 suppress the cyclooxygenase-1 (COX-1) that maintains body homeostasis. However, CUR was 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 metabolite on 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 the DMH- induced colorectal carcinogenesis of rats, HHC treatment alone markedly

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

Chapter	Page
I INTRODUCTION.....	1
Rationale and significance of the study	1
Purpose of the study	3
Scope of this study	3
Hypotheses	3
Anticipated Outcomes	4
II REVIEW OF RELATED LITERATURE AND RESEARCH.....	5
Anatomy of large intestine	5
General information of colorectal cancer	7
Risk factors of colorectal cancer	8
Mechanism of colorectal carcinogenesis	12
Aberrant crypt foci (ACF)	16
Cyclooxygenase and arachidonic metabolism.....	17
Colorectal cancer treatments	21
III RESEACH METHODOLOGY.....	33
Materials.....	33
Methods	33
Statistical analysis	41
IV RESULTS.....	42
Cytotoxic effect of single agent on growth of HT-29 human colon cancer cells	42
Combination effects of 5-FU with CUR and HHC on growth of HT-29 colon cancer cells	46

LIST OF CONTENTS (CONT.)

Chapter	Page
Combination effects of 5-FU with CUR and HHC on DMH-induced colon cancer rats.....	58
V DISCUSSION AND CONCLUSION	68
Cytotoxic effect of 5-FU on growth of HT-29 colon cancer cells.....	68
Effect of HHC alone and in combination with 5-FU on growth of HT-29 cells	68
Effect of HHC alone and in combination with 5-FU on COX-2 mRNA and protein expression in HT-29 cells	70
Effect of HHC alone and in combination with 5-FU on COX-1 mRNA and protein expression in HT-29 cells	71
Effect of HHC alone and in combination with 5-FU on apoptotic induction of HT-29 cells	72
Effect of HHC alone or in combination with 5-FU on ACF formation in DMH-induced colorectal cancer rat	73
Effect of HHC alone or in combination with 5-FU on COX-2 in DMH-induced colorectal cancer rat	76
Effect of HHC alone or in combination with 5-FU on COX-1 in DMH-induced colorectal cancer rat	77
Effect of HHC alone or in combination with 5-FU on apoptotic induction in DMH-induced colorectal cancer rat	77
REFERENCES.....	81
APPENDIX.....	105
BIOGRAPHY.....	118

LIST OF TABLES

Table		Page
1	Primers for genes amplification and expected size of PCR products.....	37
2	IC ₅₀ of CUR, HHC and 5-FU against HT-29 colon cancer cells after 24, 48 and 72h of treatment.....	46
3	Effect of CUR, HHC, 5-FU and their combined treatment on rats exposed to DMH on ACF formation.....	61

LIST OF FIGURES

Figure	Page
1 Anatomy of large intestine	5
2 Absorptive epithelium of large intestine	6
3 Incidence of colorectal cancer in 1998-2000 Thailand	7
4 Diagram showing chronic inflammation associate with development of cancer	12
5 The multi step process of human carcinogenesis	13
6 Genetic mutations in human colon cancer	14
7 Diagram showing one of APC's cellular functions	15
8 ACF in methylene blue stained-colon whole mount. ACF (circumscribed) are easily distinguished from surrounding normal colonic crypt, bars = 100 μ m.....	16
9 Production of prostaglandins by COXs and the cell-specific synthases that are involved in the conversion of prostaglandin H ₂ to the five principal prostaglandins.....	18
10 Metabolism of 5-fluorouracil.....	22
11 Pharmacology of NSAIDs and selective COX-2 inhibitors on prostaglandinsynthesis	24
12 Characteristics of <i>Curcuma Longa</i> L.....	25
13 Structures of Curcumin, Demethoxycurcumin and Bisdemethoxycurcumin	25
14 Cell cycle regulation	27
15 IKK/NF- κ B signaling pathways	29
16 CUR inhibit angiogenesis of tumor	31
17 Structure of HHC	32
18 Experimental protocol	39

LIST OF FIGURES (CONT.)

Figure	Page
19 Cytotoxic effect of 5-FU (0, 5, 10 and 25 μ M) on HT-29 cells after 24, 48 and 72 h. treatment. Each value was represented as mean \pm SEM. * indicates statistically significant values when compared to a control ($P<0.05$).....	43
20 Cytotoxic effect CUR(0, 5, 10 and 25 μ M) on growth of HT-29 after 24, 48 and 72 h. treatment.Each value was represented by mean \pm SEM. * indicates statistically significance values when compared to a control ($P<0.05$).....	44
21 Cytotoxic effect of HHC (0, 5, 10 and 25 μ M) on HT-29 after 24, 48and 72 h. treatment. Each value was represented by mean \pm SEM. * indicates statistically significance values when compared to a control ($P<0.05$).....	45
22. Growth inhibition of HT-29 after treated with 5-FU (5 μ M), CUR (5, 10, 25 μ M) alone and their combination for 24h (a), 48h (b), and 72h (c).Each value was represented by mean \pm SEM of three independent studies. * indicates statistically significance values compared to 5-FU and HHC monotherapy ($P<0.05$).....	47
23 Growth inhibition of HT-29 after treated with 5-FU (5 μ M), HHC (5,10, 25 μ M) alone and their combination for 24 (a), 48 (b), and 72 h (c). Each value was represented by mean \pm SEM of three independent studies. * indicates statistically significant values when compared to 5-FU and HHC monotherapy ($P<0.05$).....	48
24 Combination index of 5-FU combined with CUR at 24, 48 and 72 h on HT-29 colon cancer cells. Each value wasrepresented by mean \pm SEM of three independent studies.....	49
25 Combination index of 5-FU combined with HHCat 24, 48 and 72 h on HT-29 colon cancer cells. Each value was represented by mean \pm SEM of three independent studies.....	50

LIST OF FIGURES (CONT.)

Figure	Page
<p>26 RT-PCR(a) illustrate the expression of COX-2 mRNA of HT-29 cells after treated by 5-FU (5μM) combined with CUR and HHC (25 μM) for 24 h. Percent of mRNA expression (b) was determined by normalizing the band intensity of COX-2 with GAPDH. The control levels of COX-2 expression were considered as 100% and the treated levels were calculated as relative percentages for each experiment. Each bar is a mean\pmSEM of three experiments. * indicates statistically significance values compared to a control; + indicates statistically significant values compared to 5-FU and HHC monotherapy ($P<0.05$)</p>	51
<p>27 RT-PCR (a) illustrate the expression of COX-1 mRNA of HT-29 cells after treated with 5-FU (5μM) combined with CUR and HHC (25 μM) for 24 h. Percent of mRNA expression (b) was determined by normalizing the band intensity of COX-1 with GAPDH. The control levels of COX-1 expression were considered as 100% and the treated levels were calculated as relative percentages for each experiment. Each bar is a mean\pmSEM. of three experiments. * indicates statistically significance values compared to control ($P<0.05$).....</p>	52

LIST OF FIGURES (CONT.)

Figure		Page
28	Western blots (a) illustrate the expression of COX-2 (72 kDa) and β -actin (43kDa) in HT-29 colon cancer cells treated with 5-FU at 5 μ M combined with CUR and HHCat 25 μ M for 48 h. COX-2 protein level in HT-29 colon cancer cells after having been treated with control, 5-FU (5 μ M), CUR, HHC (25 μ M) and their combination for 48 h. Percent of protein expression (b) was determined by normalizing the band intensity of COX-2 with β -actin. The control levels of COX-2 expression were considered as 100% and the treatment levels were calculated as relative percentages for each experiment. Each bar is an arithmetic mean of three experiments. Statistically significant differences between the control and all treated groups are indicated by * ($P < 0.05$); + compared with 5-FU, CUR and HHC monotherapy ($P < 0.05$).....	54
29	Western blots (a) illustrate the expression of COX-1 (70 kDa) and β -actin (43kDa) in HT-29 colon cancer cells treated with 5-FU at 5 μ M combined with CUR and HHCat 25 μ M for 48 h. COX-1 protein level in HT-29 colon cancer cells after having been treated with control, 5-FU (5 μ M), CUR, HHC (25 μ M) and their combination for 48h. Percent of protein expression (b) was determined by normalizing the band intensity of COX-1 with β -actin. The control levels of COX-1 expression were considered as 100% and the treatment levels were calculated as relative percentages for each experiment. Each bar is an arithmetic mean of three experiments.....	55

LIST OF FIGURES (CONT.)

Figure	Page
30 Fluorescence images of HT-29 colon cancer cells using Hoechst 33342 staining showed apoptotic morphological changes (→) induced by various treatment. Scale bars = 25 μm	57
31 Apoptosis of HT-29 colon cancer cells after treated with 5-FU (5μM), CUR, HHC (25 μM) alone and their combination for 48 h. Each value was represented by mean ±SEM of three independent studies. * indicates statistically significance values when compared to a control (P<0.05); + indicates statistically significant values compared to 5-FU, CUR and HHC monotherapy (P<0.05).....	58
32 Mean body weight of rat in all experimental groups. Values are mean±SEM	59
33 Topographical view of aberrant crypt foci, small ACF with 2 crypts (a) and large ACF containing more than 3 crypts per ACF (b) in whole-mount colon of rats exposed to DMH. Scale bars = 25 μm....	60
34 Immunohistochemical staining of COX-2 protein labeled cell in colon mucosa of rat model. Arrow (→) indicated the COX-2 protein labeled cell. Scale bars = 50 μm.....	62
35 Percent of COX-2 proteinlabeled cell in colon mucosa of rat exposed to DMH. Each value was represented by mean±SEM. Statistically significant differences between the vehicle and treated groups are indicated by * (P<0.05); + compared with monotherapy (P<0.05)....	63
36 Immunohistochemical staining of COX-1 protein labeled cell in colonmucosa of all experimental groups. Arrow (→) indicated the COX-1 protein labeled cell. Scale bars = 100 μm.....	64
37 Percent of COX-1 protein labeled cell in colon tissues of rats treated with various agents. Each value was represented by mean±SEM of five animals per group.....	65

LIST OF FIGURES (CONT.)

Figure		Page
38	Apoptosis labeled with TUNEL method. Arrow (→) indicated the apoptosis labeled cell. Scale bar = 25μm.....	66
39	Apoptosis in colon of rat after exposed to different treatments. Each value was represented by mean±SEM. * indicates statistically significance values compared to normal ($P<0.05$); + compared with vehicle ($P<0.05$).	67
40	The possible mechanism of HHC on inhibition of colorectal carcinogenesis.....	68

ABBREVIATIONS

%	=	Percent
5-FU	=	5-Fluorouracil
ACF	=	Aberrant crypt foci
ANOVA	=	Analysis of variance
<i>APC</i>	=	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-dimethylhydrazine
DMSO	=	Dimethyl sulfoxide
DPD	=	dihydropyrimidine dehydrogenase
DSS	=	Dextran sulfate sodium
ECL	=	Enhanced Chemiluminescence
ECM	=	Extracellular matrix
EDTA	=	Ethylenediaminetetraacetic 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
μ M	=	Micromolar
VEGF	=	Vascular epithelial growth factor