EFFECT OF SERICIN AGAINST COLON CANCER IN CELL CULTURE AND ANIMAL MODELS

WARAPORN KAEWKON

A Thesis Submitted to the Graduate School of Naresuan University
in Partial Fulfillment of the Requirements
for the Doctor of Philosophy Degree
in Pharmaceutical Sciences (International Program)
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ABSTRACT

Colon cancer is one of the most common cancers in many regions of the world and could be prevented by dietary interventions. This study was aimed to examine the chemopreventive effect of silk protein, sericin, in human colorectal cancer SW480 cells compared with normal colonic mucosal FHC cells and in 1,2-dimethylhydrazine (DMH)-induced colon tumorigenesis in rats in comparison with control casein diet. Sericin was found to decrease SW480 and FHC cell viability. The small sericin possessed higher anti-proliferative effects than that of the large sericin in both cell types. Increased apoptosis of SW480 cells was correlated with increased caspase-3 activity and decreased Bcl-2 expression. In animal model, the result showed that 2 from 6 of casein fed rats developed colon tumor, whereas none of sericin fed rats exhibited tumors. Consumption of sericin prior to or during carcinogen exposure reduced the number of aberrant crypt foci (ACF). In addition to crypt number, crypt multiplicity was less progressive in sericin fed group. Sericin diet also exhibited anti-oxidative activity by reducing the level of lipid peroxidation in rat colons. These findings suggest that sericin induce apoptosis of colon cancer cell line and that consumption of sericin may reduce the progression of colon tumor development possibly by suppressing the initiation and promotion stages of colon tumorigenesis.

LIST OF CONTENTS

Chapter		Page
I	**INTRODUCTION**	1
	The rationale for the study	1
	Objectives of the study	4
	Expected outputs of the study	4
	Expected outcomes	4
II	**REVIEWS OF RELATED LITERATURE AND RESEARCH**	5
	Colon cancer	5
	Incidence	5
	Risk factors	5
	Colon cancer and diet	7
	Colon cancer development	11
	Biomarkers of colon carcinogenesis	12
	Colon cancer models	13
	Colon cancer and chemoprevention	13
	Immune system and colon cancer	16
	Sericin protein	18
	Structure and biochemical properties	18
	Application of sericin	19
III	**RESEARCH METHODOLOGY**	22
	Materials	22
	Cell lines	22
	Animals	22
	Chemical and reagents	22
	Instruments	25

LIST OF CONTENTS (CONT.)

Chapter **Page**

Methodology..... 26
Cell culture model..... 27
Animal model..... 33
Statistical analysis..... 39

IV RESULTS AND DISCUSSION..... 40
Chemopreventive effect of sericin in cell culture model..... 40
Effect of sericin on viability of SW480 cells..... 40
Effect of sericin on viability of FHC cells..... 45
Comparison the effect of sericin on SW480 and FHC cell viability..... 50
Effect of sericin on SW480 and FHC cell apoptosis..... 52
Effect of sericin on caspase-3 activity in SW480 cells..... 54
Effect of sericin on expression of Bcl-2 and Bax..... 56
Effect of sericin on cell cycle..... 59
Chemopreventive effect of sericin in animal model..... 61
Effect of sericin on body weight and food consumption..... 61
Effect of sericin on incidence and distribution of ACF and tumor in rat colons..... 64
Density of ACF in different parts of rat colons..... 66
Effect of sericin on crypt multiplicity of ACF..... 68
Area analysis of large size ACF..... 70
Effect of sericin on lipid peroxidation in rat colon..... 72
Immunohistochemical analysis of Ki67 in rat colons..... 74
Effect of sericin on immunohistochemical analysis of Bcl-2.. 79
Effect of sericin on cell surface markers in rat blood..... 82

LIST OF CONTENTS (CONT.)

Chapter		Page
V	CONCLUSIONS	85
	REFERENCES	86
	APPENDIX	104
	BIOGRAPHY	145

LIST OF TABLES

Table		Page
1	Type and molecular weight of sericin in this study	28
2	Composition of amino acids in sericin	28
3	Type of cell staining with annexin V-FITC and PI analysis by flow cytometer	30
4	Cell surface markers in rats	37
5	Effect of various concentrations of sericin on SW480 cell cycle	58
6	Effect of sericin on rat body weight	60
7	Initial and final food consumption	60
8	Effect of sericin on organ weight and serum enzymes in rat colons	61
9	Effect of sericin on incidence and distribution of ACF and tumor in rat colon	63
10	Density of ACF in different parts of colon of DMH-treated rats	65
11	Crypt multiplicity of ACF in DMH-treated rat colons	67
12	Area analysis of large size ACF	69
13	Effect of sericin on lipid peroxidation in rat colon	71
14	Total number of Ki67 positive cells in colonic crypts	74
15	Length of colonic crypts	74
16	Effect of various concentrations of sericin on SW480 cell viability after incubation for 24 h	105
17	Effect of various concentrations of sericin on SW480 cell viability after incubation for 48 h	106
18	Effect of various concentrations of sericin on SW480 cell viability after incubation for 72 h	107
19	Effect of various concentrations of sericin on FHC cell viability after incubation for 24 h	108
20	Effect of various concentrations of sericin on FHC cell viability after incubation for 48 h	109

LIST OF TABLES (CONT.)

Table **Page**

21 Effect of various concentrations of sericin on FHC cell viability after incubation for 72 h..... 110
22 Effect of various concentrations of sericin on SW480 cell apoptosis..... 111
23 Effect of various concentrations of sericin on FHC cell apoptosis..... 112
24 Effect of sericin on caspase-3 activity..... 113
25 Effect of sericin on Bcl-2 expression..... 114
26 Effect of sericin on Bax expression..... 115
27 Effect of sericin on cell cycle of SW480 cells..... 116
28 Effect of sericin on cell cycle of FHC cells..... 117
29 Effect of sericin on body weight..... 118
30 Effect of sericin on food consumption..... 119
31 Effect of sericin on ACF formation..... 120
32 Effect of sericin on distribution of ACF in proximal part of colon..... 121
33 Effect of sericin on distribution of ACF in middle part of colon..... 122
34 Effect of sericin on distribution of ACF in distal part of colon..... 123
35 Effect of sericin on area of large size of ACF in middle part of colon..... 124
36 Effect of sericin on area of large size of ACF in distal part of colon..... 125
37 Effect of sericin on lipid peroxidation in control rats..... 126
38 Effect of sericin on lipid peroxidation in DMH-treated rats..... 127
39 Effect of sericin on Ki67 proliferation marker in proximal colon..... 128
40 Effect of sericin on Ki67 proliferation marker in middle colon..... 130
41 Effect of sericin on Ki67 proliferation marker in distal colon..... 132
42 Effect of sericin on length of colonic crypts in proximal colon..... 134
43 Effect of sericin on length of colonic crypts in middle colon..... 136
44 Effect of sericin on length of colonic crypts in distal colon..... 138
45 Effect of sericin on complete blood count (CBC)..... 140

LIST OF TABLES (CONT.)

Table		Page
46	Effect of sericin on complete blood count (CBC).....	141
47	Cell surface markers in rats part I.....	142
48	Cell surface markers in rats part II.....	143
49	Cell surface markers in rats part III.....	144

LIST OF FIGURES

Figure		Page
1	Process of colon carcinogenesis	11
2	Structure of sericin	19
3	Experimental design in this study	26
4	Experimental design for DMH-induced colon cancer in rat	34
5	Viability of SW480 cells treated with various concentrations of sericin for 24 h	41
6	Viability of SW480 cells treated with various concentrations of sericin for 48 h	42
7	Viability of SW480 cells treated with various concentrations of sericin for 72 h	44
8	Viability of FHC cells treated with various concentrations of sericin for 24 h	45
9	Viability of FHC cells treated with various concentrations of sericin for 48 h	46
10	Viability of FHC cells treated with various concentrations of sericin for 72 h	47
11	Viability of SW480 and FHC cells	49
12	Effect of sericin on apoptosis of SW480 and FHC cells	51
13	Effect of sericin on caspase-3 activation of SW480 cells	53
14	Effect of sericin on Bcl-2 protein expression of SW480 cells	55
15	Effect of sericin on Bax protein expression of SW480 cells	56
16	Immunohistochemical analysis of Ki-67 expression in distal colon of control rat	75
17	Immunohistochemical analysis of Ki-67 expression in distal colon of DMH-treated rat	76

LIST OF FIGURES (CONT.)

Figure		Page
18	Immunohistochemical analysis of Bcl-2 expression in distal colon of control rat.....	78
19	Immunohistochemical analysis of Bcl-2 expression in distal colon of DMH-treated rat.....	79
20	Flow cytometry of rat surface markers.....	82

ABBREVIATIONS

α	=	alpha
β	=	beta
γ	=	gamma
kDa	=	kilodalton
nm	=	nanometer
µg	=	microgram
µl	=	micro liter
µm	=	micrometer
p	=	probability values
°C	=	degree celsius
4-HNE	=	4-hydroxynonenal
8-OHdG	=	8-hydroxydeoxyguanosine
AC-DEVD	=	acetyl-Asp-Glu-Val-Asp-7-amido-4-methylcoumarin
ACF	=	aberrant crypt foci
AITC	=	allyl isothiocyanate
AMC	=	7-amido-4-methylcoumarin
ANOVA	=	analysis of variance
AOM	=	azoxymethane
AP	=	alkaline phosphatase
APC	=	antigen-presenting cells
Apaf-1	=	apoptotic protease activating factor 1
ATCC	=	american type culture collection
Bax	=	bcl-2 associated x protein
BCA	=	bicinchoninic acid
Bcl-2	=	b-cell lymphoma 2
Bcl-X_L	=	b-cell lymphoma-extra large
BSA	=	bovine serum albumin
$CaCl_2$	=	calcium chloride
Caspase	=	cysteine-aspartic proteases

ABBREVIATIONS (CONT.)

CD	=	cluster of differentiation
cm^2	=	centimeter square
CO_2	=	carbon dioxide
Cont.	=	continued
CTLA-4	=	cytotoxic T-lymphocyte antigen 4
Cyto c	=	cytochrome c
DAB	=	3, 3'- diaminobenzidine
DC	=	dendritic cells
DMEM/F-12	=	Dulbecco's modified Eagle's medium with HamF-12
DMH	=	1,2-dimethylhydrazine
DNA	=	deoxyribonucleic acid
E	=	eosin
EDTA	=	ethylenediaminetetraacetic acid
EGCG	=	(-)-epigallocatechin gallate
EGFR	=	epidermal growth factor receptor
Em	=	emission
EtOH	=	ethanol
Ex	=	excitation
FACS	=	fluorescence-activated cell sorting
FAP	=	familial adenomatous polyposis
FBS	=	fetal bovine serum
FITC	=	fluorescein isothiocyanate
g	=	gram
h	=	hour(s)
HCl	=	hydrochloric acid
HE	=	hematoxylin
HEPES	=	N'-2-Hydroxyethylpiperazine-N'-2 ethanesulphonic acid
HER2	=	human epidermal growth factor receptor-2
HNPCC	=	hereditary non-polyposis colorectal cancer

ABBREVIATIONS (CONT.)

H_2O	=	dihydrogen monoxide
ICAM-1	=	intercellular adhesion molecule 1
IFN-γ	=	interferon-γ
IgG	=	immunoglobulin G
IL-2	=	interleukin-2
iNOS	=	inducible nitric oxide synthase
kg	=	kilogram
KCl	=	potassium chloride
KH_2PO_4	=	potassium dihydrogen phosphate
L	=	liter
Lck	=	lymphocyte-specific protein tyrosine kinase
LFA-1	=	lymphocyte function-associated antigen 1
M	=	molarity
mAb	=	monoclonal antibodies
Mac-1	=	macrophage 1 antigen
MDA	=	malondialdehyde
MHC	=	major histocompatibility complex
mg	=	milligram
min	=	minute
ml	=	milliliter
mm	=	millimeter
M-PER	=	mammalian protein extraction reagent
MTT	=	3-[4,5-dimethylthiazol-2-yl]-2,3-diphenyl tetrazolium bromide
MW	=	molecular weight
NaCl	=	sodium chloride
Na_2CO_3	=	sodium carbonate
Na_2HPO_4	=	disodium hydrogen phosphate
NBT/BCIP	=	nitro blue tetrazolium chloride/5-bromo-4-chloro-3-indolyl phosphate
NK	=	natural killer cells

ABBREVIATIONS (CONT.)

No.	=	number
OD	=	optical density
pAb	=	polyclonal antibodies
PBS	=	phosphate-buffer saline
PEN/STREP	=	penicillin/ streptomycin
pH	=	power of hydrogen ion concentration
PhIP	=	2-amino-1-methyl-6- phenyl imidazo [4,5-*b*] pyridine
PI	=	propidium iodide
PMSF	=	phenylmethylsulfonyl fluoride
PS	=	phospholipid phosphatidylserine
PTP	=	protein tyrosine phosphatases
PVDF	=	polyvinylidene difluoride
RBC	=	red blood cell
ROS	=	reactive oxygen species
S	=	sacrifice
sc	=	subcutaneous
SEM	=	standard error of the mean
SD	=	standard deviation
SDS-PAGE	=	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TBA		thiobarbituric acid
TBARs	=	thiobarbituric acid reactive substances
TBS	=	tris buffer saline
TCA	=	trichloroacetic acid
TNF	=	tumor necrosis factor
U	=	unit
UVB	=	ultraviolet B
w/v	=	weight by volume