GREEN TEA AND ITS CATECHINS MODULATE
CHOLESTEROL METABOLISM IN
CULTURED HUMAN LIVER (HEPG2) CELLS AND
THE HYPERCHEOLESTROLAEMIC RABBIT.

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# TABLE OF CONTENTS

Abstract x

Statement xiv

Acknowledgements xv

List of Figures xvii

List of Tables xxii

List of Abbreviations xxiii

Publications arising from this thesis xxiii

## CHAPTER 1

### INTRODUCTION

1.1 Cholesterol and Heart Disease 1 - 1

1.2 Cholesterol 1 - 2

1.2.1 Cholesterol synthesis 1 - 2

1.2.2 Cholesterol esterification 1 - 5

1.2.3 Cholesterol catabolism – Bile acids synthesis 1 - 5

1.3 Lipids and Lipoproteins 1 - 7

1.4 Lipoprotein Metabolism 1 - 7

1.4.1 Chylomicrons 1 - 7

1.4.2 VLDL 1 - 8

1.4.3 IDL 1 - 9

1.4.4 LDL 1 - 9

1.4.5 HDL 1 - 10
1.5 Atherosclerosis

1.6 Oxidatively Modified LDL
   1.6.1 Oxidation
   1.6.2 LDL oxidation
   1.6.3 In vivo oxidation of LDL and its role in atherosclerosis

1.7 LDL Metabolism
   1.7.1 The LDL receptor pathway
   1.7.2 Regulation of the LDL receptor
   1.7.3 Oxysterols

1.8 The LDL Receptor
   1.8.1 Importance of the LDL receptor
   1.8.2 Structure
   1.8.3 LDL receptor gene and its regulation

1.9 Sterol Regulatory Element Binding Proteins (SREBPs)
   1.9.1 Structure
   1.9.2 SREBP activation
   1.9.3 Independent regulation of SREBP-1 and -2

1.10 Antioxidants

1.11 Green tea and its Antioxidants
   1.11.1 The catechins
   1.11.2 Metabolism of catechins
   1.11.3 Antioxidant properties of the catechins
   1.11.4 Antioxidant action of the catechins
1.12 Green tea, catechins and atherosclerosis

1.12.1 Effects on LDL oxidation

1.12.2 Hypocholesterolaemic action of green tea and catechins

1.12.3 Mechanisms by which green tea and its catechins may lower plasma cholesterol:

- Cholesterol absorption

- Cholesterol synthesis

- LDL receptor

1.12.4 Effects on lesion formation

1.13 Experimental Rationale

1.14 Overall Objectives

1.15 Research Proposal

CHAPTER 2

METHODS

2.1 Cell Culture

2.1.1 Maintenance

2.1.2 Growing of cells for experiments

2.2 Test for normal LDL Receptor function before experimental intervention

2.2.1 Preparation of lipoprotein deficient-fetal calf serum (LPD-FCS)

2.2.2 Incubation with LPD-FCS
2.3 Treatment with Green Tea and EGCG

2.4 Measurement of LDL Receptor Binding Activity in HepG2 cells
   2.4.1 Preparation of colloidal gold-LDL
   2.4.2 LDL Receptor binding activity in HepG2 cells

2.5 Measurement of LDL Receptor Protein
   2.5.1 Solubilisation of cells
   2.5.2 Separation of cellular protein
   2.5.3 Immunoblotting

2.6 Measurement of hepatic LDL Receptor Binding Activity and Protein in Rabbits

2.7 Measurement of SREBP-1c
   2.7.1 Cell Culture
   2.7.2 Preparation of nuclear and membrane fractions
   2.7.3 Immunoblotting

2.8 Total Cholesterol, Unesterified Cholesterol and Cholesterol Synthesis assays
   2.8.1 Preparation of cells
   2.8.2 Preparation of media
   2.8.3 Measurement of total cholesterol, unesterified cholesterol and lathosterol
   2.8.4 Gas chromatograph conditions

2.9 Measurement of Bile Acids
CHAPTER 3

FRESHLY BREWED GREEN TEA MODULATES CHOLESTEROL METABOLISM IN CULTURED HUMAN LIVER (HEPG2) CELLS.

3.1 Introduction 3 - 1

3.2 Materials and Methods 3 - 3

3.2.1 Green tea 3 - 3

3.2.2 HepG2 cell culture 3 - 3

3.2.3 LDL receptor binding activity 3 - 3

3.2.4 SREBP-1c 3 - 3

3.2.5 Cholesterol, lathosterol and chenodeoxycholic acid 3 - 4

3.2.6 Statistical analysis 3 - 4

3.3 Results 3 - 5

3.3.1 Green tea and the LDL receptor 3 - 5

3.3.2 Green tea and cell cholesterol 3 - 5

3.3.3 Green tea and SREBP-1c 3 - 8

3.3.4 Green tea, cholesterol synthesis, media cholesterol and chenodeoxycholic acid 3 - 8

3.4 Discussion 3 - 12
CHAPTER 4

EPIGALLOCATECHIN GALLATE (EGCG) MODULATES CHOLESTEROL METABOLISM IN CULTURED HUMAN LIVER (HEPG2) CELLS.

4.1 Introduction 4 - 1

4.2 Material and Methods 4 - 2
  4.2.1 Materials 4 - 2
  4.2.2 HepG2 cell culture 4 - 2
  4.2.3 LDL receptor binding activity and LDL receptor protein 4 - 3
  4.2.4 SREBP-1c 4 - 3
  4.2.5 Cholesterol, lathosterol and chenodeoxycholic acid 4 - 3
  4.2.6 Statistical analysis 4 - 4

4.3 Results 4 - 4
  4.3.1 EGCG and the LDL receptor 4 - 4
  4.3.2 EGCG and cellular cholesterol 4 - 6
  4.3.3 EGCG and SREBP-1c 4 - 6
  4.3.4 EGCG, cholesterol synthesis, media cholesterol and chenodeoxycholic acid 4 - 9

4.4 Discussion 4 - 11

CHAPTER 5

A GREEN TEA EXTRACT LOWERS PLASMA CHOLESTEROL IN THE HYPERCHOLESTEROLAEMIC RABBIT.

5.1 Introduction 5 - 1
5.2 Materials and Methods

5.2.1 Catechin extract 5 - 3
5.2.2 Animal study 5 - 5
5.2.3 Plasma lipids 5 - 6
5.2.4 Cholesterol synthesis and the intrinsic capacity to absorb cholesterol 5 - 7
5.2.5 Hepatic LDL receptor binding assay 5 - 7
  5.2.5.1 Preparation of soluble rat liver membrane proteins 5 - 7
  5.2.5.2 Determination of LDL receptor binding activity 5 - 8
5.2.6 Quantification of LDL receptor protein 5 - 9
5.2.7 Liver lipid determinations 5 - 9
5.2.8 Artery cholesterol measurements 5 - 10
5.2.9 Statistical analysis 5 - 10

5.3 Results 5 - 11

5.3.1 Daily food consumption 5 - 11
5.3.2 Plasma lipids 5 - 11
5.3.3 Plasma lipoprotein cholesterol 5 - 12
5.3.4 Cholesterol in the arteries 5 - 19
5.3.5 Liver lipids 5 - 19
5.3.6 Cholesterol synthesis and the intrinsic capacity to absorb cholesterol 5 - 22
CHAPTER 6

GENERAL DISCUSSION

6.1 Mechanisms by which Freshly Brewed Green Tea and EGCG Modulated Cholesterol Metabolism in the HepG2 Cells

6.1.1 Lower dose treatments

6.1.2 Higher dose treatments

6.2 Mechanism by which the Crude Catechin Extract Modulated Cholesterol Metabolism in the Rabbits

6.3 Future Studies

BIBLIOGRAPHY
ABSTRACT

Hypercholesterolaemia is one of the main risk factors in the development of heart disease. Green tea and its antioxidant constituents, the catechins, have been found to be hypocholesterolaemic in both epidemiological and animal intervention studies. Previous studies in our laboratory have found that freshly brewed green tea and its most abundant catechin constituent epigallocatechin gallate (EGCG), increased the low-density lipoprotein (LDL) receptor of HepG2 cells. As an increase in the low-density lipoprotein receptor is one mechanism by which plasma cholesterol levels can be lowered, this could explain the hypocholesterolaemic effects that have been found with green tea and its catechins in the epidemiological and animal intervention studies.

The main objectives of the present studies were to investigate the mechanism by which green tea and EGCG increase the LDL receptor in HepG2 cells. The LDL receptor can be regulated through changes in cellular cholesterol content, which modulates the level of the mature active form of sterol regulatory element binding proteins (SREBP), transcription factors for the LDL receptor. These parameters were therefore investigated. Furthermore, we wanted to determine if a crude catechin extract from green tea could lower plasma cholesterol levels in the hypercholesterolaemic rabbit and ascertain if this effect was due to an increase in the LDL receptor.

Green tea and EGCG significantly decreased cellular total cholesterol (~30%) at all treatment concentrations \((p<0.05)\). There are three main mechanisms by which this could occur in liver cells: 1) an increase in the conversion of cholesterol into bile acids 2) an inhibition in cholesterol synthesis or 3) an increase in the efflux of cholesterol from the cells to the media. Chenodeoxycholic acid, the main bile acid produced by HepG2 cells, was extracted from the cell media and measured using gas chromatography (GC). No changes were noted in its production after treatment with green tea or EGCG. The reduction in cellular total cholesterol concentrations was therefore not likely to be due to an increase in the conversion of cholesterol to bile acids.
Incubation with green tea and EGCG produced a bi-phasic "down then up" effect on cholesterol synthesis as measured using the cellular concentration of lathosterol relative to cell protein. The significant decrease (-33%) in cholesterol synthesis in the lowest dose treatment group (50 μM) could explain the decrease in cellular total cholesterol in those cells. In the highest dose treatment group (200 μM) however, there was an increase in cholesterol synthesis (+40%), which did not support the decrease in cellular total cholesterol. Further studies revealed that both green tea and EGCG, in the highest dose treatment group only, increased the concentration of cholesterol in the media (+25%). This suggested that the extra cholesterol produced by the increase in cholesterol synthesis, was not remaining in the cells but was secreted into the media. The decrease in the cell cholesterol by green tea and EGCG therefore appeared to be due to a decrease in cholesterol synthesis at the lowest dose but due to an increase in the secretion of cholesterol from the cells at the highest dose.

The decrease in cellular cholesterol is consistent with the LDL receptor being upregulated via the SREBP transcription system. Measurement of SREBP-1c, using a specific polyclonal antibody and western blotting, revealed that incubation of HepG2 cells with freshly brewed green tea and EGCG increased the mature active form of SREBP-1c by 65% and 56% over control levels respectively. This increase in the mature active form of SREBP-1c is therefore consistent with the increase in the LDL receptor seen with green tea and EGCG.

To determine if the effects of green tea and EGCG on HepG2 cell cholesterol metabolism also occurred in vivo, 24 New Zealand white rabbits were initially made hypercholesterolaemic by feeding them 0.25% (w/w) cholesterol mixed in with their normal rabbit chow for a period of 2 weeks. The rabbits were then randomised into four different treatment groups based on body weight and plasma cholesterol levels. The four treatment groups were then fed the 0.25% cholesterol diet supplemented with 0, 0.5, 1 or 2% (w/w) of a crude catechin extract from green tea. At the end of the treatment period the rabbits were bled via cardiac puncture until euthanasia and their livers and aortas were excised.
The administration of the crude catechin extract (2% w/w) to cholesterol-fed rabbits produced reductions in plasma cholesterol (-57%) and cholesterol in the VLDL + IDL (-80%) and the LDL (-77%) fractions compared to the controls. There was a significant inverse linear trend between plasma, VLDL + IDL and LDL cholesterol and the dose of the crude catechin extract (p<0.05). Reductions in total and unesterified cholesterol for the liver homogenate (25% and 15%) and the liver membrane (22% and 21%) fraction were also found. There were significant inverse linear trends between total and unesterified cholesterol in both liver preparations and the dose of the crude catechin extract (p<0.05).

There also was a significant inverse linear trend (p<0.05) between cholesterol in the thoracic aorta and the dose of the crude catechin extract (-22%). Fatty streak formation was assessed by lipophilic staining using oil red O and quantified by image analysis, but the percentage lipophilic stain in the aortic arches was not different after consumption of the crude catechin extract compared to the control diet.

Cholesterol synthesis, as measured by the plasma ratio of lathosterol to cholesterol, was significantly reduced in the 1% and 2% (w/w) treatment groups (-60%) compared to the control (p<0.05). This reduction in cholesterol synthesis is consistent with the various reductions observed in plasma, aorta and liver cholesterol with the administration of the crude catechin extract. Furthermore, cholesterol synthesis was significantly correlated to plasma, VLDL + IDL, LDL and aortic cholesterol (r= 0.57, 0.56 and 0.50 respectively).

An increase was noted in LDL receptor binding activity (+80%) in the 2% (w/w) treatment group compared to the control, measured by the calcium dependant binding of colloidal gold-LDL to solubilised liver membranes. There was also an increase in the relative amounts of LDL receptor protein (+70%) in the 2% (w/w) treatment group compared to the control, measured using a polyclonal antibody and western blotting. Significant positive linear trends between LDL receptor binding activity and LDL receptor protein and the dose of the crude catechin extract were observed (p<0.05). This increase in the LDL receptor
provides another mechanism to explain the reduction in plasma lipids that occurred with the administration of the crude catechin extract. It appears however that the reduction in cholesterol synthesis may be the main driving mechanism by which the crude catechin extract produces its cholesterol lowering effects as it is more strongly correlated with plasma lipids than the LDL receptor ($r = 0.37$ with total cholesterol).

In summary, the *in vitro* studies suggest that green tea and EGCG increase the LDL receptor by decreasing the cell cholesterol concentration and increasing the mature active form of SREBP-1c. The dietary intervention study revealed that the administration of a crude catechin extract to rabbits lowered plasma and LDL cholesterol. The mechanism by which the green tea extract lowered cholesterol in the rabbit appeared to be by reducing cholesterol synthesis and increasing the LDL receptor. This study provides evidence that green tea and its catechins exhibit hypocholesterolaemic properties and may therefore provide protection against heart disease.