Short-Chain Fatty Acid Modulation of Apoptosis in Gastric and Colon Cancer Cells

A thesis submitted in fulfilment of the

Doctor of Philosophy

In

The Discipline of Physiology

The University of Adelaide

By

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February 2007

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THESIS ERRATUM

Title: Short-chain fatty acid modulation of apoptosis in gastric and colon cancer cells

In response to the markers of the current thesis:

1. It would be extremely important to extend the findings within this thesis to other cancer cell lines, to animal models of gastric carcinoma, to epithelial cells and to the association between *Helicobacter pylori* infection and gastric cancer. It would also be important to consider the contribution of intestinal flora and their metabolites in the activities of SCFAs, however this was outside of the scope of this project.
2. G6PDH is the rate limiting enzyme of the oxidative pentose pathway and thus its measurement implies the overall activity of this pathway. This is explicitly described in the introduction chapter (Page 3, line 13).
3. This thesis does not have the subheading "Aims", however the specific aims of each chapter are stated in the final paragraph of each chapter introduction.
4. The lack of a consistent time response between 1-^{13}C-D-glucose oxidation and G6PDH activity does not disturb the validation of its measurement and this is discussed in chapter 2 (Page 40, line 15). We propose that both methods of measurement are required to completely appreciate the movement of glucose through the OPP and/or the TCA cycle.
5. The titles above each of the tables describing cell viability within this thesis should state that viability is measured as a percentage of total cell numbers.
6. Line 1 of page 74 should read "G6PDH activity was not altered with any concentration of butyrate".
7. Line 18 of page 134 should read: "1mM butyrate increased the percentage of TA greater than 1mM propionate".
8. Line 19 of page 193 should read: "expression of many genes, including ornithine decarboxylase (differentiation marker)".

ABBREVIATIONS

AIF	Apoptosis inducing factor
ANOVA	Analysis of variance
Apaf-1	Apoptotic protease-activating factor-1
ATCC	American type culture collection
ATP	Adenosine triphosphate
Cdx-2	Caudal related homeobox-2
CO_2	Carbon dioxide
DEM	Diethyl maleate
DHEA	Dehydroepiandrosterone
DMEM	Dulbeccos modified Eagles medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
cDNA	Complimentary DNA
dsDNA	Double-stranded DNA
DOB	Delta over baseline
DTNB	5,5'-dithiobis (2-nitrobenzoic acid)
EA	Early apoptosis
EDTA	Ethylenediaminetetraacetic acid
FACS	Flow assisted cell sorting
FBS	Foetal bovine serum
FDG-PET	2-fluoro-2deoxy-D-glucose-positron emission tomography
FITC	Fluroscein isothiocyanate
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase

G0-G1	Gap phase 0 and gap phase 1
G2-M	Gap phase 2 and mitosis
G6P	Glucose-6-phosphate
G6PDH	Glucose-6-phosphate dehydrogenase
GPX	Glutathione peroxidase
GSH	Glutathione (reduced)
GSSG	Glutathione (oxidised)
GST	Glutathione-S-transferase
Hes-1	Hairy and enhancer of split-1
IGF-I	Insulin-like growth factor I
IRMS	Isotope ratio mass spectrometry
LA	Late apoptosis
M	Molar concentration
mM	Millimolar concentration
μM	Micromolar concentration
M-MLV	Moloney murine leukaemia virus
Msi-1	Musashi-1
$NADP^+$	Nicotinamide adenine dinucleotide phosphate (oxidised)
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced)
NEAA	Non-essential amino acids
NOPP	Non-oxidative pentose pathway
ODC	Ornithine decarboxylase
OPP	Oxidative pentose pathway
$^{\bullet}OH^-$	Hydroxyl radical

$^{\bullet}O_2^-$	Superoxide dismutase
PCR	Polymerase chain reaction
PTS	Phosphatidyl serine
PI	Propidium iodide
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RPMI	Roswell park memorial institute
RTPCR	Reverse transcription polymerase chain reaction
S	Synthesis phase
SCFA	Short-chain fatty acid
SEM	Standard error of the mean
TA	Total apoptosis
TCA	Tricarboxylic acid cycle
TCF	Temperature correction factor
TNF	Tumour necrosis factor

ABSTRACT

Introduction: Gastric and colon cancer are major causes of mortality and morbidity worldwide. Gastric cancer is often detected at an advanced stage and current chemotherapeutics are only modestly effective against this neoplasm. Novel chemotherapeutics, chemopreventive agents and treatment strategies are required to prevent and treat gastric cancer. The ideal method to eliminate cancer cells may be the induction of apoptosis, further preventing cell proliferation and tumour growth. Recently, short-chain fatty acids (SCFAs) butyrate and propionate have been investigated as potential chemotherapeutic agents, particularly in colon cancer. Butyrate is reported to induce apoptosis in colon cancer cells and is demonstrated to modulate intracellular redox state by altering the levels of an antioxidant, glutathione (GSH). GSH availability is controlled by the oxidative pentose pathway (OPP). Very few studies have investigated the effects of butyrate on cell types other than colon cancer cells, and even less is known regarding the effects of propionate. This thesis investigated the potential for SCFAs to induce apoptosis in a gastric cancer cell line, Kato III, compared to the colon cancer cell line, Caco-2. Cell cycle regulation, OPP activity, GSH availability and glucose metabolism were also assessed. **Methods:** Initial studies developed a new technique to measure 1-^{13}C-D-glucose metabolism. Following this, Kato III and Caco-2 colon carcinoma cells were treated with butyrate or propionate (1mM, 5mM or 10mM) or a 5mM combination of both SCFAs. The induction of apoptosis and cell cycle alterations by these SCFAs were assessed using flow cytometry. OPP activity and GSH availability were assessed in both cell lines using colorimetric techniques. Butyrate metabolism was assessed using ^{13}C-butyrate. **Results:** Butyrate and propionate significantly induced apoptosis and G2-M arrest in Kato III and Caco-2 cells, although to a significantly greater extent in the latter cell line. Moreover, butyrate induced apoptosis to a significantly greater extent than propionate, in both cell lines. SCFA treatment led to the

significant up-regulation of OPP activity in both cancer cell lines while GSH availability was significantly reduced. Glucose metabolism was initially increased by all SCFA treatments, however, 72hr butyrate treatment led to its reduction. Importantly, glucose metabolism was measured using a new technique developed within this thesis. The rate of butyrate metabolism was demonstrated to correlate with the sensitivity of each cell line to this SCFA. **Conclusions:** This thesis provides evidence that SCFAs, particularly butyrate, induce apoptosis in gastric and colon cancer cells *in vitro*. The response of cancer cells to SCFAs appears complex, and involves multiple distinct mechanisms and pathways, including p53, Fas, changes to intracellular redox state and glucose metabolism. The capability of butyrate to induce apoptosis also appears to be directly related to the rate of its metabolism. Butyrate has the potential to be utilised as an adjunctive therapy for the treatment of gastric cancer and colon cancer.

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis being made available in the University Library.

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Geoffrey Mark Matthews

February 2007

ACKNOWLEDGEMENTS

I would firstly like to thank my PhD supervisors, Assoc Prof Ross Butler and Assoc Prof Gordon Howarth for their support, guidance and encouragement throughout my PhD. Without their continued positivity and trust, this project would never have run so smoothly. Many thanks also go to Prof Geoff Davidson and Dr David Moore for allowing me to undertake my research in their excellent department.

I would like to thank Esther Staunton and Betty Zacharakis for analysing the many $^{13}CO_2$ samples that just seemed to keep piling up. I need to thank Greg Hodge for his help and expertise in Flow Cytometry and Fiona Campbell for her help with cell culture and its problems. I would like to thank everyone else within the Gastroenterology Department who have had some input into my PhD project and/or social life (Dr Cuong Tran, Mark Geier, Stamatiki Kritas, Su San Lee, Roger Yazbeck, Erin Symonds, Pat Westin). Many thanks also go to my friends and mates, both in Australia and throughout the world, who have helped me through this exciting but challenging period of my life with beverages aplenty.

I would like to thank my parents and brothers for their help and advice throughout my PhD. They have always been there for me, have always been interested in what I'm doing and have always supported me in my choices, for that I am forever thankful. Finally I would like to thank all of the other people that have been part of my life during the undertaking and writing up of my PhD.