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Flinders University, South Australia

Faculty of Health Sciences, Department of Gastroenterology

AOU: Professor Graeme P. Young

CONSEQUENCES OF THE REGULATION OF DNA DAMAGE AND OTHER HOST RESPONSES BY FISH OIL FOR COLORECTAL ONCOGENESIS.

A Ph.D. Thesis

By

Laura Sophia Nyskohus, B.S (Hons)

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ABSTRACT

The acute cellular responses to DNA damaging agents are critical in determining the long term outcome of disease. A cell's susceptibility to damage, or its capacity to remove or repair this damage, all contributes to the eventual health or disease of tissues. This process is especially crucial in colonic epithelial cells and in the development of colorectal oncogenesis. The colonic lumen is constantly subjected to different environmental compounds that may have genotoxic properties that can initiate mutational events and possibly carcinogenesis. Therefore, the study of a regulatory dietary agent that improves the colonic cells ability to withstand damage, improve repair and retain its general health is a significant and practical tool in the fight against colorectal cancer.

The health benefits of fish oil, including its potential chemopreventative properties, have been reported in numerous studies. However, the mechanism by which this protective effect occurs remains unclear. A gap in current literature exists that fails to explore the effect of fish oil on the early cellular responses to carcinogenic agents. Therefore, this thesis aims to firstly, better understand the specific host responses to an insult of carcinogen *in vivo*; secondly, to determine if regulation of these responses can be achieved by dietary fish oil; and lastly, to explore the potential consequences of this regulation for colorectal oncogenesis.

All experimental work was carried out using a rat – azoxymethane (AOM) animal model of colorectal carcinogenesis. The key host responses to the carcinogen that were measured included the formation of acute O^6methyldeoxyGuanosine (O^6medG) DNA damage, the acute apoptotic response to genotoxic carcinogen (AARGC) and cell proliferation rates. A novel immunochemical assay was designed to detect both the levels and distribution of O^6medG in colonic cells. With this established, a pattern of these host responses were mapped out over time. A dietary intervention study trialling a range of fish oil diets containing different doses and forms was then carried out to determine if modulation of responses occurred. This study was then followed on by a longer term study that explored the consequences of regulation by fish oil on pre-neoplastic lesions in the colon.

The acute host responses to an insult of AOM showed that colonic O^6medG formation began 2h post AOM administration and peaked at 6h. The AARGC response followed

the pattern of O^6medG by a 2h delay, peaking at 8h post AOM administration, while cell proliferation rates decreased significantly after 6h.

The inclusion of tuna oil in the diet did not affect either the AARGC or cell proliferation rates when given in any form or at any dose. Animals fed a diet with 15% free tuna oil and 7% encapsulated tuna oil did however have significantly reduced levels of O^6medG DNA damage in the distal colon ($p<0.05$). This reduction in O^6medG levels did not translate into a reduction of ACF lesion, with a protective effect against ACF lesions only being observed in animals fed the high dose fish oil groups.

Analysis of the data suggest that the acute host responses to an insult of DNA damaging agent appear to be closely related, all reaching their peak level of response 6-8h after the insult. The short time frame between both O^6medG and apoptosis also did not support the current popular theory which explains O^6medG mediated apoptosis. An alternate hypothesised BER mediated apoptotic pathway was also not supported.

Regulation of the acute apoptotic response or the cell proliferation rate was not achieved by dietary fish oil. However, a high dose fish oil diet did regulate the level of O^6medG in colonic epithelial cells by significantly reducing the total O^6medG DNA damage load. This reduction of O^6medG by a high fish oil diet however, was not translated into a protective effect against the formation of pre-neoplastic lesions. These data suggests that regulation of the acute O^6medG response to a damaging agent does not necessarily imply protection for longer term colorectal oncogenesis. Additional studies exploring both the effect of fish oil on AOM metabolising enzymes and also a longer term cancer study may help to answer some pertinent questions evolving from this thesis.

DECLARATION

I certify that this thesis does not incorporate without acknowledgement any material previously submitted for a degree or 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 other person except where due reference is made in the text.

Laura Sophia Nyskohus

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LIST OF ABBREVIATIONS

8-OH-dG	8 –hydroxy-2-deoxy Guanosine
AA	Arachnidonic Acid
AARGC	Acute Apoptotic Response to Genotoxic Carcinogen
ACF	Aberrant Crypt Foci
AOM	Azoxymethane
APC	Adenomatous Poliposis Coli
BER	Base Excision Repair
BNF	Beta - naphthoflavone
CRC	Colorectal cancer
CSIRO	Commenwealth Scientific Industrial Research Organisation
DAB	3,3` Diaminobenzidine
DHA	Docosahexaenoic acid
DHM	Dimethylhydrazine
ENU	N-ethyl-N-nitrosourea
EPA	Eicosapentaenoic acid
FAP	Familial Adenomatous Polyposis
FIT	Faecal Immunohistochemical Test
FOBT	Faecal Occult Blood Test
FSA	Food Sciences Australia
GC	Gas Chromatography
HNPCC	Hereditary Non-Polyposis Colorectal Cancer
LA	Linoleic Acid
LCFA	Long Chain Fatty Acid
LNA	Linolenic Acid
LOH	Loss of Heterozygosity
MAM	Methylazoxymethanol
MBN	Methylbenzylnitrosamine
MCFA	Medium Chain Fatty Acids
ME	Microencapsulated
MEMO	Microencapsulated menhaden oil
MESO	Microencapsulated sunflower oil
METO	Microencapsulated tuna oil
MO	Menhaden oil
MGMT	O^6-methylguanine-DNA methyltransferase
MMR	Mismatch Repair

MMS	Methyl methane-sulfonate
MNU	N-Nitroso-N-methylurea
MUFA	Monounsaturated fatty acid
N7meG	N7methyldeoxyGuanosine
O^6medG	O^6methyldeoxyGuanosine
PCNA	Proliferating nuclear cell antigen
PICR	Patterson Institute of Cancer Research
PPAR	Peroxisome Proliferators Activated Receptors
PUFA	Polyunsaturated Fatty Acid
ROI	Reactive Oxygen Intermediates
SCFA	Short Chain Fatty Acid
SFA	Saturated fatty acid
SO	Sunflower oil
TO	Tuna oil

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