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Thesis title: *In vitro* chemically-induced DNA damage in cancer patients and healthy individuals

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Comet assay, micronucleus assay, sister chromatid exchanges, FISH, food mutagens, nanoparticles

Abstract

In the present study DNA damage was measured in peripheral blood lymphocytes from polyposis coli and colorectal cancer patients, treated with different dietary and environmental compounds and compared with lymphocytes from healthy individuals. In addition, confounding factors such as age, gender, alcohol intake and smoking habits were taken into consideration. The assays used in this study included the Comet assay, the Micronucleus assay, the Micronucleus – FISH assay and the sister chromatid exchange assay.

The food mutagens, PhIP and IQ, as well as titanium dioxide nanoparticles (TiO_2 NPs) induced a dose dependent increase in the DNA damage and chromosomal abnormalities in all tested groups regardless of confounding factors. Prior to experiments physicochemical characterisation of nanoparticles was conducted. In the presence of the flavonoids, quercetin and rutin that were acting in an antioxidant manner, the DNA damage resulting from the highest doses of food mutagens was significantly reduced. Thus, dietary supplementation with flavonoid-rich vegetables and fruits may prove very effective in protection against oxidative stress.

The polyposis coli and colon cancer patients were more susceptible to food mutagens, PhIP and IQ, as well as TiO_2 NPs, and in the majority of cases had a higher level of DNA damage in the Comet assay and higher cytogenetic damage in the Micronucleus assay.

In the final project, twelve frequently encountered (NewGeneris) chemical compounds were evaluated to establish their damaging potential in lymphocytes and spermatozoa from healthy donors. The highest damage was produced by DNA reactive aldehydes, food mutagens and benzo[*a*]pyrene when assessed with the neutral and alkaline Comet assay with and without metabolic activation.

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LIST OF ABBREVIATIONS

8-MeIQx	2-amino-3,8-dimethylimidazo[4,5 –*f*]quinoxaline
8-OHdG	8-hydroxydeoxyguanosine
AA	acrylamide
AJCC	American Joint Committee on Cancer
ALS	alkali labile sites
APC	adenomatous polyposis coli gene
ATB1	aflatoxin B1
Au	gold
BaP	benzo[*a*]pyrene
BiBuds	nuclear buds in binucleated cells
BiMN	micronuclei in binucleated cells
BiNC	binucleated cells
BiNPBs	nucleoplasmic bridges in binucleated cells
Bmpr1	Bone morphogenetic protein receptor
BN	binucleated
BPDE	benzo[*a*]pyrene diol epoxide
BRAF	v-Raf murine sarcoma viral oncogene homolog B1
BrdU	bromodeoxyuridine
BRI	Bradford Royal Infirmary
C+MN	centromere positive micronucleus
CA	chromosomal aberrations
Caco-2	human epithelial colorectal adenocarcinoma cell line
CBMN	the Cytochalasin B/ Cytokinesis Block Micronucleus assay
CHO	Chinese hamster ovary cell line
C-MN	centromere negative micronucleus
CRC	colorectal cancer
CTC	CT colonography
CYP1A1/2	cytochrome P450 enzymes
CYP1B1	

Cyt-B	cytochalasin B
Cytb5Rd	cytochrome b5 reductase
DABCO	1,4-Diazabicyclo[2,2,2]octane
DAPI	4',6-diamidino-2-phenylindole (binds to DNA)
dATP	2'-deoxyadenosine 5'-triphosphate
DBPs	disinfection by-products
DCBE	double contrast barium enema
dCTP	2'-deoxycytidine 5'-triphosphate
ddH_2O	double distilled water
dGTP	2'-deoxyguanosine 5'-triphosphate
DLS	dynamic light-scattering
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DON	deoxynivalenol
DSB	double strand breaks
DTT	dithiothreitol
dTTP	2'-deoxythymidine 5'-triphosphate
EDTA	ethylenediaminetetraacetic acid
ER	estrogen receptor
EtBr	ethidium bromide
EtOH	ethanol
FAP	Familial adenomatous polyposis
FBS	foetal bovine serum
FCS	foetal calf serum
FISH	fluorescence in-situ hybridisation assay
FIT	faecal immunochemical test
FITC	Fluorescein isothiocyanate
FKHRL1	forkhead transcription factor
FPG	flurescence plus Giemsa
FSIG	flexible sigmoidoscopy
G-6-P DH	glucose-6-phosphate dehydrogenase
G-6-P	glucose-6-phosphate
GA	glycidamide
gFOBT	guaiac faecal occult blood test

Hb	haemoglobin
HCA	heterocyclic amines
Hep3B	human hepatoma cell line
HepG2	human derived hepatoma cell line
HNE	4-hydroxynonenal
HNPCC	hereditary non-polyposis colorectal cancer
HSP	heat shock protein
IARC	the International Agency for Research on Cancer
IL6	Interleukin 6
IQ	2-amino-3-methylimidazo-[4,5-*f*]quinoline
IWGTP	the International Workshop on Genotoxicity Test Procedures
KCl	potassium chloride
KRAS	V-Ki-ras2 Kirsten rat sarcoma viral oncogene homologue
L5178Y	mouse lymphoma cell line
LMPA	low melting point agarose
MAP	MUTYH attenuated FAP
MCF10A	mammary epithelial cells
MCF-7	breast cancer cell line
MDA	malondialdehyde
MLH1	MutL homolog 1, colon cancer, nonpolyposis type 2 (E. coli)
MMC	Mitomycin C
MN	micronucleus
MonoMN	micronuclei in mononucleated cells
MSH2/6	MutS homolog 2/6, colon cancer, nonpolyposis type 1 (E. coli)
MUTYH	Mut Y homolog gene (MYH)
NADP	nicotynamide adenine dinucleotide phosphate
NaOH	sodium hydroxide
NDMA	N-nitrosodimethylamine
NIH	National Institute of Health
NMPA	normal melting point agarose
NO_2^-	nitrites
NO_3^-	nitrates
NOC	N-nitroso compounds
NPBs	nucleoplasmic bridges

NSAIDs	nonsteroidal anti-inflammatory drugs
OTM	Olive tail moment
p53	tumour protein 53
p63	tumour protein 63
PCB77	3,3,4,4-tetrachlorobiphenyl
PCBs	polychlorinated biphenyls
PCR	polymerase chain reaction
PHA-M	phytohaemaglutinin M
PhIP	2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine
PIK3CA	phosphoinositide-3-kinase
PK	proteinase K
PLP	polyposis coli
PMS2	Mismatch repair endonuclease
PTEN	phosphatase and tensin homolog
Q	quercetin
R	rutin
RNA	ribonucleic acid
ROS	reactive oxygen species
SCEs	sister chromatid exchanges assay
SCGE	Single Cell Gel Electrophoresis
sDNA	stool DNA test
SEM	Scanning Electron Microscopy
SMAD2/4	Mothers against decapentaplegic homolog 2/4
SS	salmon sperm
SSB	single strand breaks
TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
TiO_2 NPs	titanium dioxide nanoparticles
TNM	tumour-node-metastasis classification
TP53	gene encoding p53
V79	Chinese hamster lung fibroblast cell line

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