Flavonoids inhibit the genotoxicity of hydrogen peroxide (H$_2$O$_2$) and of the food mutagen 2-amino-3-methylimidazo[4,5-\textit{f}]-quinoline (IQ) in lymphocytes from patients with inflammatory bowel disease (IBD)

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Inflammatory bowel disease (IBD) including Crohn’s disease (CD) and ulcerative colitis (UC) is a chronic inflammatory gastrointestinal autoimmune condition with an inappropriate immune response. We investigated DNA damage induced in vitro in lymphocytes from IBD patients caused by oxidative stress through H$_2$O$_2$ and 2-amino-3-methylimidazo[4,5-\textit{f}]quinoline (IQ) and whether the plant flavonoids, quercetin and epicatechin, found in fruits, tea and soybeans could effectively reduce such stress. Lymphocytes from IBD patients and healthy volunteers were treated with 50 &mu;g/ml H$_2$O$_2$ or IQ in the presence of quercetin (0–250 &mu;g/ml) or epicatechin (0–100 &mu;g/ml). Flavonoid supplementation (250 &mu;M quercetin or 100 &mu;M epicatechin) caused an overall significant decrease of induced DNA damage resulting in a 48.6% ($P < 0.001$) reduction of H$_2$O$_2$-induced and a 43% ($P < 0.001$) reduction of IQ-induced DNA damage within the patient groups; for the control groups, reductions in DNA damage were 35.2 and 57.1%, respectively (both, $P < 0.001$). There was less induced DNA damage within lymphocytes from UC patients compared to CD patients for both series of experiments (H$_2$O$_2$ and quercetin, IQ and epicatechin). In conclusion, flavonoids dramatically reduced oxidative stress in vitro in lymphocytes from IBD patients and healthy individuals. Thus, flavonoids could be very effective in the treatment of oxidative stress and encouraged in the diet of IBD patients.

Introduction

The generation of DNA damage by environmental, medical or lifestyle factors is considered to be an important initial event in carcinogenesis. Despite various cellular mechanisms to counteract these detrimental events, the sheer number of potentially carcinogenic compounds leading to oxidative stress can negatively affect the DNA integrity of cells.

A very important group of promutagenic/carcinogenic chemicals, the heterocyclic amines (HCA), is widely produced when cooking food, especially during the pyrolysis of creatinine, amino acids and proteins (1). Major subclasses of HCA found in the human diet comprise of aminoimidazoazaarenes (AIA), 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), 2-amino-3,4-dimethylimidazo[4,5-f]quinoline, 2-amino-3, 8-dimethylimidazo[4,5-f]quinoxaline, 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (1). There is increasing evidence for the involvement of HCA as pyrolysis products of high-protein diets in the aetiology of human cancer (2,3), which has stimulated strong efforts to identify exogenous and endogenous factors that modify health risks caused by HCA. More than 600 individual compounds and complex dietary mixtures have been studied for protective effects towards HCA (4) and numerous articles have been published regarding mammalian enzymes involved in the bioactivation and detoxification of these compounds (5).

In addition to the formation of DNA adducts as a major causal factor in the carcinogenesis of HCA, oxidative stress plays a crucial role in further damaging the DNA (6). IQ also induces unscheduled DNA synthesis in liver cells and shows strong mutagenic properties in the Salmonella typhimurium test system, which classifies it as a potent carcinogen (7). HCA like IQ are able to generate free radicals in the presence of reduced nicotinamide adenine dinucleotide phosphate and cytochrome b5 reductase (8). Also in addition to the formation of DNA adducts, oxidative damage to the DNA itself plays a crucial role in the carcinogenic process of food mutagens (6).

Dietary flavonoids acting as antioxidants (9) have been identified to be capable of counteracting these adverse oxidative effects. Flavonoids are classified as polyphenolic compounds that are ubiquitous in nature and categorized according to their chemical structure into flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones. So far, thousands of flavonoids have been found and identified in plants showing antioxidative qualities (9). The antioxidative potency of several widespread dietary flavonoids showed a dose-dependent reduction of induced oxidative DNA damage in vitro, highlighting an even higher protective effect than vitamin C (10). It has also been shown that flavonoid intake can lower the mortality rate caused by coronary heart disease (11).

Ulcerative colitis (UC) and Crohn’s disease (CD) are inflammatory disorders of the gastrointestinal tract, which are unevenly distributed within the populations throughout the world. Although the exact cause of inflammatory bowel disease (IBD) remains unknown, the epidemiology of IBD has provided an insight into the pathogenesis of the disease by examining geographic, ethnic and other IBD risk factors (genetic, environmental, etc.) as well as their natural history (12). Interestingly, reactive oxygen species (ROS) (13) are produced in abnormally high levels in cells from IBD patients (14) leading to oxidative stress and thus to DNA damage due to an imbalance between innate and exogenous antioxidants and ROS (15,16). Oxidative stress has been linked to cancer, ageing, atherosclerosis, ischaemic injury, inflammation and neurodegenerative diseases (17). Medically, IBD is characterized by the infiltration of CD4+ T-lymphocytes and other mononuclear cells into inflamed mucosal regions (13). During this process, interleukin (IL)-16 exerts a strong chemoattractant

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activity towards CD4+ cells. Moreover, IL-16 activates the expression and production of pro-inflammatory cytokines such as IL-1 beta, IL-6, IL-15 and tumour necrosis factor alpha (TNF-alpha) in human monocytes (13). The cytokine TNF-alpha plays a crucial role in the pathogenesis of IBD (18). To inhibit TNF-alpha activity, biotechnologically manufactured agents such as the chimeric monoclonal anti-TNF antibody (infliximab), a human monoclonal anti-TNF antibody (CDP571) and a recombinant TNF receptor fusion protein (etanercept) have been used (19). Important insights have also been gained into the function of 'caspase-activating and recruitment domain-15' (CARD15/NOD2, the first cloned susceptibility gene for CD (20,21). Patients carrying one of the NOD2 mutations have a 2- to 4-fold increased risk, while those carrying two mutations have a 20- to 40-fold increased risk of developing CD (22). While a basic inflammatory reaction of the intestinal mucosa as a response of the innate immune system may be essential for the maintenance of gut homeostasis (20,23,24), CD shows hyperresponsiveness of the mucosal immune system. Nevertheless, additional ‘innate’ pathways by which commensal and pathogenic bacteria are able to directly interact with cells of the intestinal mucosa exist (e.g. Toll-like receptors) more and more converging microbial and genetic influences within IBD pathophysiology (20,25,26).

In this present study, we used the comet assay, as a fast and reliable method that is able to detect genotoxicity in virtually any mammalian cell type without the requirement for cell culture (27), on human lymphocytes from IBD patients and healthy individuals. The antioxidative effects of the flavonoids, quercetin and epicatechin (28), were tested in the presence of an exogenous oxidative insult (H2O2 co-treated with quercetin and IQ with epicatechin) to show that these two flavonoids are able to reliably protect cells against the damaging effects of ROS, even in the context of a disease like IBD where levels of ROS are already highly increased.

Materials and methods

Lymphocyte isolation from peripheral blood

Before each experiment, whole blood was collected by venipuncture from 40 individuals (two groups of 10 patients and 10 controls, each). The IBD patients have been treated at the Gastroenterology Department of St Luke’s Hospital and Bradford Royal Infirmary (BRI). Twenty healthy volunteers were recruited within the Division of Biomedical Sciences at the University of Bradford (West Yorkshire, UK). Ethical permission was obtained from both the BRI Local Ethics Committee and the University of Bradford’s subcommittee of Research Ethics Involving Human subjects. The heparinized blood was diluted with 0.9% ethylenediaminetetraacetic acid (EDTA), 10 mM Tris, 10% DMSO, 1% Triton X-100, pH 10 and incubated at 4°C overnight. Then, the slides were placed on the tray of an electrophoresis tank, filled with cold alkaline electrophoresis buffer (300 mM NaOH, 1 mM EDTA, pH < 13) and incubated for 30 min at 4°C in the dark to allow the unwinding of DNA and expression of alkali-labile sites. Electrophoresis was conducted at the same temperature for 30 min using 25 V. The current was adjusted to 300 mA by raising or lowering the buffer level. After electrophoresis, the slides were removed from the tank and soaked three times for 5 min each with neutralizing buffer. Cellular DNA was stained with 20 µg/ml ethidium bromide and coverslips applied. Slides were examined using a fluorescence microscope equipped with a charge-couple device monochrome camera and a computerized image analysis system, Komet 4.0 (Kinetic Imaging, Liverpool, UK) to measure the comet parameters. The median Olive tail moment (OTM) as a qualitative measure of the fraction of DNA in the comet tail and its length as well as the % tail DNA as a quantitative measure were used as arbitrary units of assessment (30–32) for statistical analysis. All slides were coded by an independent person ensuring that scoring took place completely randomized and in a ‘blind’ manner (33). For each replicate slide, 25 cells were scored (50 cells in total) for each of 10 individuals in each group making 500 observations per experimental point, allowing a more than adequate statistical power to detect effects (31).

Statistical analysis

Gaussian normality was violated for many of the scale variables as endorsed by the Kolmogorov–Smirnov test. Therefore, non-parametric test procedures were adopted wherever necessary, such as the Kruskal–Wallis and the Mann–Whitney (M–W) tests for independent samples. When testing intra-subject differences in DNA damage, the Wilcoxon signed-rank test was applied. For the binary response variables, Fisher’s exact test was applied. Throughout the analyses, a significance level of 5% was used and unilateral alternative hypotheses preferred to bidirectional tests (wherever appropriate). Graphical evidence in support of findings was described in terms of medians and quartiles and illustrated using box plots. The SPSS package version 16 was used to compare patient and control groups at different doses of H2O2/quercetin and IQ/epicatechin.

Results

Patient versus control groups

As shown in Figures 1 and 2, there was a significant difference in basic DNA damage within lymphocytes of IBD patients when comparing them with healthy individual controls (2.5-fold in the groups treated with H2O2 and quercetin and 5.2-fold in the groups treated with IQ and epicatechin) (M-W, P < 0.001) before in vitro treatment.

The study groups as well as the control groups showed significant increases in DNA damage induced by H2O2 (for the study group 2.1-fold and for the control group 3.2-fold) (P < 0.001) and IQ (for the study group 2.0-fold and for the control group 3.6-fold) (P < 0.001). The induced damage caused by the in vitro treatment with H2O2 or IQ decreased significantly...
Flavonoids, food mutagen, H₂O₂, IQ, lymphocytes, IBD

The induced DNA damage (M-W, \( P = 0.174 \); \( t \)-test, \( P = 0.134 \)). Percentage tail DNA and OTM data were evaluated. There was a significant increase within the control and patient groups after inducing DNA damage with H₂O₂. For the patient/study group treated with H₂O₂ and quercetin, a 1.5-fold decrease in damage was seen; when treated with IQ and epicatechin, it was 1.4-fold. The patterns for % tail DNA and for OTM were the same. The responses for OTM and % tail DNA are shown in Table I.

**Differences in IBD subgroups**

As shown in Figures 3 and 4, there was less baseline damage in the UC patient group (\( n = 4 \)) than in the CD group (\( n = 4 \)) each being significantly different (\( P < 0.001 \)) (2.0-fold in the study group which was treated with H₂O₂ and quercetin and 3.8-fold in study group treated with IQ and epicatechin) when compared with the combined patient groups, which also included the indeterminate group where it was difficult to differentiate into UC or CD (\( n = 2 \)). Also there was less induced DNA damage in the study group treated with H₂O₂ and quercetin compared with the study group treated with IQ and epicatechin, although the patients were selected randomly (Figures 3 and 4). There was less induced damage of DNA in the lymphocytes from UC patients compared to CD patients in both series of experiments (H₂O₂ with quercetin and IQ with epicatechin; \( P < 0.001 \)) (see Table II and Figures 3 and 4).

**Confounding factors**

Ethnicity, age, gender, smoking and drinking habits. There were small differences of median levels of DNA damage in Caucasians (\( n = 13 \)) and Asians (\( n = 7 \)) as well as in males and females. However, these differences were not found to be statistically significant (M-W, \( P = 0.170 \)). There were also no statistically significant differences in the age distributions between patients as well as between control individuals (H₂O₂ with quercetin experiment: patients’ mean age = 42.4 years ± SD 11.6 and control individuals’ mean age = 28.9 years ± SD 9.0; IQ with epicatechin experiment: patients’ mean age = 39.2 years ± SD 10.3 and control individuals’ mean age = 22.6 years ± SD 9.2). No major differences were seen due to smoking and/or drinking habits (Table II).

**Previous medication in the IBD group as a confounding factor.** Patients had been treated with a range of drugs for IBD, namely, azathioprine, mesalazine and pentasa, asacol, prednisolone, mercaptopurine alone or in combination prior to taking part in the study. Azathioprine and pentasa, azathioprine and mesalazine, mercaptopurine and balsalazide (\( n = 6 \)); asacol (\( n = 1 \)); pentasa and prednisolone, prednisolone and mesalazine (\( n = 3 \)). Within the treatment groups, there appeared to be differences but they were not significant.

**Discussion**

CD and UC, known as IBD, are fairly common chronic inflammatory conditions of the gastrointestinal tract. Although the exact aetiology of IBD remains uncertain, dysfunctional immunoregulation of the gut is believed to be the main cause. Among the immunoregulatory factors, ROS (13) are produced in abnormally high levels in IBD (14). An imbalance between antioxidants and ROS results in oxidative stress, leading to cellular damage (14).
Food-derived HCA like IQ have been shown to be mutagenic in the Ames test inducing gene mutations and tumours in vivo (34,35). Food mutagens may cause different types of DNA damage from chromosomal aberrations to subtle nucleotide alterations. Most food mutagens like HCA are able to form reactive DNA adducts by covalently binding to nucleotides. However, the effect of food mutagens in carcinogenesis can be modified by heritable traits, namely, low-penetrant genes that affect exposure of the mutagen to DNA through metabolic activation and detoxification or other cellular responses to DNA damage. Also DNA damage seems to be indirectly triggered by oxidative stress. When considering the human diet, it should be recognized that food contains both mutagens and components that decrease cancer risk such as antioxidants (8,36).

The present study demonstrates that H$_2$O$_2$ and IQ are capable of inducing significant DNA damage as a result of oxidative stress (Figures 1 and 2). There was a significant increase of DNA damage after treating lymphocytes from healthy and IBD patients with H$_2$O$_2$ and IQ, while a significant protective effect was found in the presence of the flavonoids, quercetin and epicatechin (Figures 1 and 2). There was a significant increase in % tail DNA after treating with H$_2$O$_2$ and IQ as is shown in Table I. Flavonoids are known to have antioxidative properties in vivo (9) and modulate effects of food mutagens in vitro in human lymphocytes and sperm (37).

Cooking fish and beef inevitably generate HCA especially at high temperatures (1), which are carcinogenic in mice, rats and monkeys producing hepatic, intestinal and mammary tumours (38). For instance, AIA, categorized as a subclass of HCA, can be found in the human diet (1) and is only genotoxic after being activated to electrophilic derivatives that form DNA adducts (39). A variety of host drug-metabolizing enzymes are able to activate and detoxify HCA including enzymes like CYP1A2, Table I.

<table>
<thead>
<tr>
<th>Different concentrations</th>
<th>H$_2$O$_2$ + quercetin (group one)</th>
<th>IQ + epicatechin (group two)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OTM % tail DNA</td>
<td>OTM % tail DNA</td>
</tr>
<tr>
<td>Study group Control group</td>
<td>Study group Control group</td>
<td>Study group Control group</td>
</tr>
<tr>
<td>A1</td>
<td>Study group Control group</td>
<td>Study group Control group</td>
</tr>
<tr>
<td>B1</td>
<td>Study group Control group</td>
<td>Study group Control group</td>
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<tr>
<td>C1</td>
<td>Study group Control group</td>
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</tr>
<tr>
<td>D1</td>
<td>Study group Control group</td>
<td>Study group Control group</td>
</tr>
<tr>
<td>E1</td>
<td>Study group Control group</td>
<td>Study group Control group</td>
</tr>
</tbody>
</table>

A1 and A2, no treatment; B1, H$_2$O$_2$, 50 µg/ml + quercetin 0 µg/ml; B2: IQ 50 µg/ml + epicatechin 0 µg/ml; C1, H$_2$O$_2$, 50 µg/ml + quercetin 100 µg/ml; C2, IQ 50 µg/ml + epicatechin 25 µg/ml; D1, H$_2$O$_2$, 50 µg/ml + quercetin 100 µg/ml; D2, IQ 50 µg/ml + epicatechin 50 µg/ml; E1, H$_2$O$_2$, 50 µg/ml + quercetin 250 µg/ml; E2, IQ 50 µg/ml + epicatechin 100 µg/ml.
Flavonoids, food mutagen, H$_2$O$_2$, IQ, lymphocytes, IBD

Table II. Details of patient and control groups relating to confounding factors and their significant differences

<table>
<thead>
<tr>
<th>Confounding factors</th>
<th>Type of IBD</th>
<th>Defining factors</th>
<th>n</th>
<th>OTM</th>
<th>P value</th>
<th>IQ (0 μM) + quercetin (0 μM)</th>
<th>Defining factors</th>
<th>n</th>
<th>OTM</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Female 10</td>
<td>3.6 ± 0.1</td>
<td>&lt;0.001</td>
<td>UC 4</td>
<td>3.5 ± 0.1</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Male 10</td>
<td>3.6 ± 0.1</td>
<td>&lt;0.001</td>
<td>CD 4</td>
<td>7.3 ± 0.7</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0–25 Female 7</td>
<td>3.6 ± 0.1</td>
<td>0.298</td>
<td>Indeterminate 2</td>
<td>7.0 ± 0.2</td>
<td>0.395</td>
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<tr>
<td></td>
<td>26–45 Female 8</td>
<td>3.6 ± 0.1</td>
<td>0.298</td>
<td>Indeterminate 2</td>
<td>7.0 ± 0.2</td>
<td>0.395</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethnic origin</td>
<td>Caucasian 10</td>
<td>3.6 ± 0.1</td>
<td>&lt;0.001</td>
<td>CD 4</td>
<td>7.3 ± 0.7</td>
<td>&lt;0.001</td>
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<tr>
<td></td>
<td>Asian 10</td>
<td>3.6 ± 0.1</td>
<td>&lt;0.001</td>
<td>Indeterminate 2</td>
<td>7.0 ± 0.2</td>
<td>0.395</td>
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<tr>
<td>Smoking</td>
<td>Active smokers 9</td>
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<td>0.357</td>
<td>Active smokers 9</td>
<td>7.4 ± 3.2</td>
<td>0.090</td>
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<tr>
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<td>Ex-smokers 3</td>
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<td>&lt;0.001</td>
<td>Ex-smokers 3</td>
<td>1.55 ± 0.3</td>
<td>&lt;0.001</td>
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<tr>
<td>Drinking habit</td>
<td>Non-smokers 8</td>
<td>3.6 ± 0.1</td>
<td>&lt;0.001</td>
<td>Non-smokers 8</td>
<td>1.24 ± 1.1</td>
<td>&lt;0.001</td>
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<tr>
<td></td>
<td>Non-alcoholic 10</td>
<td>3.6 ± 0.1</td>
<td>0.704</td>
<td>Non-alcoholic 10</td>
<td>1.27 ± 1.0</td>
<td>0.123</td>
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</tr>
<tr>
<td></td>
<td>Moderate 8</td>
<td>3.6 ± 0.1</td>
<td>&lt;0.001</td>
<td>Moderate 8</td>
<td>7.6 ± 4.2</td>
<td>&lt;0.001</td>
<td></td>
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<tr>
<td></td>
<td>Severe 5</td>
<td>3.6 ± 0.1</td>
<td>&lt;0.001</td>
<td>Severe 5</td>
<td>15.0 ± 0.7</td>
<td>0.487</td>
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<tr>
<td>Ethnic origin</td>
<td>Caucasian 10</td>
<td>3.6 ± 0.1</td>
<td>&lt;0.001</td>
<td>Caucasian 10</td>
<td>9.8 ± 0.9</td>
<td>0.487</td>
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<tr>
<td></td>
<td>Asian 8</td>
<td>3.6 ± 0.1</td>
<td>&lt;0.001</td>
<td>Asian 8</td>
<td>11.6 ± 2.7</td>
<td>0.487</td>
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<tr>
<td>Gender</td>
<td>Female 10</td>
<td>3.6 ± 0.1</td>
<td>&lt;0.001</td>
<td>Female 10</td>
<td>10.3 ± 3.0</td>
<td>0.838</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Male 10</td>
<td>3.6 ± 0.1</td>
<td>&lt;0.001</td>
<td>Male 10</td>
<td>9.7 ± 2.3</td>
<td>0.838</td>
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N-acetyltransferase, sulphotransferase, prolyl tRNA synthetase, phosphorylase and COX isomers (40).

In a recent case–control study, no associations were found between colorectal cancer (CRC) risk and polymorphisms within the genes of these enzymes (28). This comprehensive analysis, however, failed to consider commensal bacteria and their potential impact on HCA activation, an effect independent of the host genotype. The pro-carcinogenic IQ is predominantly produced through the pyrolysis of creatinine with sugars and becomes significantly mutagenic in the presence of hepatic microsomes (41). Anaerobic colonic bacteria can convert IQ to 2-amino-3-methyl-3H-imidazo[4,5-f]quinoline-7-one (HOIQ), a direct-acting mutagen (42). Intestinal anaerobic bacteria like *Eubacterium* spp. specifically metabolize IQ to HOIQ along with yet undefined commensal bacteria in mice, rats and humans (43–45). These commensal bacteria can strongly influence IQ-induced DNA damage in colonic cells and also in hepatocytes as measured by the alkaline comet assay (46). DNA from axenic rats exhibited significantly fewer alkaline-labile breaks than rats colonized with conventional murine or human commensal bacteria. In contrast, other intestinal commensal bacteria including *Bifidobacterium longum* and lactobacilli appear to be antagonistic to the mutagenic effects of IQ (46,47). Mechanisms underlying these observations are unclear but may involve inactivation of IQ or direct binding of IQ to bacteria (45). Judgement on the significance of IQ or HOIQ in promoting CRC, however, still awaits appropriately designed clinical studies (45).

Modulating effects of two flavonoids, quercetin and rutin, on the mutagenic anticancer drug mitomycin C have been found using the comet assay with human lymphocytes (48). Quercetin significantly reduced DNA strand breakage induced by mitomycin C displaying protective effects supporting other findings where flavonoids modulated effects of food mutagens (37). Flavonoids have not only a long history of use in preventing capillary fragility and bruising, but also some of the research has focused on their anticancer benefits (49,50). Green tea and, to a lesser extent, black tea are a rich source of still another group of flavonoids called catechins. These catechins—including the closely related epigallocatechin-3 gallate (EGCG), epigallocatechin and epicatechin-3 gallate—form ~30% of the dry weight of tea leaves (49). It was found that drinking green tea could prevent cancer: EGCG in green tea has been identified as a potent inhibitor of urokinase, an enzyme used by cancer cells to invade and metastasize. A single cup of green tea contains enough EGCG to temporarily inhibit urokinase activity—and is safer than synthetic drugs that block urokinase activity (51). To evaluate the *in vivo* antioxidative activity after intake, it is essential to know the bioavailability of flavonoids involving intestinal absorption, metabolic conversion and urinary excretion (52). Oxidative stress leads to a variety of pathophysiological events; therefore, this antioxidative activity accounts, at least partly, for their potential health effects. For epicatechin and quercetin, two typical flavanol- and flavonol-flavonoids present in vegetables, fruits and tea, it is known that their metabolic conversion begins in the intestinal mucosa where the activity of uridine-5'-diphosphoglucuronosyltransferase is highest. Both flavonoids accumulated mostly as glucuronide and sulphate conjugates in the blood plasma of rats after oral administration. No intact quercetin was found in the circulation. However, upon oral administration, the antioxidative ability of these flavonoids within the blood plasma was enhanced indicating that conjugated metabolites participate in the antioxidative defence (52).

Genotoxic properties of dihydroquercetin were previously studied *in vivo* by counting chromosome aberrations and employing the comet assay for DNA damage. Dihydroquercetin administered repeatedly (five times, up to 1.5 mg/kg) or once up to 2000 mg/kg induced no DNA damage in mouse bone marrow, blood, liver and rectal cells. Also, no chromosome aberrations in mouse bone marrow cells were seen (53). Thus, no pro-oxidant activity, even when given high doses, was observed for this flavonoid. The activity of quercetin is believed to be due to its antioxidative properties; however, it has been suggested that quercetin may also have pro-oxidative activities, which might then directly affect genotoxicity (54). Consequently, quercetin acts as a strong antioxidant and scavenger of free radicals while it might simultaneously undergo an oxidation process giving rise to the formation of the semiquinone radical (55).

In the present study, we report for the first time the protective *in vitro* effect of quercetin and epicatechin against oxidative stress in lymphocytes from IBD patients and healthy.
individuals (Figures 1 and 2). We were able to show that untreated lymphocytes from IBD patients had significantly increased DNA damage when compared to healthy individuals (56). Flavonoids dramatically reduced the basic DNA damage in lymphocytes from IBD patients treated with H2O2 and IQ. In vitro treatment with H2O2 and IQ significantly induced DNA damage by oxidative stress in both groups. When co-treated with flavonoids, a significant protective effect was shown against free radical damage to the DNA generated by H2O2 or IQ (Figures 1 and 2). There was a very high level of damage in the patient group without any treatment because of their background inflammation and the IBD therapeutic drugs which they had taken, but both patients and controls showed a parallel and gradual reduction in DNA damage after treating with flavonoids (Figures 1 and 2).

Also in the present study, lymphocytes from CD patients in two series of study groups appeared to have a greater level of baseline DNA damage than those from UC patients when compared to the whole patient group \((P < 0.001)\), suggesting that lymphocytes from CD patients are more exposed to oxidative stress than the other IBD subgroup (Figures 3 and 4). It becomes obvious that an excessive production of ROS and radical nitrogen metabolites occurs during the inflammation of the intestine from IBD patients (57). It seems that a misbalanced production of pro-inflammatory and anti-inflammatory cytokines is characteristic of IBD and severely affects the immune homeostasis in peripheral blood cells, even more in CD than in UC patients (58). However, all subgroups react in the same way towards exogenous oxidative stressors as well as towards the inhibition of oxidative stress by flavonoids.

In conclusion, flavonoids significantly reduce oxidative stress \textit{in vitro} in lymphocytes of IBD patients as well as healthy individuals. Thus, a diet containing flavonoids could be very effective in reducing baseline and exogenously induced oxidative DNA damage of IBD patients.

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**References**


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