EFFECT OF ANTIOXIDANT-DIETARY FIBER MIXTURES ON CANCER GROWTH IN COLORECTAL CANCER-INDUCED RATS

By

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ABBREVIATIONS USED IN THIS THESIS

ACF	Aberrant Crypt Foci
AGRF	Australian Genome Research Facility
AI	Apoptotic Index
AOM	azoxymethane
AP	Alkaline phosphatase
APC	Adenomatous Polyposis Coli
ATCC	American Type Culture Collection
ATP	Adenosine triphosphate
BARF	*B-type Raf kinase*
BLAST	Basic Local Alignment Search Tool
C	Cellulose
CFU	Colony Forming Units
CIMP	CpG Island Methylator Phenotype
CIN	Chromosomal Instability Pathway
Cin	Cincau
CinL	Cincau Leave
COX-2	cyclooxygenase-2
CRC	Colorectal Cancer
CtBP1	C-terminal Binding Protein-1
DCC	Deleted in Colorectal Carcinoma
DE	Degree of Esterification
DF	Dietary fiber
DGGE	Denaturing gel gradient electrophoresis
DMEM	Dulbecco's Modified Eagle's Medium
DMH	dimethyhydrazine
DNA	Deoxyribonucleic acid
DP	degree of polymerization
DSS	Dextran Sulphate Sodium
EGCG	(-)-epigallocatechin-3-gallate
FAP	Familial Adenomatous Polyposis
FB	Faecal Blank
FOS	fructose oligosaccharide
FS	fermentation supernatant
HCA	Heterocyclic Aromatic Amines
HDAC	Histone deacetylase
I	Inulin
IAP	Intestinal Alkaline Phosphatase
IBD	Inflammatory Bowel Disease
iNOS	inducible nitric oxide synthases
LDH	Lactate dehydrogenase
LSD	Less significant difference
MDA	Malondialdehide
MDF	mucin-depleted foci
MSI	Microsatelite Instability
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium

NAD	nicotinamide adenine dinucleotide
NEEA	non essential amino acid
NOx	Nitrogen oxide
NSAIDs	non steroidal anti-inflammatory drugs
P	pectin
PARP	Poly(ADP-ribose) Polymerase
PC	Pectin-Cellulose
PCNA	proliferating cell nuclear antigen
PCR	polymerase chain reaction
PGE2	prostaglandin E (2)
PGE3	prostaglandin E (3)
ROS	Reactive Oxygen Species
RNA	Ribonucleic acid
RNS	Reactive Nitrogen Species
SCFA	short-chain fatty acids
SEM	Standard error of mean
SHIME	Simulator of the Human Intestinal Microbial Ecosystem bromide
UPGMA	Unweighted pair group with mathematical averages

ABSTRACT

Colorectal cancer (CRC) incidence is rising significantly in most Countries due to increasing prosperity. Epidemiological studies indicate that dietary fiber and antioxidants may protect against CRC. Dietary fiber is thought to suppress colorectal cancer growth via the production of short chain fatty acids (SCFA) in the colon, where specific compounds are produced via bacterial breakdown of the fiber. Colonic bacteria are also involved in antioxidant metabolism in the colon, and they can increase antioxidant bioavailability and activity. My research aimed to study the beneficial effect of different combinations of dietary fiber and antioxidant sources including dried green cincau extracts in the colon, these effects were examined in both *in vitro* and *in vivo* models of colon cancer. Green cincau (*Premna oblongifolia* Merr) is an Indonesia plant where the extract has high dietary fiber and antioxidant activity and was also tested in this thesis.

SCFA significantly inhibited proliferation while inducing differentiation of Caco-2 cells irrespective of the media pH. Caspase 3 and 7 (key mediators in the extrinsic and intrinsic apoptotic pathway) activities were affected by both pH and SCFA, but there was no interaction between them. Caco 2 cells were less proliferated in low media pH as this condition induced cell apoptosis. Butyrate induced cell death was observed through both caspase3/7-dependent and -independent pathways as indicated by increased caspase 3/7 activity.

Fermentation experiments using anaerobic batch cultures inoculated with human fecal slurries showed that soluble fiber (pectin and inulin)

resulted in significantly higher SCFA production than that observed with insoluble (cellulose) fiber. In Caco-2 cells, inhibition of cell growth was dependent on the amount of SCFA generated during fermentation in particular butyrate. However, the effect of fermentation supernatant (FS) on cell differentiation and apoptosis was not able to be explained by the butyrate content, as high butyrate in the FS did not always promote differentiation and the apoptotic process. Apoptotic, necrotic and autophagic pathways might all be involved in cell death in response to FS treatment. The ability of the supernatant to modulate parameters of cell growth, differentiation and apoptosis was dependent on butyrate concentration and, possibly, unidentified compounds.

Using the Azoxymethane (AOM)-induced rat model of CRC it was found that 0.1% epigallocatechin-3-gallate (EGCG) increased some individual SCFA concentrations (acetate and butyrate) in digesta when the dietary fiber source was cellulose (CE), and an opposite effect was observed when the dietary fiber source was pectin (PE). Pectin-EGCG combined induced cancer progression, characterized by an increase in total number of aberrant crypt foci (ACF), and also an increase in the proliferating cell nuclear antigen (PCNA) labelling index and PCNA positive cells. This effect was associated with increasing lipid peroxidation in the liver. The protective effect of antioxidant EGCG consumption against colon cancer development appears to be dependent on the type of the dietary fiber source in the diet and the mechanism particularly through the modification of antioxidant/prooxidant properties of the EGCG.

The beneficial effect of individual dietary fibers does not automatically synergize with the positive effects of potential antioxidants, and their combined effect will depend on how they interact with the colon microbiota of the individual. Natural mixtures of dietary fiber and antioxidant sources (as found in fruits, vegetables and plant extracts) may exhibit protective effect against CRC, and utilization of these sources should consider the processing method such as the drying process to protect their potency. In conclusion the work presented in this thesis suggests that the consumption of fresh dietary fiber antioxidants sources may pose the greatest protection against CRC.

DECLARATION

I certify that this thesis does not incorporate without acknowledgement any material previously submitted for a degree of diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Adelaide, 29 August 2012

Samsu Udayana Nurdin

PREFACE

This thesis is based on the three major research projects that I have conducted during my PhD. It has 7 chapters arranged chronologically including general introduction (Chapter 1), three journal articles (Chapter 2, 3 and 4), Conclusions and Future direction (Chapter 5), Appendix (Chapter 6) and References (Chapter 7). I present this thesis mainly as three journal articles prepared for submission, so there will be some repetition of concepts, abbreviations and definitions, but all references are listed in one chapter (Chapter 7). Although these papers will be eventually submitted to different journals for ease of reading they all conform to the same style in the thesis. Some data and information supporting the main thesis can also be found in Appendix (Chapter 6).

Samsu Udayana Nurdin

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