

THE EFFECT OF THE FLAVONOID QUERCETIN ON PHASE 1 AND PHASE 2 ENZYME
ACTIVITIES IN THE SW-480 HUMAN COLON CARCINOMA CELL LINE

by

ELIZABETH ANN BUCK

(Under the Direction of JAMES L. HARGROVE)

ABSTRACT

Many cases of colon cancer, the third leading site of cancer in the United States, are diet-related. Fruit and vegetable intake is inversely associated with colon cancer risk, and the flavonoids may have an important role. One mechanism by which flavonoids, including quercetin, may prevent colon cancer is the regulation of Phase 1 and Phase 2 enzymes, which protect the body from foreign substances. Quercetin, the most abundant flavonoid, has been described as a monofunctional enzyme inducer, which is optimal for cancer prevention. This study tested the effect of quercetin on Cytochrome P450 1A1, Glutathione S-transferase, and Quinone reductase activity, and cell viability and proliferation in the SW-480 cell line. Quinone reductase activity decreased significantly with the highest concentration of quercetin. There was no significant effect on the other enzyme activities, cell viability or proliferation. The results of this study cannot confirm that quercetin acts as a monofunctional enzyme inducer.

INDEX WORDS: Flavonoids, Quercetin, SW-480, Glutathione-S-Transferase,
Quinone Reductase, Cytochrome P450 1A1

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DEDICATION

I would like to dedicate my thesis to my family – my parents, Geoff and Denise, and my sisters, Carolyn, Lauren, and Meredith, as well as my extended family. Their loving support, prayers, and encouragement throughout my life and educational career have enabled me to succeed to this point. They have made me the person that I am, and I hope they are as proud of me as I am of them! I love you!

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CHAPTER 1

INTRODUCTION

Although less publicized than other types of cancer, such as breast and lung cancers, colon cancer remains a prevalent disease in our country. According to the American Cancer Society, colon cancer is the third most common cancer in both men and women (ACS, 2002b). In 2002, there were an estimated 148,300 new cases and 56,600 deaths due to colorectal cancer (ACS, 2002a), and experts predict 147,500 new cases of colorectal cancer and 57,100 deaths in 2003 (ACS, 2003). Genetic factors, such as a family or personal history of colorectal cancer, polyps, or inflammatory bowel disease, cause just 10% of colorectal cancers (Armstrong and Mathers, 2000; Go et al., 2001), so environmental factors are involved in the disease process development as well. Physical inactivity, obesity, red meat intake, smoking, alcohol intake, and low fruit and vegetable consumption are all lifestyle-related risk factors for colorectal cancer. Up to 80% of all colorectal cancer cases and deaths are attributable to diet (Bingham, 2000), and many cases of colorectal cancer and related deaths may be preventable by dietary modifications (Giovannucci and Willett, 1994).

The majority of studies in this area suggest that an inverse relationship exists between fruit and vegetable intake and the risk of colorectal cancer (Fund, 1997; Steinmetz and Potter, 1996). Individual components of plant foods are being investigated, and a new area of nutrition research involves studying the effects of phytochemicals on colon cancer risk. Phytochemicals are non-nutritive compounds produced by plants that may contribute to the health benefits of fruits and vegetables, beyond their vitamin and mineral content (Bloch, 1995).

Plants produce phytochemicals mainly for protection from herbivores, parasites, and oxidative stress, and the only way for humans to get the health benefits of these compounds is through dietary intake. The flavonoids are the most widely distributed group of polyphenols, a class of phytochemicals characterized by their phenolic ring structures, with over 5,000 unique compounds found in various fruits, vegetables, grains, nuts, teas, and wines. The flavonoids are especially promising in the area of cancer prevention and are among the most thoroughly studied classes of phytochemicals. Several studies have shown a link between intake of specific flavonoids, including quercetin, and a reduction in colon cancer risk (Cook and Samman, 1996; Yang, 2001).

One possible mechanism by which flavonoids could prevent colon cancer is by regulation of Phase 1 and Phase 2 enzyme activity. The human body is constantly exposed to potential carcinogens through the environment, and the body deals with these compounds through a system of xenobiotic-metabolizing enzymes, called Phase 1 and Phase 2 enzymes. These enzymes work together to metabolize foreign substances, such as drugs, carcinogens, and endogenous compounds, which enter or make contact with the body. The Phase 1 enzymes (i.e. cytochrome P450) are responsible for bioactivation of xenobiotics in the body by adding a polar functional group to the original molecules, which produces reactive compounds that can bind to DNA and cause mutations. The Phase 2 enzymes (i.e. glutathione S-transferase and quinone reductase) conjugate these reactive intermediates by adding a hydrophilic functional group and making them water-soluble and more easily excreted from the body (Jones and DeLong, 2000).

Two classes of chemopreventive agents have been identified: bifunctional inducers concurrently stimulate Phase 1 and Phase 2 enzymes, while monofunctional inducers increase Phase 2 enzyme activity without affecting Phase 1 enzyme activity. Quercetin, a flavonoid, has

been described as a monofunctional inducer (Henderson et al., 2000). Many of the cytochrome P450 enzymes (CYP450s) are tissue- and substrate-specific, allowing for metabolism of the numerous drugs, toxins, and possible carcinogens that the body encounters every day. The flavonoids and other phytochemicals may prevent cancer by decreasing the activity of CYP450s, which are necessary for the detoxification of xenobiotics by the Phase 2 enzymes. However, higher levels of Phase 1 enzyme activity compared to Phase 2 enzyme activity can cause negative effects, and the ratio between these two enzyme classes is very important. Therefore, monofunctional inducers, which increase the activity of Phase 2 enzymes but not Phase 1 enzymes, may be more effective chemopreventive agents than bifunctional inducers, which increase both Phase 1 and Phase 2 enzyme activity (Manson et al., 1997).

The glutathione S-transferases (GSTs) are an example of the Phase 2 enzymes, which detoxify carcinogens and promote their excretion by promoting the conjugation of electrophilic compounds with glutathione. Many studies show that flavonoids such as quercetin can stimulate GST, which may be a promising strategy for prevention of colon cancer (Hayes and Pulford, 1995). Quinone reductase (QR), another Phase 2 enzyme, catalyzes two-electron reductions on free radicals and toxic oxygen metabolites, which deactivates them and protects the surrounding tissues from mutagenesis. Both GST and QR are induced by dietary components, including the flavonoids, thus reducing cancer risk (Benson et al., 1980). Colon cancer is characterized by uncontrolled growth and proliferation of abnormal cells. Controlling the rate of cell proliferation may be an important mechanism for cancer prevention. Flavonoids such as quercetin have been shown to inhibit cell proliferation in animals and in human cell lines, thereby potentially protecting against colon cancer (Yang et al., 2000).

Quercetin is the most widely distributed flavonoid in the food supply, and is found abundantly in apples, onions, black tea, and red wine (Hollman et al., 1997). The total flavonoid intake, comprised mostly of quercetin, has been estimated to be between 20-70 mg/day (Beecher, 2003; Manach et al., 1997). In both *in vitro* and *in vivo* studies, quercetin has demonstrated a protective role in breast (Choi et al., 2001), lung (Khanduja et al., 1999), liver (Denda et al., 1998), ovarian (Scambia, 1994), and colon cancers (Huber et al., 1997). Much research exists on the role of quercetin in regulating Phase 1 and Phase 2 enzymes, although the results of these studies are not consistent. Previous studies on quercetin and CYP1A1, GST, and QR activity in the colon and liver will be discussed.

Much of the research conducted on quercetin and cancer prevention has used higher concentrations than could feasibly be achieved in blood plasma by humans through dietary intake. *In vitro* studies have used concentrations of quercetin from 1-200 μM , whereas only 1 μM is typically found in plasma. Many rat studies have used diets containing 1-2% quercetin (10-20 g/kg diet), while the average human intake of total flavonoids in the diet is only 25-50 mg/day. Recent studies have focused on the direct uptake of flavonoids, including quercetin, by human intestinal cells. Therefore, the debate over the concentrations of quercetin that could be achieved in the blood may not be relevant, since higher concentrations could be obtained in the lumen of the GI tract.

The present study tested the hypothesis that quercetin treatment would monofunctionally induce the Phase 2 enzymes glutathione S-transferase and quinone reductase in the SW-480 human colon carcinoma cell line. The objectives of this study were: 1) to explore whether quercetin could mono-functionally increase the activity of the Phase 2 enzymes, GST and QR, without affecting the activity of the PI enzyme CYP1A1, 2) to determine if each enzyme activity

increases in a dose response manner, and 3) to observe the effect of quercetin on cell viability and proliferation.

The results of this study did not support the original hypothesis. Quinone reductase activity was significantly decreased with the highest dose of quercetin (40 μM), and glutathione S-transferase activity showed this same pattern, although the results were not statistically significant. We were unable to measure cytochrome P450 1A1 activity in this model, due to either an absence of the isoform in this cell line or insufficient sample to measure enzyme activity. We were able to show that quercetin (0-40 μM) does not exhibit cytotoxic effects on these cells, since there was no significant difference between treatment groups in the cell viability and cell proliferation studies. Therefore, it is unlikely that the decline in enzyme activity noted at higher concentrations of quercetin were attributable to cell death or toxicity.

This is the first *in vitro* study to use the SW-480 cell line to test the effect of quercetin on the activity of these Phase 1 and Phase 2 enzymes – glutathione S-transferase, quinone reductase, and cytochrome P450 1A1 – simultaneously in colon tissue. This study also utilized lower concentrations of quercetin than previous research to demonstrate the effect of physiological, rather than pharmacological, doses of flavonoids on enzyme activity. The doses used in this study were shown not to have cytotoxic effects on the SW-480 cells.

CHAPTER 2

LITERATURE REVIEW

Colon Cancer

Cancer is the uncontrolled growth of abnormal cells in the body, which usually begins in a single organ or tissue (such as the colon) and then spreads to other parts of the body. Almost all cancers are caused by a mutation or some other anomalous activation of the cellular genes that control cell growth and mitosis. The main stages of cancer development are initiation, promotion, progression, and metastasis. The first stage of carcinogenesis is called initiation, which begins when a reactive intermediate alters the genetic make-up of the cell and produces a mutation by modifying oncogenes, tumor suppressor genes, and DNA-repair genes. Without successful repair or cell death, the mutated cell enters the stage of promotion, which is characterized by rapid proliferation to convert the initiated cells into a population of cancer cells. This stage is reversible by the same mechanisms as the initiation stage: cell repair or death. Failure to cease the proliferation of the mutated cells allows the cells to enter the third stage, called progression. During this last stage the cancerous cells of the tumor invade surrounding tissues throughout the body. Metastasis, the final stage of carcinogenesis, is associated with widespread DNA damage, and the loss of cell function leads to organ dysfunction, which could eventually result in death.

Although less publicized than other types of cancer, such as breast and lung cancers, colon cancer remains a prevalent disease in our country. According to the American Cancer Society, colorectal cancer (colon and rectal cancers combined) is the third most common cancer

in both men and women (ACS, 2002b). Colorectal cancer accounts for approximately 11% of all cancer-related deaths in the United States. In 2002, there were an estimated 148,300 new cases and 56,600 deaths due to colorectal cancer (ACS, 2002a). These figures are consistent with trends over the past twenty years, and experts expect similar results in the future. The projected numbers for 2003 are 147,500 new cases and 57,100 deaths (ACS, 2003). Georgia is ranked 27th among the states in overall cancer mortality rates, and there are expected to be 3,300 new cases of colorectal diagnosed and 1,300 deaths due to colorectal cancer in Georgia in 2003 (CDC, 2003). Due to increased public awareness, early detection methods, and improved treatments, the colorectal cancer rates had decreased over time but have now leveled off.

Genetic factors, such as a family or personal history of colorectal cancer, polyps, or inflammatory bowel disease, can increase the risk for colorectal cancer. Age and race are also risk factors; individuals over age 50 and African Americans are more likely to develop colorectal cancer. Gender does not appear to be a risk factor, since men and women have almost identical rates of occurrence and death (ACS, 2002b). However, since inherited gene mutations cause just 10% of colorectal cancers (Armstrong and Mathers, 2000; Go et al., 2001), environmental factors must be involved in the disease process development as well. This conclusion is supported by epidemiological evidence, including both cohort and case-control studies. The incidence of colorectal cancer can vary by as much as 20 times in different regions throughout the world (Giovannucci, 2003; Slattery and Caan, 2001). In addition, migration studies have revealed remarkable changes in the occurrence of colorectal cancers in populations, following relocation from a low-risk to high-risk area (Gomez et al., 2003).

Physical inactivity, obesity, red meat intake, smoking, alcohol intake, and low fruit and vegetable consumption are all lifestyle-related risk factors for colorectal cancer. Studies also

indicate that consuming a typical Western diet – high in fat and animal protein, low in fruits, vegetables, and fiber – can increase colon cancer risk. Epidemiological studies show that Americans have a higher risk of colon cancer compared to other countries with different dietary patterns (Slattery et al., 1998).

Diet and Colon Cancer

The impact of diet on overall health is significant, and this fact has become better recognized in recent years. In fact, five of the ten leading causes of death in the United States are diet-related: heart disease, cancer (including colorectal cancer), stroke, diabetes and atherosclerosis. Obesity is also gaining prominence as an important risk factor for all of these conditions, and diet is a primary prevention and treatment method for obesity as well. The importance of nutrition in addressing chronic disease prevention cannot be overstated; the evidence is mounting that diet is among the most important predictors of disease risk.

Up to 80% of all colorectal cancer cases and deaths are attributable to diet, based on the majority of the epidemiological evidence, which shows strong correlations between trends in food intake and incidence of colorectal cancer (Bingham, 2000). Therefore, many cases of colorectal cancer and related deaths may be preventable by dietary modifications (Giovannucci and Willett, 1994). Much recent research has focused on the role of specific food groups, including dietary fat, animal protein, and fruits and vegetables, in colorectal cancer development.

In 2002, the American Cancer Society released a comprehensive list of recommendations for healthy lifestyle choices, focusing on nutrition and physical activity, to reduce cancer risk in the general population (ACS, 2002c). The report encourages positive lifestyle choices, such as increasing intake of plant-based diets rich in whole grains, fruits, and vegetables, increasing

physical activity both through moderate exercise and everyday activities, and maintaining a healthy body weight through caloric balance. The recommendations also encourage individuals to limit consumption of certain foods associated with increased cancer risk, such as high-fat foods, fried products, refined carbohydrates and sugary foods, excessive alcohol, red meats, and large amounts of charbroiled or grilled meats, which may contain cancer-promoting components (nitrites and polyaromatic hydrocarbons).

One of the most studied dietary aspects of colon cancer prevention is fruit and vegetable intake. The overall conclusion of these studies is that an inverse relationship exists between fruit and vegetable intake and the risk of colorectal cancer (Fund, 1997; Steinmetz and Potter, 1996). The association between dietary patterns and risk for chronic diseases is complicated, due to the many variables involved, which makes proving a causative relationship between any two components very difficult. Several studies have found a more convincing correlation between vegetable intake and cancer risk, as opposed to fruit intake (Voorrips et al., 2000), and some have found no evidence of an association between fruit and vegetable intake and colorectal cancer (Flood et al., 2002; Michels et al., 2000). Other studies suggest that overall diet diversity – the variety of food consumed – is associated with decreased colorectal cancer risk (Fernandez et al., 2000). However, the majority of the evidence supports total fruit and vegetable intake as a negative risk factor for cancer, including colorectal cancer.

In order to elucidate the relationship between fruit and vegetable intake and colorectal cancer risk, the role of individual components of plant foods are being thoroughly investigated. Vitamins, minerals, fiber, and antioxidants, are all dietary components that may have a role in reducing cancer risk, but these components alone can not explain the anticarcinogenic effects of fruits and vegetables. A new area of nutrition research involves studying the effects of

phytochemicals on chronic disease risk. Phytochemicals are non-nutritive compounds produced by plants that may contribute to the health benefits of fruits and vegetables, beyond their vitamin and mineral content (Bloch, 1995). Several groups are developing databases that include the chemical structure, reported functions, and food content of phytochemicals that will most certainly prove to be very useful as the research in this area continues (Beecher, 2003; Dwyer et al., 2003).

Flavonoids and Colon Cancer

Plants produce phytochemicals mainly for protection from herbivores, parasites, and oxidative stress. Mammals do not synthesize these compounds, and the only way for humans to get the health benefits of these compounds is through dietary intake. The polyphenols are a class of phytochemicals characterized by their phenolic ring structures. All polyphenols are derived from a common intermediate, phenylalanine, and can be divided into ten classifications based on structural differences (Yang, 2001). The flavonoids are the largest class of polyphenols, with over 5,000 unique compounds in existence. The flavonoids are also the most widely distributed class of polyphenols in the food supply, and they are found in various fruits, vegetables, grains, nuts, teas, and wines. The estimated dietary intake of all flavonoids in humans is approximately 1 g per day (Scalbert and Williamson, 2000). Other studies have reported slightly higher or lower values, since the flavonoid content of plants is highly variable depending on the plant species, growth, conditions, and maturity (Cook and Samman, 1996).

The flavonoids are especially promising in the area of cancer prevention and among the most thoroughly studied classes of phytochemicals. Several studies have shown a link between specific flavonoids, including quercetin, and a reduction in colon cancer risk (Cook and

Samman, 1996; Yang, 2001). A large cohort study of 9,959 men and women in Finland from 1967-1991 found an inverse association between total flavonoid intake and cancer incidence at all sites (Knekt et al., 1997).

The mechanism of action by which the flavonoids may prevent colon cancer is a popular research area, utilizing both animal and cell culture models. Possible preventive mechanisms being studied include: 1) antioxidant activity, 2) estrogenic activity, 3) inhibition of cell proliferation, 4) inhibition of lipid peroxidation, 5) stimulation of cell cycle arrest, 6) stimulation of apoptosis (programmed cell death), and 7) regulation of the detoxification enzymes responsible for carcinogen metabolism (Birt et al., 2001). Research is ongoing in each of these areas, and it is likely that each of these mechanisms is used by different flavonoids in different tissues.

Many variables affect the efficacy of polyphenols on prevention of carcinogenesis. The method of administration – through dietary intake, topical application, or injection – can have great impact on the outcome of a study. The chemical form given, glycone or aglycone, can also alter the results. In addition, the stage of carcinogenesis during which the flavonoids were given can influence the effectiveness of the treatment. Flavonoids given earlier in the process, prior to the initiation stage, were more effective than those administered during the promotion or progression phases (Yang, 2001). Flavonoids have been classified as “blocking agents” because they are believed to act before the mutagenic step of carcinogenesis, preventing the initiation of cancer. Other dietary compounds, such as retinoids, indoles, and carotenoids, are referred to as “suppressing agents,” which act after the mutation occurs to prevent further progression of cancer (Henderson et al., 2000; Wattenberg, 1997). This reinforces the belief that nutrition can

play an important role in chronic disease prevention. Although diet also has limited use in the treatment of these diseases, it is much more beneficial in the prevention stage.

Phase 1 and Phase 2 Enzymes

The human body is constantly exposed to potential carcinogens – through air, water, pollution, cigarette smoke, and even foods. One mechanism used by the body to deal with these possibly harmful substances is a system of carcinogen-metabolizing enzymes. This system consists of two separate classes of enzymes: the Phase 1 enzymes and the Phase 2 enzymes. These two enzyme groups work together to metabolize any foreign substances, or xenobiotics, that enter or make contact with the body. Xenobiotics include prescription drugs, over-the-counter medications and supplements, environmental contaminants (pesticides, air and water pollution, diesel exhaust), naturally-occurring plant compounds, and substances formed by cooking certain foods (i.e. heterocyclic aromatic amines found in cooked meat products).

The Phase 1 and Phase 2 enzymes are essential to the body's defenses against cancer, and much research recently has focused on how the enzyme activity and concentration in the body influence cancer risk. These two types of enzymes work in cooperation, and each is necessary to facilitate the elimination of carcinogens from the body. Since most xenobiotics occur in a hydrophobic form in nature, the Phase 1 and Phase 2 enzymes must convert them to a hydrophilic form in order to leave the body (Jones and DeLong, 2000; **Figure 1**).

The Phase 1 enzymes, including the cytochrome P450 enzymes, are responsible for bioactivation of xenobiotics in the body. These enzymes work by adding a polar functional group to the original molecules, producing reactive compounds. These reactive intermediates can then bind to DNA and cause a mutation, or they can become a substrate for the second class

of detoxification enzymes. The Phase 2 enzymes, including glutathione S-transferase and quinone reductase, conjugate these reactive intermediates, adding a hydrophilic functional group and making them water-soluble. These secondary products are then more easily excreted from the body (Jones and DeLong, 2000).

The GI tract is the first line of defense against environmental toxins in the diet (Carriere et al., 2001). Epithelial tissues, such as the gastrointestinal tract, lungs, and skin, are the most exposed and vulnerable to exogenous carcinogens, and 95% of cancers occur in epithelial tissues. The Phase 1 and Phase 2 enzymes are especially important to prevention of colon cancer, since the gastrointestinal (GI) tract is constantly exposed to potential carcinogens through the food we eat. In addition, these enzymes are abundant in epithelial tissues and can be induced by dietary compounds found in the GI tract (Yang et al., 1992).

Two classes of chemopreventive agents have been identified: bifunctional inducers and monofunctional inducers. Bifunctional inducers concurrently stimulate Phase 1 and Phase 2 enzymes, while monofunctional inducers increase Phase 2 enzyme activity without affecting Phase 1 enzyme activity. Quercetin, a flavonoid, has been described as a monofunctional inducer (Henderson et al., 2000). Monofunctional inducers are usually considered optimal for cancer prevention, since they tend to balance the Phase 1 and Phase 2 enzyme activities in the body. According to Manson et al. (1997), “the result of exposure to an environmental toxin in terms of acute or chronic toxicity largely depends on the balance between these two processes.”

A third class of chemopreventive agents, known as dual-acting agents, has been described to stimulate Phase 2 enzymes but inhibit Phase 1 enzymes. According to Birt et al. (2001), these dual-acting agents would be the ideal compounds for preventing carcinogen activation, although none of these agents has yet been identified. Research is currently in progress to confirm the

existence and the identity of these dual-acting agents, as well as to test their effectiveness in preventing cancer development.

Cytochrome P450 Enzymes

The cytochrome P450s (CYP450) are a large family of Phase 1 enzymes, with over 1,000 unique isoforms known. Different CYP450 isoforms (i.e. CYP2E1, CYP3A4) exist in separate body tissues; some localize exclusively in one area and some exist in many areas. For example, CYP1A1 occurs in the liver and in several tissues of the gastrointestinal tract, including the colon. In contrast, CYP2C9 exists only in the liver (McKinnon et al., 1995). One well-known cytochrome P450 isoform, CYP3A4, is most abundant in the liver and small intestine, and is responsible for the metabolism of > 60% of all clinically used drugs (Anzenbacher and Anzenbacherova, 2001; Engman et al., 2001).

Many of the cytochrome P450 enzymes are not only tissue-specific, but substrate-specific as well, allowing for metabolism of the numerous drugs, toxins, and possible carcinogens that the body encounters every day. Some isoforms have only a few specialized substrates, while others metabolize many similar substrates. CYP1A1 shows a specificity for polycyclic hydrocarbons, and CYP1A2 is focused on arylamines and arylamides. The main drug-metabolizing isoform, CYP3A4, has many substrates – dihydropyridines, steroids, cyclosporin, lovastatin, and other drugs (Guengerich, 1992).

One concern about the ability of dietary components to stimulate or inhibit these enzymes is that they metabolize drugs as well as carcinogens, and the effect of enzyme modulation on drug interactions is largely unknown. Studies have documented that grapefruit juice consumption decreases the activity of CYP3A4, which can result in dangerously high serum

levels of drugs, such as lovastatin, in patients consuming both (Kantola et al., 1998). Although the methods of this particular study have been called into question, the concerns about dietary modulation of carcinogen-metabolizing enzymes and the resulting effects on drug metabolism are still valid.

In animal studies, dietary components, such as indoles and isothiocyanates, increased CYP1A1 activity in the presence of a carcinogen. This protective effect may be due to increased metabolism of carcinogens in the intestine, thereby reducing the exposure of the rest of the body to the carcinogen (He et al., 2000; Tan et al., 1999). Conversely, when the same dietary components were introduced without a carcinogen present, CYP1A1 enzyme activity was unchanged (Breinholt et al., 1999; Canivenc-Lavier et al., 1996). Other variables that may affect the induction of CYP1A1 and other cytochrome P450 isoforms are: 1) the tissue in which the enzyme is located, 2) the structure of the dietary component, and 3) whether the dietary component is introduced before or after the carcinogen (Yang et al., 1992).

One proposed mechanism by which the flavonoids and other phytochemicals may prevent cancer is by decreasing the activity of CYP450 enzymes. Large variations in CYP450 enzyme activity exist between individuals, and the deviation may be due to genetic differences and the inducibility of these enzymes by drugs and dietary components. Genetic testing has revealed that colon cancer patients have increased expression of the CYP1A1 allele (Kiss et al., 2000). Thus, the effect of diet on these enzymes and the resulting impact on cancer risk is of great interest (Guengerich, 1992).

The Phase 1 enzymes are necessary for the detoxification of xenobiotics by the Phase 2 enzymes, but higher levels of Phase 1 enzyme activity compared to Phase 2 enzyme activity can cause negative effects. Some experts believe that the ratio between Phase 1 and Phase 2 enzyme

activity is more important than the specific activity of either category of enzymes (Yang, 2001). For this reason, monofunctional inducers, which increase the activity of Phase 2 enzymes but not Phase 1 enzymes, may be more effective chemopreventive agents than bifunctional inducers, which increase both Phase 1 and Phase 2 enzyme activity (Manson et al., 1997). Several studies using animal models have documented that flavonoids such as quercetin, act as monofunctional inducers in the liver, kidney, and colon, which allows for metabolism of carcinogens by both classes of enzymes to promote excretion of most xenobiotics (Siess et al., 2000).

Glutathione S-transferase Enzymes

The glutathione S-transferases (GSTs) are an example of the Phase 2 enzymes, which detoxify carcinogens and promote their excretion from the body. The GSTs promote the conjugation of electrophilic compounds, often the byproducts of Phase 1 enzymes, with glutathione. This conjugation of xenobiotics with glutathione is considered to be among the most important mechanisms for protecting tissues from the damaging effects of these reactive intermediates (Jones and DeLong, 2000). There are several isoforms of glutathione S-transferases, which are divided into four main classes – Alpha, Mu, Pi, and Theta. However, these isoforms are not as distinctly localized as the cytochrome P450's and are often measured as a group. The glutathione S-transferase enzymes also function as antioxidants, which is another possible mechanism for their chemopreventive properties (Clapper and Szarka, 1998).

In addition to modulation of cytochrome P450 enzymes, another mechanism by which the flavonoids may prevent cancer is by increasing GST activity in the body. GST expression and activity can vary widely between individuals, and studies have documented that persons with a genetic propensity to deficient levels of GST are at increased risk for colorectal cancers (Zhang

et al., 1999). Other studies have shown that rats with induced colon carcinoma developed significantly lower GST activity compared to controls during the course of cancer initiation and progression, which supports the use of GST as a biomarker for cancer risk (Clapper and Szarka, 1998). A large body of data also supports the ability of chemopreventive agents to induce GST and other Phase 2 enzymes, which may be a promising protective strategy for high-risk individuals. Therefore, consuming dietary components that increase GST activity could be a therapeutic approach to cancer prevention for individuals who are predisposed to colon cancer. According to Hayes et al. (1995), “evidence suggests that the level of expression of GST is a crucial factor in determining the sensitivity of cells to a broad spectrum of toxic chemicals.”

Other studies have been conducted on the expression of GST in colon tumors compared to normal colon mucosa. Compared to the normal colon, the colon tumors were found to have higher levels of GST activity, which may produce drug-resistance in the tissues. Tumors that express high levels of GST are correlated with decreased effectiveness of chemotherapy and poor patient outcomes and survival rates (Sutoh et al., 2000). Research has shown that polyphenols found in red wine and black tea decrease GST expression in colon tumors; this suggests a possible role for polyphenols in preventing drug resistance in these tissues. Therefore, dietary components may have both chemopreventive and chemotherapeutic roles in the development of colon cancer through the regulation of GST expression and activity (Luceri et al., 2002).

Quinone Reductase Enzyme

Like the glutathione S-transferases, NAD(P)H:quinone oxidoreductase, also known as DT-diaphorase, is a Phase 2 enzyme found in the human colon. Quinone reductase, as it is

commonly known, is a useful representative of the Phase 2 enzymes due to the wide distribution found in mammalian tissues, the relative ease of measurement for enzyme activity, and the large induced response normally seen with dietary components (Talalay et al., 1995). Unlike the cytochrome P450 enzymes and the glutathione S-transferase enzymes, which have many isoforms and classes that exist and have effects in multiple locations, quinone reductase is a single, multi-functional enzyme that exerts its effects in different body tissues, including the liver, lung, colon, and breast (Benson et al., 1980).

Quinone reductase works by catalyzing two-electron reductions on free radicals and toxic oxygen metabolites, which deactivates them and protects the surrounding tissues from mutagenesis and carcinogenesis. The cytochrome P450s and other Phase 1 enzymes activate these reactive compounds through one-electron reductions, and quinone reductase acts on the products of the Phase 1 enzymes to inactivate them and promote their excretion (Wang and Higuchi, 1995).

Like glutathione S-transferase, increased expression of quinone reductase has been observed in tumors compared to normal tissues from the same organ, which can cause drug-resistance and poor response to chemotherapy (Belinsky and Jaiswal, 1993). Colorectal tumors with metastasis also expressed higher quinone reductase activity than tumors without metastasis (Mikami et al., 1998). In addition, non-cancerous tissues that express high levels of quinone reductase have decreased numbers of DNA adducts, a known biomarker for cancer susceptibility (Uda et al., 1997). This evidence supports the dual role of quinone reductase in both cancer prevention and therapy.

Quinone reductase, like the glutathione S-transferase enzymes, can be induced by dietary components, which has been shown to have beneficial effects on cancer risk (Benson et al.,

1980). Due to the differences in their mechanisms of action, however, quinone reductase can be induced by antioxidants, such as vitamins A, C, and E, which do not affect the glutathione S-transferases. Other chemopreventive agents, such as the flavonoids, have been shown to induce both classes of Phase 2 enzymes (Wang and Higuchi, 1995) (Valerio et al., 2001).

Cellular Proliferation

Cancers, including colon cancers, are characterized by uncontrolled growth and proliferation of abnormal cells. Therefore, cellular proliferation is an important aspect of cancer prevention to be studied and understood. The first stage of cancer development, the initiation stage, begins with a single genetic mutation, which then multiplies and spreads through the promotion and progression stages. The Phase 1 and Phase 2 enzyme systems work at this first stage to prevent potential carcinogens from entering the body and causing the initial mutation from occurring. In contrast, the control of cellular proliferation would occur at one of the later stages; the initial mutation has already occurred, but slowing the rate of abnormal cell growth and division would decelerate the progression of the cancer (Gee et al., 2002; Krishnan et al., 2000).

Flavonoids such as quercetin have been shown to inhibit cell proliferation in animals and human cell lines, and a greater effect has been shown in cancerous models compared to normal models (Yang et al., 2000). This effect may be explained by several mechanisms: 1) suppression of mitosis, 2) inhibition of cell proliferation, 3) stimulation of apoptosis, and 4) down-regulation of the cell cycle (Gee et al., 2002). Flavonoids may have different levels of effectiveness on the inhibition of cell proliferation, based on their structural characteristics. As mentioned earlier, flavonoids may act as anticarcinogens by several mechanisms, including the regulation of Phase

1 and Phase 2 enzymes and inhibition of cell proliferation. Unique flavonoids may act by separate mechanisms (Kuo, 1996).

There are several proposed mechanisms of action by which quercetin affects Phase 1 and Phase 2 enzyme activity. The first mechanism involves quercetin binding with specific receptors inside the cells, which can activate one of two gene promoter regions: the antioxidant response element (ARE) or the xenobiotic response element (XRE; Jaiswal, 2000). Depending on which of these regions is activated, a compound may be a monofunctional inducer (increases Phase 2 enzyme activity, and decreases or does not affect Phase 1 enzyme activity) or a bifunctional inducer (increases both Phase 1 and Phase 2 enzyme activity). A bifunctional inducer enters the cell and binds to the aryl hydrocarbon receptor (AhR), and this complex will then dimerize with the aryl hydrocarbon receptor nuclear transferase (ARNT) and enter the cell nucleus. The complex then binds to a specific region of the gene called the xenobiotic response element (XRE), which enables the transcription of proteins for both Phase 1 and Phase 2 enzymes (Rushmore and Kong, 2002). Alternatively, a monofunctional inducer bypasses the AhR and binds directly to a region of the gene called the antioxidant response element (ARE). This promoter region allows the transcription of the proteins for Phase 2 enzymes, but not Phase 1 enzymes (Guengerich, 1993).

Another mechanism by which quercetin may affect the body's response to xenobiotics is through the regulation of inflammatory agents, such as tumor necrosis factor alpha (TNF- α) and nuclear factor kappa B (NF- κ B). NF- κ B is a transcription regulation factor, which is inhibited by a protein called I kappa B (I κ B). Activation by a cytokine causes I κ B to be degraded, ceasing the inhibition of NF κ B, which enters the nucleus. NF κ B then forms a transcription complex with two proteins called p50 and p65. The complex binds to a response element in the promoter

regions of many pro-inflammatory cytokines, including TNF- α . Inflammatory cytokines are associated with cancer (Abraham, 2000). The human adenocarcinoma cell line, Caco-2, has been shown to produce TNF- α as a result of NF- κ B activation, in response to xenobiotics (Hosoi et al., 2003). Ganey and Roth (2001) suggest that these pro-inflammatory cytokines can cause cells to become more sensitive to the damaging effects of xenobiotics. This negative effect may be due to concurrent activation of Phase 1 and Phase 2 enzymes and NF- κ B in response to the presence of xenobiotics in the intestinal tract. Transcriptional activation of both CYP1A1 and NF- κ B are mediated by the AhR, so the bifunctional inducers that activate Phase 1 enzyme activity could also stimulate NF- κ B and the release of pro-inflammatory cytokines, such as TNF- α (Ciolino et al., 1999). Quercetin has been shown to inhibit TNF- α transcription, which would reduce the inflammatory response and theoretically decrease colon cancer risk (Moon et al., 2003; Wadsworth et al., 2001).

Human Colon Carcinoma Cell Lines

Cell lines are an appropriate experimental model for mechanistic studies, such as the effect of quercetin on Phase 1 and Phase 2 enzymes, because they are simpler than a complete organism. Isolating the effects of specific chemicals or compounds on certain tissues can be accomplished easily in cell models, as opposed to complete animal or human systems that have too many variables to control. The disadvantage of cell models is that compounds may not behave the same as they would in a complete living system, in the absence of hormones, buffers, and other regulating factors, so results from cell studies cannot be applied directly to humans.

Several human colon carcinoma cell lines exist, including SW480, HT-29, and Caco-2 cells. Among the different cell lines that exist, SW480 cells are the least differentiated and most

rapidly proliferating, which make them very similar to cancerous colon cells in the human body. The more differentiated cells, like Caco-2 cells, are actually more representative of small intestinal cells than colon cells (Izuishi et al., 2000; Meunier et al., 1995). In addition, since the nature of cancer is the increased proliferation, the more rapidly the cell lines divide, the more realistic the model becomes (Wang et al., 2000).

The SW-480 cell line was initiated in 1974 and taken from a 51-year-old Caucasian male with a Grade 4 primary colon tumor (Leibovitz et al., 1976). This cell line is positive for several oncogene mutations – including myc, ras, fos, and p53 – which makes it genetically similar to tumors found in the human colon. The SW-480 cell line is also poorly differentiated, which is also typical of cancer cells, and this characteristic also makes this an appropriate model to study colon cancer (Brattain et al., 1999; Park et al., 1994). Other colon tumor cell lines may have various degrees of differentiation and diverse mutations, and these characteristics must be taken into account when choosing an experimental model.

Quercetin

Quercetin is the most widely distributed flavonoid found in the food supply. The main dietary sources of quercetin are apples, onions, black tea, and red wine (Hollman et al., 1997). The daily intake of quercetin in Western countries has been estimated as 25-50 mg/day, out of a total flavonoid intake of approximately one gram/day (Hollman et al., 1997). However, other studies have suggested the actual numbers might be lower, with estimates for dietary flavonoid intake ranging from 20-70 mg/day, with the majority (60-75%) as quercetin (Beecher, 2003; Manach et al., 1997). Hertog et al. (1993) measured the daily intake of flavonoids in the Netherlands using HPLC (high-performance liquid chromatography) as 23 mg/day, including 16

mg of quercetin. The average intake of flavonoids in U.S. adults, based on data from a food frequency questionnaire used in the US Health Professionals Study, was 20-22 mg/day (Sampson et al., 2002). Other studies have documented large variations in flavonoid intake between individuals – up to an 80 mg/day difference depending on the type of diet consumed (de Vries et al., 1997). Research also demonstrates that cooking and processing can affect the flavonoid content of foods through losses or transformation of the original compounds (Beecher, 2003). An accurate measurement of quercetin intake is complicated by several factors: 1) the concentration of quercetin in a few foods, 2) the uneven distribution of quercetin in plant tissues (i.e. in the apple peel, not the rest of the apple), and 3) the variation of quercetin content in the same foods, depending on growing conditions, seasons, and cooking or processing methods (Scalbert and Williamson, 2000).

The flavonoids, as mentioned earlier, are a class of polyphenols whose structure includes two phenolic benzene rings linked by a heterocyclic pyrone ring containing an oxygen molecule. In nature, flavonoids are usually found in the glycosylated form, meaning they are linked to a sugar moiety. The function of flavonoids in plants include pigmentation, signal conduction, and repelling pests. Quercetin belongs to a class of flavonoids called flavonols, which are characterized by a 3-OH group on the pyrone ring (Sampson et al., 2002).

Many studies indicate that the structure of quercetin is directly responsible for its functions (**Figure 2**). Siess et al. (2000) demonstrated that only nonpolar flavonoids with free hydroxyl groups, such as quercetin, showed anticarcinogenic properties in an animal model. Other studies have confirmed this finding; quercetin has been shown to modulate activity of carcinogen-metabolizing enzymes, which are important for prevention of colon cancer (Guengerich, 1992). Flavonoids with free hydroxyl groups – quercetin, kaempferol, and

naringenin – inhibit Phase 1 enzyme activity, and flavonoids without free hydroxyl groups – flavone, tangeretin, and nobiletin – stimulate Phase 1 enzyme activity (Yang et al., 1992). In addition, the authors postulated that the presence of free hydroxyl groups may increase absorption and bioavailability of flavonoids in the body. The evidence for quercetin's effect on Phase 1 and Phase 2 enzyme activity in human, animal, and cell culture studies will be reviewed in the next section.

Quercetin and Cancer

In both *in vitro* and *in vivo* studies, quercetin has demonstrated a protective role in breast (Choi et al., 2001), lung (Khanduja et al., 1999), liver (Denda et al., 1998), ovarian (Scambia, 1994), and colon cancers (Huber et al., 1997). This effect may be due to one of several mechanisms, which quercetin has been shown to exert on cancer cells. These mechanisms include: antioxidant activity, anti-inflammatory action, stimulation of apoptosis, decreased cell proliferation, and regulation of Phase 1 and Phase 2 enzyme activity (Bravo, 1998; Nijveldt et al., 2001). Many other mechanisms of action for the role of quercetin in cancer prevention are currently under investigation and will undoubtedly continue to be studied in the future.

Much research exists on the role of quercetin in regulating Phase 1 and Phase 2 enzymes, although the results of these studies are not always consistent (**Table 1**). The effectiveness of quercetin as a modulator of these carcinogen-metabolizing enzymes varies, depending on several factors: 1) the model used (*in vitro* vs. *in vivo* studies), 2) the tissue involved (such as lung, liver, kidney, colon, or breast), 3) the doses of quercetin used for treatment (varies from 1 – 200 μM *in vitro* and 0.3-25% of diet *in vivo*), 4) the form of quercetin given *in vivo* (glycone or aglycone), and 5) the time period in which the quercetin was administered (before, after, or in the absence

of a carcinogen). In order to elucidate the mechanisms of action by which quercetin may decrease the risk for specific cancers, many studies using various models and tissues, several doses and forms of quercetin, at different time points must be conducted and the results analyzed, in order to form a strong conclusion.

Animal studies in our lab have shown that quercetin increases the activity of Phase 2 enzymes, including GST, at high doses (1-2% of the diet; 10-20 g/kg diet) in rat colon and liver (Fischer and Fisher, 2000; Mikulcik and Fischer, 2001). Quercetin also stimulated apoptosis but did not affect lipid peroxidation, when given at high concentrations (40-80 μM) in the presence of iron (10-50 $\mu\text{M/L}$) in the human hepatoma HepG2 cell line (Mayer Jr, 2001). More recently, quercetin has been shown to monofunctionally induce Phase 1 (CYP1A1) and Phase 2 (GST and QR) enzyme activity, in a dose-dependent manner at lower concentrations (0.3-0.9% of diet; 3-9 g/kg diet) in the liver and colon of rats (Penn, 2003).

Several rat studies have investigated the effect of quercetin (0.001-2.0% of diet) on colon GST activity, and the research suggests that a dose of at least 1% of the diet must be given to significantly increase GST activity in the colon (Breinholt et al., 1999; Breinholt et al., 2003; Huber et al., 1997). *In vitro* studies using cell lines (COLO 320HSR) and primary colon carcinoma cells have shown a contradictory effect – a dose-dependent decrease in GST activity at concentrations of 40 μM and higher (Zhang and Wong, 1997; Zhang et al., 2003).

The research on quercetin and liver GST activity is less convincing; studies using rat models and doses of quercetin ranging from 0.001-1% of the diet have shown no significant effect on GST activity in the liver (Breinholt et al., 2003; Brouard et al., 1988; Canivenc-Lavier et al., 1996). Musonda et al. (1997) used doses of quercetin from 0.5-20 μM to study GST

activity in the HepG2 liver carcinoma cell line, and they also found no effect of quercetin on liver GST activity.

Fewer studies have been conducted on the relationship between quercetin and QR activity. Breinholt et al. (1999) fed rats a diet containing 0.01% of the diet as quercetin and measured its effect on colon QR activity, but they found no significant changes in activity compared to controls. Several *in vitro* studies have investigated the effect of quercetin (1-50 μM) on QR activity in the liver using the Hepalclc7 murine hepatoma cell line, and the overall conclusion of these studies was that quercetin increases liver QR activity in a dose-dependent manner (Uda et al., 1997; Williamson et al., 1996; Yannai et al., 1998). Yannai et al. (1998) further investigated the role of the aryl hydrocarbon receptor (AhR) in the stimulation of QR activity and concluded that quercetin directly and monofunctionally induces QR activity in the liver.

Since the majority of CYP1A1 is found in the liver, most of the studies involving quercetin and CYP1A1 activity used liver tissue. Presently, the only study on quercetin and CYP1A1 activity in colon mucosa is by Penn (2003). This study, as mentioned earlier, showed a dose-dependent decrease in CYP1A1 activity in rat colon using doses of quercetin from 0.3-0.9% of the diet.

Rat studies on liver CYP1A1 activity used doses from 0.3-1.0% of the diet as quercetin, and these found no effect on CYP1A1 in the liver (Brouard et al., 1988; Canivenc-Lavier et al., 1996). Siess et al.(1995), however, fed 0.3% of the diet as quercetin to rats and found a significant decrease in CYP1A1 activity in the liver. Sousa et al. (1985) studied the relationship between quercetin (50-300 nM) in isolated rat liver microsomes, and found a dose-dependent decrease in liver CYP1A1 activity. In contrast, several *in vitro* studies on quercetin (0.5-20 μM)

and liver CYP1A1 activity in the HepG2 cell line have shown a dose-dependent increase in CYP1A1 activity (Allen et al., 2001; Musonda et al., 1997). Other studies using cloned human liver CYPs and isolated human liver microsomes and quercetin concentrations of 10-100 μM have found decreases in CYP1A2 and CYP2C8 activity, but not CYP1A1 (Masimirembwa et al., 1999).

Quercetin Absorption and Metabolism

Much of the research conducted on quercetin and cancer prevention has used higher concentrations than could feasibly be achieved in blood plasma by humans through dietary intake, and possibly even through supplementation. In animals, the quercetin concentrations found in plasma have ranged from 45 $\mu\text{mol/L}$ to 120 $\mu\text{mol/L}$ (Manach et al., 1997; Manach et al., 1995). In humans, plasma concentrations ranging from 0.1 $\mu\text{mol/L}$ to 5.0 $\mu\text{mol/L}$ have been reported (Olthof et al., 2000) (Hollman et al., 1995; Hollman et al., 1997). The pronounced differences in plasma concentrations between animals and humans may be due to: 1) the higher amounts fed to the animals through the prepared diet, and 2) interspecies differences in quercetin absorption and metabolism.

The concentrations of quercetin used in many previous *in vitro* research studies have far exceeded the amount found in the plasma of animals and humans. *In vitro* studies have used concentrations of quercetin from 1-200 μM , whereas only 1 μM is typically found in plasma. Many rat studies have used diets containing 1-2% quercetin (10-20 g/kg diet), while the average intake of total flavonoids in the diet is only 25-50 mg/day. Manach et al. (1995) fed rats a 0.5% quercetin diet and measured quercetin concentration in the plasma (100 $\mu\text{mol/L}$) and the cecum (1400 $\mu\text{mol/L}$). This large concentration gradient between the cecal contents and the circulating

blood results in passive diffusion from the colonic lumen directly into the bloodstream, most likely via the portal vein.

The extent of the absorption of dietary quercetin in humans is unclear, and may vary depending on the form of quercetin given. Hollman et al. (1995) studied quercetin absorption in ileostomy patients, in order to correct for any degradation by colonic bacteria. They found that 52% of quercetin glycosides (from onions), 17% of quercetin rutinoid (from tea), and 24% of pure quercetin aglycone (the isolated form used in many studies) was absorbed. They concluded that humans can absorb each of these forms, and that absorption of quercetin from the small intestine is increased in the glycosylated form (Hollman et al., 1995). In another study, the results suggested that the bioavailability of quercetin from onions was three times greater than from apples or tea (Hollman et al., 1997). Studies by Walle et al. (2000), using a realistic intake of 10.9 to 51.6 mg quercetin and HPLC analysis of the food eaten and the blood plasma levels after the meal, revealed that 65 to 81% of the quercetin was absorbed. Although the majority of the quercetin given was in the glucoside form (from onions), only the aglycone form was observed in the plasma. The authors concluded that glycosylated quercetin is hydrolyzed in the human small intestine to the aglycone form, which is then effectively absorbed into the blood stream (Walle et al., 2000). This study confirmed the work of Walgren et al. (Walgren et al., 2000), who found that quercetin glucosides are poorly absorbed by the cell monolayer in human carcinoma Caco-2 cells, a well-documented model of human intestinal absorption. Other studies have suggested that quercetin glycosides may be actively transported across the epithelial membrane of the intestinal tract by sodium-dependent glucose transporter (SGLT-1), although quercetin aglycone remains the primary form absorbed (Walgren et al., 1998).

Recent studies have focused on the uptake of flavonoids, including quercetin, by human intestinal cells directly (**Figure 3**). This research has shown that quercetin is absorbed from the lumen of the GI tract, via basal to apical cell contact and bi-directional transport (Murota et al., 2000; Murota et al., 2002). Therefore, the debate over the concentrations of quercetin that could be achieved in the blood may not be relevant, since higher concentrations would be found in the lumen of the GI tract. The exact mechanisms by which quercetin is absorbed into the human epithelial cells of the intestine are not completely understood, and more research is needed in this area to explore the methods, efficiency, and metabolic effects of this transport. Quercetin aglycone appears to be absorbed more efficiently by the intestinal cell monolayer, compared to the various quercetin glycosides (Murota et al., 2000). The absorption rate of quercetin is enhanced by its solubility in different solvents, such as water, alcohol, or dimethyl sulfoxide (DMSO), which may account for variation among quercetin bioavailability from food sources that are water-based or oil-based (Piskula and Terao, 1998).

Figures

FIGURE 1

The actions of Phase 1 and Phase 2 enzymes on carcinogenesis.
(Modified from “Detoxification and Protective Functions of Nutrients” in Biochemical and Physiological Aspects of Human Nutrition by Jones and Delong, 2000)

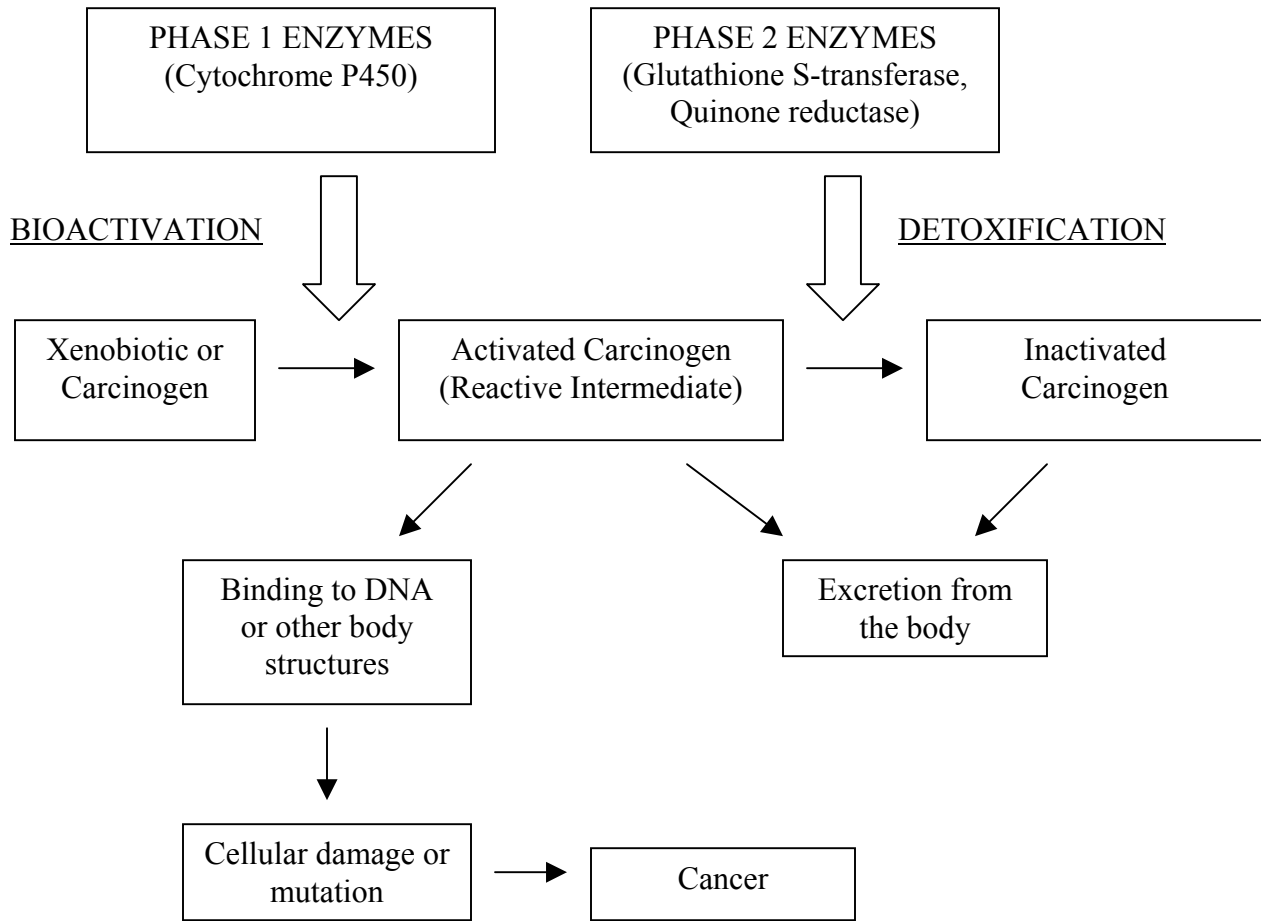


FIGURE 2

The chemical structure of the flavonoid quercetin.

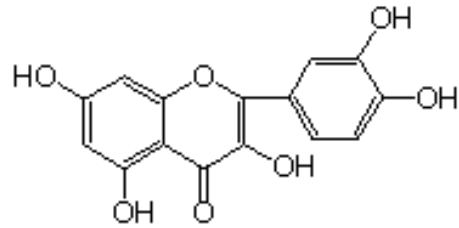
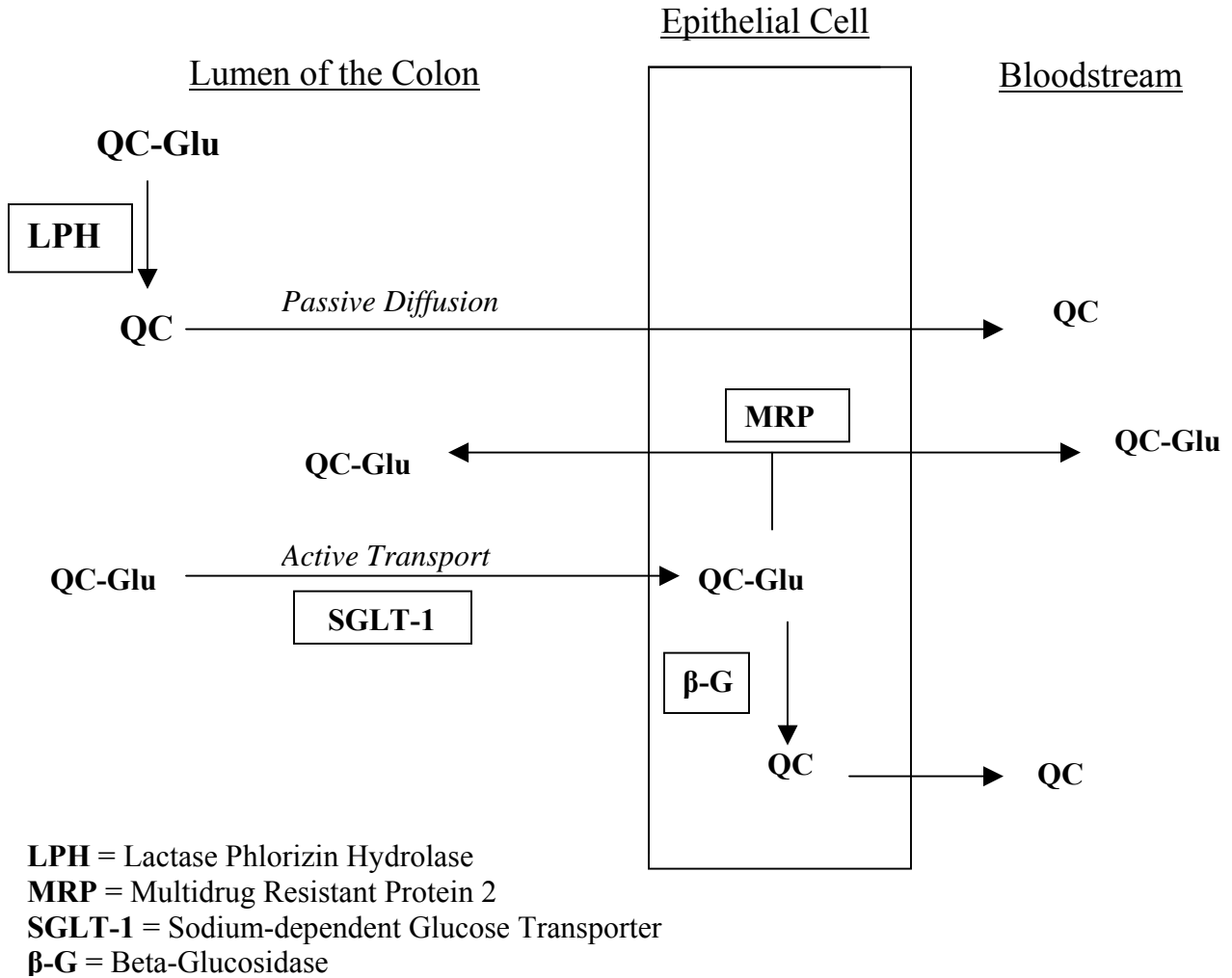


FIGURE 3

Mechanisms involved in the direct uptake of quercetin via the colonic epithelium.



(Walgren et al., 2000; Petri et al., 2003)

Tables

TABLE 1

Previous human, animal, and cell culture studies on the effect of the flavonoid quercetin on Phase 1 (Cytochrome P450 1A1) and Phase 2 (Glutathione S-transferase and Quinone reductase) enzyme activity in colon and liver tissue.

| Author | Model Used | Quercetin Dose | GST | QR | CYP1A1 |
|----------------------------|---------------------------------------------|-------------------------------|----------------------|-------------------|-------------------|
| COLON | | | | | |
| Breinholt et al., 1999 | Female Wistar rats | 0.01% (0.1 g/kg diet) | No effect | No effect | |
| Breinholt et al., 2003 | Male F344 rats | 0.001-0.1% (1-100 mg/kg diet) | No effect | | |
| Fischer and Fisher, 2000 | Male Sprague-Dawley (SD) rats | 1-2% (10-20 g/kg diet) | ↑↑ GST at both doses | | |
| Huber et al., 1997 | Male F344 rats | 1% (10 g/kg diet) | No effect | | |
| Mikulcik and Fischer, 2001 | Male SD rats | 0.2-1% (2-10 g/kg diet) | ↑↑ GST at 1% dose | | |
| Penn, 2003 | Male SD rats | 0.3-0.9% (3-9 g/kg diet) | No effect | Dose-dependent ↑↑ | Dose-dependent ↓↓ |
| Zhang et al., 1997 | COLO 320HSR human colon carcinoma cell line | 5-200 μM | Dose-dependent ↓↓ | | |
| Zhang et al., 2003 | Primary human colon adenocarcinoma cells | 40 μM | ↓↓ GST | | |
| LIVER | | | | | |
| Allen et al., 2001 | HepG2 human liver carcinoma cell line | 5-20 μM | | | ↑↑ at 20 μM dose |
| Breinholt et al., 2003 | Male F344 rats | 0.001-0.1% (1-100 mg/kg diet) | No effect | | |
| Brouard et al., 1988 | Male Wistar rats | 1% (10 g/kg diet) | No effect | | No effect |
| Canivenc- | Male Wistar | 0.3% (3 | No effect | | No effect |

| | | | | | |
|---------------------------|-------------------------------------------------|--------------------------|---------------------------|---------------------------------|--------------------------------|
| Lavier et al., 1996 | rats | g/kg diet) | | | |
| Kang et al., 1999 | HepG2 human liver carcinoma cell line | 0.1-10 μ M | | | No effect |
| Masimirembwa et al., 1999 | Cloned human liver CYPs in <i>S. cerevisiae</i> | 10 μ M | | | ↓↓ CYP2C8, not CYP1A1 |
| Musonda et al., 1997 | HepG2 human liver carcinoma cell line | 0.5-20 μ M | No effect | | Dose-dependent ↑↑ |
| Penn, 2003 | Male SD rats | 0.3-0.9% (3-9 g/kg diet) | ↑↑ at 0.6% and 0.9% doses | ↑↑ at 0.9% dose | No effect |
| Reid et al., 1999 | Isolated human liver microsomes | 20-200 μ M | | | ↓↓ CYP1A2, not CYP1A1 |
| Siess et al., 1995 | Human and rat liver | 0.3% (3 g/kg diet) | | | ↓↓ CYP1A1 |
| Sousa et al., 1985 | Rat liver microsomes | 50-300 nM | | | Dose-dependent ↓↓ CYP1A1 |
| Uda et al., 1997 | Hepalclc7 murine hepatoma cell line | 1-50 μ M | | Dose-dependent ↑↑ QR | |
| Williamson et al., 1996 | Hepalclc7 murine hepatoma cell line | 20 μ M | | ↑↑ QR | |
| Yannai et al., 1998 | Hepalclc7 murine hepatoma cell line | 20 μ M | | ↑↑ QR directly (monofunctional) | |

CHAPTER 3

THE EFFECT OF THE FLAVONOID QUERCETIN ON PHASE 1 AND PHASE 2 ENZYME ACTIVITIES IN THE SW-480 HUMAN COLON CARCINOMA CELL LINE¹

¹ Buck, E.A., Fischer, J.G., Grider, A.F., Power, J.D., Mouat, M.F., Hargrove, J.L. To be submitted for publication in The Journal of Nutrition.

Abstract

Colon cancer is the third leading site of cancer in the United States, and many of these cases may be prevented through dietary modification. High fruit and vegetable intake has been shown to have a strong inverse relationship with colon cancer risk, and the specific compounds responsible for this phenomenon are under investigation. The flavonoids, including quercetin, are a class of phytochemicals that show much promise in colon cancer prevention. One mechanism by which flavonoids may prevent colon cancer is the regulation of Phase 1 (CYP1A1) and Phase 2 (GST, QR) enzymes, which protect the body from the harmful effects of foreign substances, such as drugs, carcinogens, or endogenous compounds. Quercetin, the most widely distributed flavonoid found in the food supply, has been described as a monofunctional inducer that increases Phase 2 enzyme activity without affecting Phase 1 enzyme activity. This study tested the effect of physiological concentrations of quercetin (5-40 μM) on CYP1A1, GST, and QR activity, as well as cell viability and proliferation in the SW-480 human colon carcinoma cell line. The only significant change in enzyme activity was decreased QR activity at the highest dose of quercetin ($p = 0.0393$). There was no effect on GST or CYP1A1 activity in this study, although GST also showed a decreasing trend as quercetin level increased. The viability and proliferation of the cells were not significantly affected by quercetin. More studies in this area are needed to further investigate the effect of quercetin on Phase 1 and Phase 2 enzyme activity. The concentrations of quercetin used in this study could feasibly be attained in the colonic lumen through dietary means, but they might not be effective in preventing colon cancer via the regulation of Phase 1 and Phase 2 enzyme activity.

Introduction

According to the American Cancer Society, colon cancer is the third most common cancer in both men and women (ACS, 2002b), although it receives less media attention than breast or lung cancers. In 2002, there were an estimated 148,300 new cases and 56,600 deaths due to colorectal cancer (ACS, 2002a), and experts predict 147,500 new cases of colorectal cancer and 57,100 deaths in 2003 (ACS, 2003). Genetic factors only account for 10% of colorectal cancers (Armstrong and Mathers, 2000; Go et al., 2001), so environmental factors are also involved in colon cancer development. Physical inactivity, obesity, red meat intake, smoking, alcohol intake, and low fruit and vegetable consumption are all lifestyle-related risk factors for colorectal cancer. Up to 80% of all colorectal cancer cases and deaths are attributable to diet (Bingham, 2000), and many cases of colorectal cancer and related deaths may be preventable by dietary modifications (Giovannucci and Willett, 1994).

The majority of studies in this area suggest that an inverse relationship exists between fruit and vegetable intake and the risk of colorectal cancer (Fund, 1997; Steinmetz and Potter, 1996). Individual components of plant foods are being investigated, and a new area of nutrition research involves studying the effects of phytochemicals on colon cancer risk. Phytochemicals are non-nutritive compounds produced by plants that may contribute to the health benefits of fruits and vegetables, beyond their vitamin and mineral content (Bloch, 1995).

Plants produce phytochemicals mainly for protection from herbivores, parasites, and oxidative stress, and the only way for humans to get the health benefits of these compounds is through dietary intake. The flavonoids are and most widely distributed group of polyphenols, a class of phytochemicals characterized by their phenolic ring structures, with over 5,000 unique compounds found in various fruits, vegetables, grains, nuts, teas, and wines. The flavonoids are

especially promising in the area of cancer prevention and are among the most thoroughly studied classes of phytochemicals. Several studies have shown a link between intake of specific flavonoids, including quercetin, and a reduction in colon cancer risk (Cook and Samman, 1996; Yang, 2001).

One possible mechanism by which flavonoids could prevent colon cancer is by regulation of Phase 1 and Phase 2 enzyme activity. The human body is constantly exposed to potential carcinogens through the environment, and the body deals with these compounds through a system of xenobiotic-metabolizing enzymes, the Phase 1 and Phase 2 enzymes. These enzymes work together to metabolize foreign substances, such as drugs, carcinogens, and endogenous compounds, which enter or make contact with the body. The Phase 1 enzymes (i.e. cytochrome P450) are responsible for bioactivation of xenobiotics in the body by adding a polar functional group to the original molecules, which produces reactive compounds that can bind to DNA and cause mutations. The Phase 2 enzymes (i.e. glutathione S-transferase and quinone reductase) conjugate these reactive intermediates by adding a hydrophilic functional group and making them water-soluble and more easily excreted from the body (Jones and DeLong, 2000).

Two classes of chemopreventive agents have been identified: bifunctional inducers concurrently stimulate Phase 1 and Phase 2 enzymes, while monofunctional inducers increase Phase 2 enzyme activity without affecting Phase 1 enzyme activity. Quercetin, a flavonoid, has been described as a monofunctional inducer (Henderson et al., 2000). Many of the cytochrome P450 enzymes (CYP450s) are tissue- and substrate-specific, allowing for metabolism of the numerous drugs, toxins, and possible carcinogens that the body encounters every day. The flavonoids and other phytochemicals may prevent cancer is by decreasing the activity of CYP450s, which are necessary for the detoxification of xenobiotics by the Phase 2 enzymes, but

an imbalance between the two enzyme classes can cause negative effects. Therefore, monofunctional inducers, which increase the activity of Phase 2 enzymes but not Phase 1 enzymes, may be more effective chemopreventive agents than bifunctional inducers, which increase both Phase 1 and Phase 2 enzyme activity (Manson et al., 1997). The glutathione S-transferases (GSTs) are an example of the Phase 2 enzymes, which detoxify carcinogens and promote their excretion by promoting the conjugation of electrophilic compounds with glutathione. Many studies show that flavonoids such as quercetin can stimulate GST, which may be a promising strategy for prevention of colon cancer (Hayes and Pulford, 1995). Quinone reductase (QR), another Phase 2 enzyme, works by catalyzing two-electron reductions on free radicals and toxic oxygen metabolites, which deactivates them and protects the surrounding tissues from mutagenesis and carcinogenesis. Like GST, QR can be induced by dietary components, including the flavonoids, which has been shown to have beneficial effects on cancer risk (Benson et al., 1980). Colon cancer is characterized by uncontrolled growth and proliferation of abnormal cells, so the rate of cell proliferation is important for cancer prevention. Flavonoids such as quercetin have been shown to inhibit cell proliferation in animals and human cell lines, which may be another mechanism by which these compounds protect against colon cancer (Yang et al., 2000).

Quercetin is the most widely distributed flavonoid found in the food supply, found abundantly in apples, onions, black tea, and red wine (Hollman et al., 1997). The total flavonoid intake, which is composed mostly of quercetin, has been estimated to be between 20-70 mg/day (Beecher, 2003; Manach et al., 1997). In both *in vitro* and *in vivo* studies, quercetin has demonstrated a protective role in breast (Choi et al., 2001), lung (Khanduja et al., 1999), liver (Denda et al., 1998), ovarian (Scambia, 1994), and colon cancers (Huber et al., 1997). Much

research exists on the role of quercetin in regulating Phase 1 and Phase 2 enzymes, although the results of these studies are not always consistent. Previous studies on quercetin and CYP1A1, GST, and QR activity in the colon and liver will be discussed.

Much of the research conducted on quercetin and cancer prevention has used higher concentrations than could feasibly be achieved in blood plasma by humans through dietary intake. *In vitro* studies have used concentrations of quercetin from 1-200 μM , whereas only 1 μM is typically found in plasma. Many rat studies have used diets containing 1-2% quercetin (10-20 g/kg diet), while the average intake of total flavonoids in the diet is only 25-50 mg/day. Recent studies have focused on the uptake of flavonoids, including quercetin, by human intestinal cells directly. Therefore, the debate over the concentrations of quercetin that could be achieved in the blood may not be relevant, since higher concentrations could be obtained in the lumen of the GI tract. Manach et al. (1995) fed rats a 0.5% quercetin diet and measured quercetin concentration in the plasma (100 $\mu\text{mol/L}$) and the cecum (1400 $\mu\text{mol/L}$).

The present study tested the hypothesis that quercetin treatment would monofunctionally induce the Phase 2 enzymes glutathione S-transferase and quinone reductase in the SW-480 human colon carcinoma cell line. The objectives of this study were: 1) to explore whether quercetin could mono-functionally increase the activity of the Phase 2 enzymes, GST and QR, without affecting the activity of the PI enzyme CYP1A1, 2) to determine if each enzyme activity increases in a dose response manner, and 3) to observe the effect of quercetin on cell viability and proliferation.

Methods

Cell culture, media, and experimental design

Human colon carcinoma cells (SW-480; ATCC, Rockville, MD) were grown in filtered Leibovitz's L-15 Medium with 2 mM L-glutamine (ATCC; Rockville, MD), supplemented with 10% fetal bovine serum (FBS; Equitech-Bio, Inc; Kerrville, TX). Cells were maintained in 75 cm² canted-neck flasks in 15 ml media, incubated at 37°C in air, without CO₂ (Harris Model# HWO701T-ABA, Norwalk, CT). Cells were passed at a 1:4 ratio once per week.

When cells were approximately 90% confluent at 7 days after passage, the old media was discarded, and 0, 5, 10, 20, or 40 μM quercetin-loaded media (FW = 338.3; Sigma; St. Louis, MO) was filtered and added to cell flasks. After a 48-hour incubation period, the cells were washed with Hanks' BSS solution (0.3% EDTA) and harvested using Hanks' BSS with Trypsin (0.25% Trypsin-0.3% EDTA solution). The trypsinization was stopped by adding media to form a cell suspension. For cell viability and cell proliferation assays, the cell suspension was taken directly and used in the experiment. For the enzyme activity assays, the cell suspension was prepared following the procedure described in the section below.

The cell viability study included three replicates per treatment group, for a total of 15 samples. The enzyme activity assays – glutathione S-transferase, quinone reductase, and cytochrome P450 1A1 – each included four replicates per treatment group, with all samples run in triplicate, for a total of 60 samples. The protein analysis assay included four replicates per treatment group, with all samples run in duplicate, for a total of 40 samples. The cell proliferation study included five replicates per treatment group, for each of five time points, for a total of 125 samples.

Cell Preparation for Enzyme Activity Assays

Following trypsinization and harvesting, the cell suspension was centrifuged at 750 X G for 5 minutes (Beckman T-J6; Palo Alto, CA). The supernatant was discarded. The cells were resuspended in 2 ml phosphate-buffered saline (PBS) and centrifuged again at 750 X G for 5 minutes. The cells were then disrupted by using a sonicator (Branson Sonifier 450; St. Louis, MO) for 30 seconds at 20% power. The homogenate was combined with an equal amount of homogenizing buffer and centrifuged (Beckman J2HS, JS-7.5 swinging bucket rotor; Palo Alto, CA) at 10,000 X G for 20 minutes at 4°C. The supernatant was transferred to a polycarbonyl centrifuge tube, and the weight-matched tubes were ultracentrifuged (Beckman Optima LE-80K Ultracentrifuge; Palo Alto, CA) at 100,000 X G for 1 hour and 10 minutes at 4°C. The supernatant (cytosol) was divided into 3 tubes and frozen at -80°C. The pellet (microsomes) was resuspended in homogenizing buffer, divided into 2 tubes, and frozen at -80°C until analysis.

Cell Viability

Cell viability in this study was measured using the Trypan Blue Exclusion Assay. When cells were approximately 100% confluent (~7 days after passage), three flasks per treatment group were trypsinized and harvested per usual procedure and mixed with 2 ml media for a total volume of 5 ml. Each cell suspension was aspirated 8-10 times with an electric pipettor to prepare it for counting. A 10 µl aliquot of each cell suspension was taken and mixed with 10 µl of 0.4% Trypan Blue solution (Sigma, St. Louis, MO). Directly before counting, the cell suspension was shaken gently to evenly distribute cells and ensure an accurate cell count. A 10 µl aliquot of the Trypan Blue/cell suspension mixture was placed on a hemocytometer, and

viable (unstained) and non-viable (stained) cells were counted under an Olympus ULWCD Phase Contrast microscope (Olympus America Inc; Melville, NY). The percent viable cells, or cell viability, was calculated as total viable cells / total cells (viable and unviable) x 100.

Glutathione S-Transferase Activity

Glutathione S-transferase (GST) activity was measured as described by Habig et al (1974), using 10 mM 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate. Briefly, an aliquot of cytosolic sample, potassium-phosphate buffer (0.1 mol/L), and the reduced form of glutathione (GSH; MW = 307.3) were added to each tube, vortexed and placed in the spectrophotometer. To initiate the assay, 0.1 ml CDNB was added to each tube and stirred.

The rate of change in absorbance of each sample was read at 340 nm using a Beckman DU-650 spectrophotometer (Beckman Instruments, Fullerton, CA). This assay indirectly measures enzyme activity by measuring the conjugation of CDNB with glutathione by GST. All samples were run in triplicate.

Quinone Reductase Activity

Quinone reductase activity was measured using the method of Benson et al (1980), with some modifications based on the work of Kore et al (1993). Samples of cytosol were diluted 3X with Tris HCl buffer (pH = 7.4), vortexed, and placed in a cuvette with a 1% Tween 20 stock solution, and either Dicumarol (0.6 mM) or Tris buffer (25 mM). The catalyst for the reaction, 2,6-dichlorophenolindophenol solution (12 mM), was added; the cuvette was then shaken vigorously until the mixture appeared homogeneous. The rate of change in absorbance of samples was read at 600 nm using a Beckman DU-650 spectrophotometer

(Beckman Instruments, Fullerton, CA). Samples were run in duplicate: two samples containing Tris buffer and one containing Dicumarol.

Cytochrome P450 1A1 Activity

Cytochrome P450 1A1 (CYP1A1) activity was determined as described by Thomas et al (1998). A serial dilution of resorufin (0.4 mM) was prepared from 200 nM to 0 nM. An aliquot of microsomal sample, KPO₄ buffer (0.1 mol/L), and ethoxyresorufin (0.75 mM) were added to a glass culture tube and vortexed. The tube was incubated in a water bath for 4 minutes at 37°C, while shaking at low speed. After incubation, 100 µL of NADPH solution was added, and the tube was vortexed. The sample was then quickly poured into a 3 mL cuvette and read in a spectrofluorimeter (Model RF-5301, Shimadzu, Columbia, MD). The excitation wavelength was 550 nm, and the emission wavelength was 581 nm. All samples were run in triplicate.

Protein Analysis

The amount of protein in the cells was measured by the method of Lowry et al. (1951) with bovine serum albumin (BSA; Sigma; St. Louis, MO) as a standard. Samples were read using a Beckman DU-650 spectrophotometer (Beckman Instruments, Fullerton, CA), and enzyme activities for glutathione S-transferase and quinone reductase were expressed per mg protein. All samples were run in duplicate.

Cell Proliferation

Cell proliferation was measured using the Promega CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega Corporation; Madison, WI). When the cells were

approximately 90% confluent at 7 days after passage, they were harvested via trypsinization and added to sterile microplates for incubation. Prior to our study on the effects of quercetin on cell proliferation, a pilot study was done to determine the optimum seeding density for this application. Based on the literature and our pilot study results, a seeding density of 2.5×10^4 cells/ml was used for this study.

The cell suspension was added to 25 wells/plate for each of 5 plates. One plate was used for each time period (0, 24, 48, 72, and 96 hours). Each plate contained 6 rows of 5 wells each; one for each level of quercetin being studied (0, 5, 10, 20, and 40 μM). The first row of wells in each plate contained media alone as a control. The plates were incubated at 37°C without CO₂. One plate was removed from the incubator each day at the same time. The cell proliferation reagent (Promega Corporation; Madison, WI) was added to each well, and the plates were incubated for one hour. The samples were read using a microplate reader (Dynex Technologies, Model MRX; Chantilly, VA) and Revelation Software Version 4.02 (Dynex Technologies; Chantilly, VA) at 490 nm with a reference wavelength of 630 nm.

Statistical Analysis

Treatment means, standard error of the mean, analysis of variance (ANOVA), and least significant different tests were determined using the statistical package SAS (SAS version 6.10, SAS Institute, Cary, NC). The overall effects of quercetin supplementation on glutathione S-transferase, quinone reductase, and cytochrome P450 1A1 enzyme activities, as well as cell viability, were analyzed using analysis of variance (ANOVA). The differences among the means for each treatment group were assessed using Fisher's least significant difference test. The enzyme activity data was assessed using non-parametrical analysis to test for normality, and the

data was normally distributed. The effect of quercetin on cell proliferation was analyzed using multiple regression, in order to control for the multiple time points and multiple doses. All differences with $p < 0.05$ were considered significant.

Results

Quinone Reductase Activity

Quercetin exhibited a significant effect on QR activity ($p = 0.0393$). Compared to the control group, the 5 μM quercetin treatment group was the only one in which mean QR activity was elevated above control, but this increase was not significant. As the concentration of quercetin increased from 10-40 $\mu\text{mol/L}$ quercetin, QR activity decreased gradually (**Table 2**), although post-hoc tests showed that the 40 μM treatment was the only one which was significantly different than control. Despite the lack of statistical significance, in the lower concentration ranges, the trend suggests a dose-response relationship in which increased quercetin concentration causes a decline in QR activity in SW480 cells (**Figure 4**).

Glutathione S-transferase Activity

As seen with QR activity, the 5 μM quercetin treatment increased mean GST activity above the mean control activity, while the other treatment groups (10-40 μM quercetin) decreased GST activity in a stepwise fashion (**Figure 5**). The results of this study, however, were not statistically significant ($p = 0.4804$; **Table 3**). The striking similarity to the effect of quercetin on QR activity suggests that quercetin in the 0-40 $\mu\text{mol/L}$ range may alter Phase 2 enzyme activity, but a larger study may be necessary to demonstrate this effect.

Cytochrome P450 1A1 Activity

No CYP1A1 activity was detected in this study in any of the control or treatment groups. This could be due to the absence of the enzyme in this particular cell line or an inadequate amount of sample used for the assay. In either case, we were not able to obtain any results on the effect of quercetin treatment in CYP1A1 activity.

Cell Proliferation

The effects of quercetin treatment at each time point on cellular proliferation (the number of cells) are shown in **Table 4**. Analysis of variance showed no significant effect of quercetin on cell proliferation ($p = 0.5892$). The trends in the data seen in **Figure 6** show that at most time points, the 40 μM quercetin treatment group had lower values than the control group. However, this result was not significant, and we conclude that at these levels quercetin has no effect on cellular proliferation in these cells.

Cell Viability

The results of the Trypan Blue Exclusion assay for cellular viability support the data from the cell proliferation study. No difference was seen between treatment groups in the percentage of viable cells remaining ($p = 0.3948$; **Table 5**). No evidence of toxicity to the as a result of these levels of quercetin treatment is observed from this data.

Discussion

This study was designed to test the effectiveness of quercetin as a monofunctional enzyme inducer – to increase Phase 2 enzyme activity, while decreasing or not affecting Phase 1 enzyme activity – in a human colon carcinoma cell line. The results of this study were not able to confirm this hypothesis. Quinone reductase activity was significantly decreased at the highest dose of quercetin (40 μ M), and glutathione S-transferase activity showed a similar pattern, although the results were not statistically significant. We were unable to measure cytochrome P450 1A1 activity in this model, due to either an absence of the isoform in this cell line or insufficient sample to measure enzyme activity. However, we did measure the microsomal protein in the sample, and these values appeared to be adequate compared to other similar samples that showed activity. We were able to show that quercetin does not exhibit cytotoxic effects on these cells, since there was no significant difference between treatment groups in the cell viability and cell proliferation studies. Therefore, it is unlikely that the decline in enzyme activity noted at higher concentrations of quercetin were attributable to cell death or toxicity.

The finding that quercetin significantly decreased QR activity in colon mucosa cells opposes the results of Penn (2003), who found that quercetin markedly increased QR activity in rat colon tissue, and Breinholt et al. (1999), who found that quercetin had no effect on QR activity in rat colon tissue. Both of these studies used animal models (0.1-0.9% quercetin), and the obvious differences in complexity and metabolism of *in vivo* and *in vitro* models is a possible reason for this disparity. Cell cultures are much simpler systems than living organisms, and this simplicity allows for close examination of mechanisms and structures but does not account for the many metabolic and chemical interactions that one would observe in a complete living system. In addition, the cells used in this study were of human origin, while the colon mucosa

used in the other experiments was of animal origin. Although animals are often used as a model for humans, they are distinct species and the differences between them cannot be overlooked. Another difference between these studies is the presence or absence of a carcinogen. Penn measured QR activity in animals that were not exposed to a carcinogen, and Breinholt et al. measured QR activity after the animals had been exposed to a carcinogen – 2-amino-1 methyl-6-phenylimidazo{4,5-b}pyridine – for 24 hours but did not necessarily have cancer. In contrast, the cell culture model used in this study is an advanced carcinoma, which may express different levels of these enzymes or respond to inducers in different ways.

The lack of effect on GST activity by quercetin differs from the results of Penn (2003) and Mikulcik and Fischer (2001), which showed that quercetin significantly increased GST activity in the colon tissue of rat models. Again, several differences may account for these contrasting results: 1) the type of model used (in vivo vs. in vitro), 2) the origin of the tissues used (human vs. animal), 3) the dose or concentration of quercetin given, and 4) the presence or absence of a carcinogen. In contrast, Zhang et al. (2003) found that quercetin, at the highest dose used in this study (40 μ M), inhibited GST activity in isolated human adenocarcinoma cells. Although our results were unable to show a significant decrease in GST activity as a result of quercetin treatment, the trend was a steady decrease in GST activity as quercetin concentration increased.

We were unable to measure the effect of quercetin on cytochrome P450 1A1 activity. We have not found any other studies done on this particular enzyme in this specific cell line, so there is a possibility that CYP1A1 is not expressed in the SW-480 cells. Alternatively, the cell line may express the enzyme, but there was not a sufficient amount of protein in the sample to

detect the enzyme activity. Due to limitations of time and sample volume, we were not able to have a larger sample size or replicate experiments, so this would be an area for future study.

The cell viability and cell proliferation studies both indicated that concentrations of quercetin up to 40 $\mu\text{mol/L}$ had no toxic effect on these cells. These results are similar to the results of Salucci et al. (2002), who found that concentrations of quercetin up to 70 μM had no cytotoxic effect on the Caco-2 cell line, another model of human colon carcinoma. Previous studies by Kuo (1996), Gee et al. (2002), and Luceri et al. (2002) have shown that quercetin inhibits cell proliferation of colon mucosa in both *in vitro* and *in vivo* models. However, these studies used doses of quercetin much higher than those in our study – up to 100 μM in the *in vitro* studies (compared to 40 μM in this study), and up to 50 mg/kg in the animal studies (compared to 3-9 mg/kg in other studies from our lab). These levels are much higher than what would be obtainable by diet, but these high levels may be required to achieve the desired effects on decreasing cell proliferation.

One difficulty in analyzing the large number of studies on quercetin and colon cancer development is the variety of doses used in each model system. There is not a consensus on the definition of a physiological dose of quercetin, especially with the recent research into the uptake of quercetin by the colon mucosa. Previously, the plasma levels of quercetin were used to decide which level of supplementation would be appropriate and obtainable by the diet. However, since quercetin is taken up through the colonic epithelial cells, the luminal concentration of quercetin may be relevant. Manach et al. (1995) measured the cecal contents of rats fed 16 mmol quercetin/kg body weight, and found that the circulating concentration of quercetin was 115 $\mu\text{mol/L}$. In another study, volunteers were given an oral supplement containing 500 mg of quercetin twice a day, which in a liter volume would give an intestinal concentration of 1.5 mM,

much higher than any plasma concentrations (Katske et al., 2001). Similarly, if a person were to eat an average size onion (4 oz.), which contains approximately 165 mg quercetin, assuming that all of this remains in the GI tract and arrives in the colon, the concentration of quercetin in colon would be 49 $\mu\text{mol/L}$. Although this hypothetical example is based on several assumptions, one can see that the highest dose used in our study (40 μM) is physiological in the sense that it is obtainable through the diet.

This issue of the uptake of quercetin through the colonic epithelium raises questions about the possible mechanisms by which this process occurs. *In vitro* studies have demonstrated that colon carcinoma cell lines are capable of cellular uptake of quercetin over time (Murota et al., 2002; Salucci et al., 2002). Research by Petri et al. (2003) indicate an intestinal absorption rate of 60% of the quercetin glucosides given to humans orally as onion extract (Petri et al., 2003). Human and animal studies have also shown that quercetin is transported from the lumen of the colon directly into the epithelial lining, without entering the bloodstream (Gee et al., 2000; Olthof et al., 2003; Walgren et al., 2000). Several mechanisms have been discussed in the literature: 1) the active transport of quercetin glycosides by the sodium-dependent glucose transporter (SGLT1; Wolffram et al., 2002), 2) the hydrolysis of quercetin glycosides to quercetin aglycone by lactase phlorizin hydrolase (LPH; Day et al., 2003) and subsequent passive diffusion into the intestinal mucosa, and 3) transcellular efflux of quercetin by multidrug resistance-associated protein (MRP2; Walgren et al., 2000). Each of these mechanisms may be involved in the colonic uptake of quercetin and transport into the epithelial layer, perhaps in specific parts of the colon or under unique circumstances. Future research in this area needs to be conducted to determine the primary transporter of quercetin in the colon.

There are several proposed mechanisms of action by which quercetin affects Phase 1 and Phase 2 enzyme activity. The first mechanism involves quercetin binding with specific receptors inside the cells, which can activate one of two gene promoter regions: the antioxidant response element (ARE) or the xenobiotic response element (XRE; Jaiswal, 2000). Depending on which of these regions is activated, a compound may be a monofunctional inducer (increases Phase 2 enzyme activity, and decreases or does not affect Phase 1 enzyme activity) or a bifunctional inducer (increases both Phase 1 and Phase 2 enzyme activity). A bifunctional inducer enters the cell and binds to the aryl hydrocarbon receptor (AhR), and this complex will then dimerize with the aryl hydrocarbon receptor nuclear transferase (ARNT) and enter the cell nucleus. The complex then binds to a specific region of the gene called the xenobiotic response element (XRE), which enables the transcription of proteins for both Phase 1 and Phase 2 enzymes (Rushmore and Kong, 2002). Alternatively, a monofunctional inducer bypasses the AhR and binds directly to a region of the gene called the antioxidant response element (ARE). This promoter region allows the transcription of the proteins for Phase 2 enzymes only, not Phase 1 enzymes (Guengerich, 1993).

Another mechanism by which quercetin may affect the body's response to xenobiotics is through the regulation of inflammatory agents, such as tumor necrosis factor alpha (TNF- α) and nuclear factor kappa B (NF- κ B). NF- κ B is a transcription regulation factor, which is inhibited by a protein called I kappa B. Activation by a compound (i.e. a cytokine) causes I κ B to be degraded, ceasing the inhibition of NF κ B, which enters the nucleus. NF κ B then forms a transcription complex with two proteins called p50 and p65. The complex binds to a response element in the promoter regions of many pro-inflammatory cytokines, including TNF- α . Inflammatory cytokines are associated with cancer (Abraham, 2000). The human

adenocarcinoma cell line, Caco-2, has been shown to produce TNF- α as a result of NF- κ B activation, in response to xenobiotics (Hosoi et al., 2003). Ganey and Roth (2001) suggest that these pro-inflammatory cytokines can cause cells to become more sensitive to the damaging effects of xenobiotics. This negative effect may be due to concurrent activation of Phase 1 and Phase 2 enzymes and NF- κ B in response to the presence of xenobiotics in the intestinal tract. Transcriptional activation of both CYP1A1 and NF- κ B are mediated by the AhR, so the bifunctional inducers that activate Phase 1 enzyme activity could also stimulate NF- κ B and the release of pro-inflammatory cytokines, such as TNF- α (Ciolino et al., 1999). Quercetin has been shown to inhibit TNF- α transcription, which would reduce the inflammatory response and theoretically decrease colon cancer risk (Moon et al., 2003; Wadsworth et al., 2001).

Tables

TABLE 2

Mean quinone reductase (QR) activity of SW-480 cells after quercetin (QC) treatment. ¹

| Treatment Group | Mean QR Activity (U/mg protein) |
|------------------------|--------------------------------------------|
| 0 μ M QC | 3.27 \pm 0.31 ^{a,b} |
| 5 μ M QC | 3.92 \pm 0.90 ^a |
| 10 μ M QC | 2.59 \pm 0.77 ^{a,b,c} |
| 20 μ M QC | 1.99 \pm 0.63 ^{b,c} |
| 40 μ M QC | 0.96 \pm 0.25 ^c |
| ANOVA | P = .0393 |

¹Means \pm SEM (n = 4/group); values with different superscript letters differ significantly (p<0.05).

TABLE 3

Mean glutathione S-transferase (GST) activity of SW-480 cells after quercetin (QC) treatment. ¹

| Treatment Group | Mean GST Activity (U/mg protein) |
|------------------------|---------------------------------------------|
| 0 μ M QC | 0.093 \pm 0.008 ^a |
| 5 μ M QC | 0.149 \pm 0.039 ^a |
| 10 μ M QC | 0.109 \pm 0.035 ^a |
| 20 μ M QC | 0.108 \pm 0.030 ^a |
| 40 μ M QC | 0.078 \pm 0.011 ^a |
| ANOVA | P = .4804 |

¹Means \pm SEM (n = 4/group); values with different superscript letters differ significantly (p<0.05).

TABLE 4

Cell proliferation, measured by absorbance, of live SW-480 cells during quercetin (QC) treatment over 96 hours. ¹

| Treatment Group | 0 hours (abs) | 24 hours (abs) | 48 hours (abs) | 72 hours (abs) | 96 hours (abs) |
|------------------------|----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| 0 μ M QC | .0452 | .0670 | .0970 | .0890 | .1140 |
| 5 μ M QC | .0522 | .0810 | .1180 | .0730 | .1240 |
| 10 μ M QC | .0712 | .0650 | .0960 | .0920 | .1430 |
| 20 μ M QC | .0960 | .1180 | .0760 | .1550 | .0532 |
| 40 μ M QC | .0532 | .0460 | .1120 | .0610 | .1090 |
| ANOVA | P = .5892 | | | | |

¹ Mean absorbance at each time point (n = 5/group); differences are considered significant if p < 0.5.

TABLE 5Cell viability of live SW-480 cells measured by Trypan Blue Exclusion. ¹

| Treatment Group | Mean % Cell Viability |
|------------------------|------------------------------|
| 0 μ M QC | 97.4 \pm 0.6 ^a |
| 5 μ M QC | 97.7 \pm 0.1 ^a |
| 10 μ M QC | 97.5 \pm 0.1 ^a |
| 20 μ M QC | 97.9 \pm 0.2 ^a |
| 40 μ M QC | 97.0 \pm 0.3 ^a |
| ANOVA | P = .3948 |

¹Means \pm SEM (n = 3/group), values with different superscript letters differ significantly (p<0.05).

Figures

FIGURE 4

Mean quinone reductase (QR) activity of SW-480 cells after quercetin treatment.

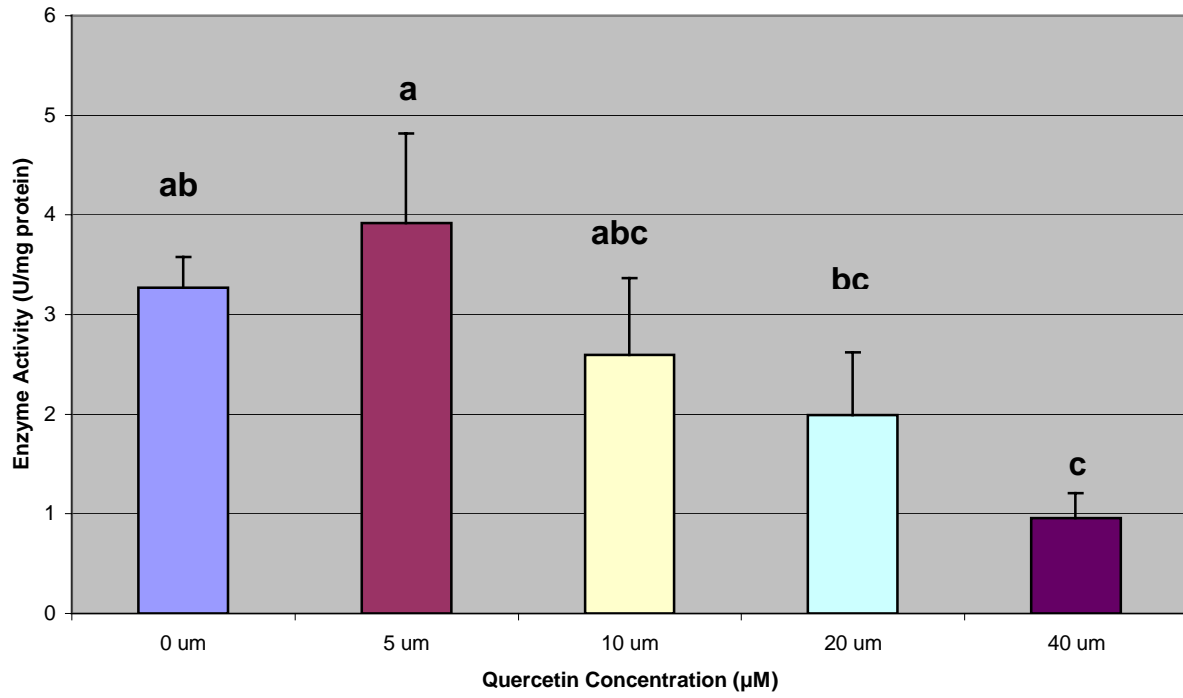


FIGURE 5

Mean Glutathione S-transferase (GST) activity of SW-480 cells after quercetin treatment.

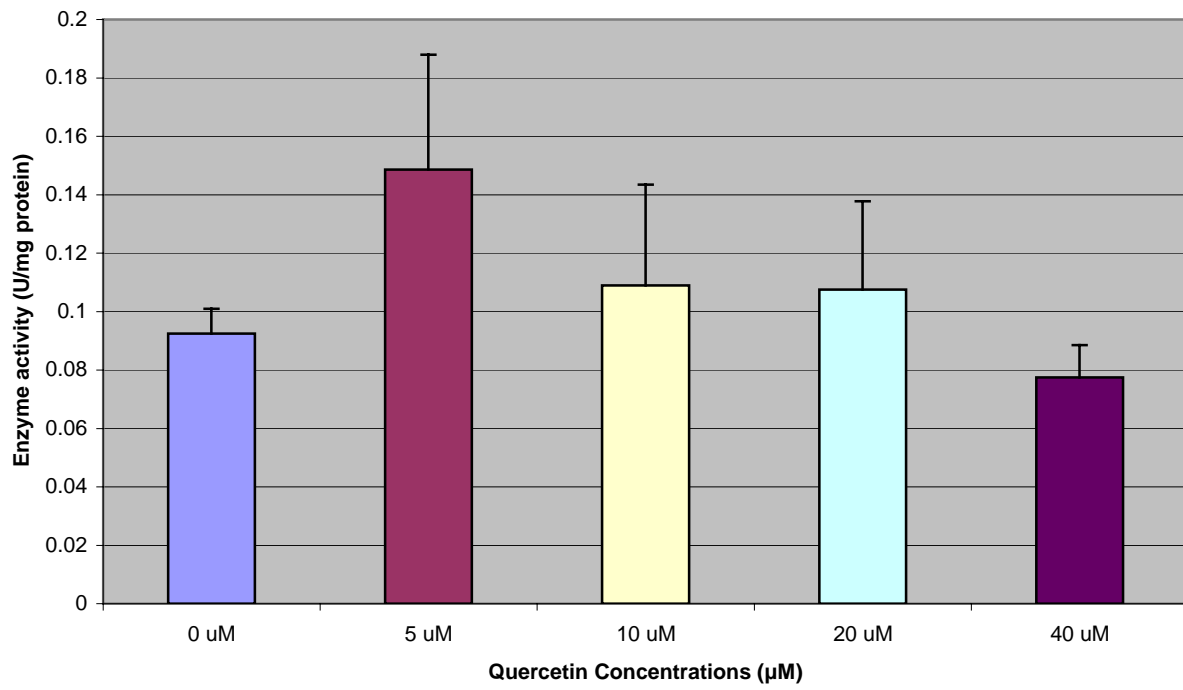
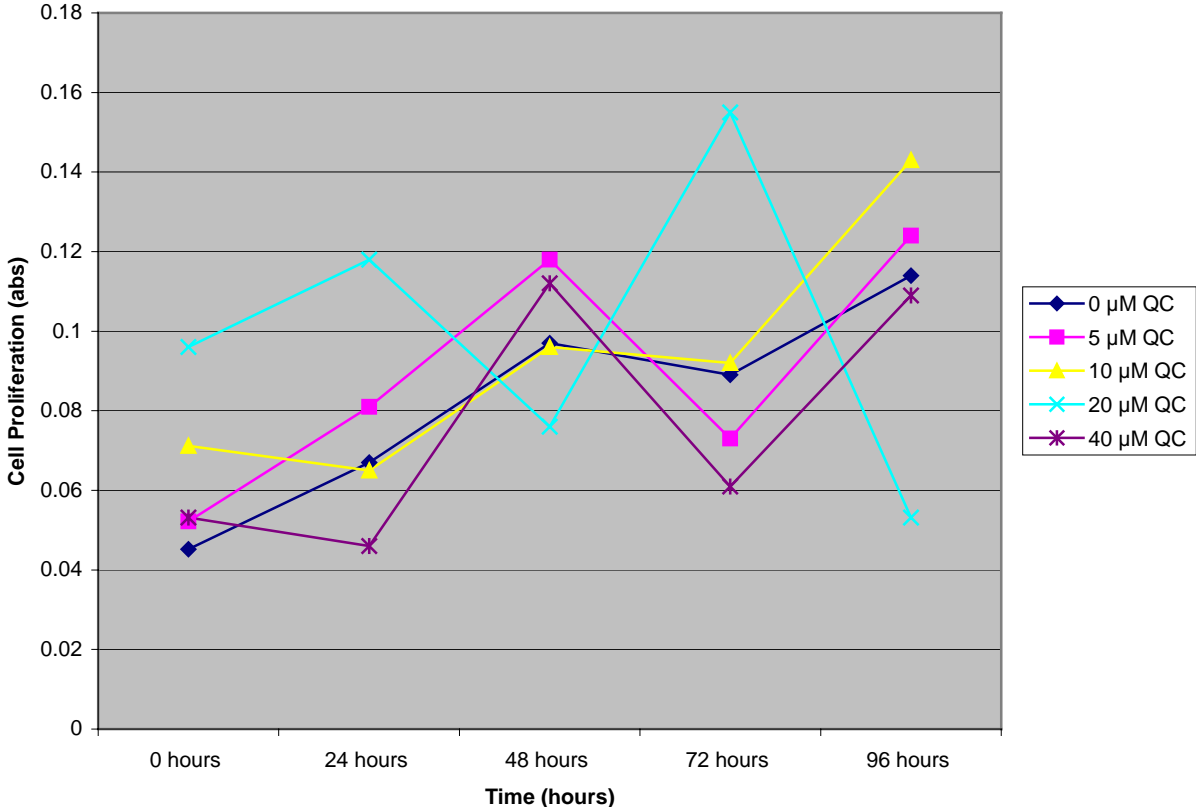


FIGURE 6

Cell proliferation, measured by absorbance, of live SW-480 cells during quercetin treatment over 96 hours.



CHAPTER 4

SUMMARY

Major Findings

The aim of this study was to test the effectiveness of the flavonoid quercetin as a monofunctional inducer of xenobiotic-metabolizing enzymes. We measured the effect of four concentrations of quercetin (5, 10, 20, and 40 μM) on Phase 1 and Phase 2 enzyme activity, along with cell proliferation and viability, in the SW-480 human colon carcinoma cell line. We found that the lowest dose slightly increased QR activity, while the higher doses systematically decreased activity, although only the highest concentration of quercetin significantly decreased QR activity. We found a similar pattern of increasing and then decreasing activity with GST, although the results from this study were not significant. We were unable to determine the effect of quercetin on Phase 1 activity, because we did not detect any CYP1A1 activity in this cell line. The cell viability and cell proliferation studies both indicated that quercetin had no effect at any of the concentrations used on cytotoxicity in this cell line. The results of this study do not support the previous research on quercetin's role as a monofunctional enzyme inducer, although these differences may be due to the form or doses of quercetin used, the *in vitro* model, the use of a human cell line, and the presence of a carcinogen.

Implications

Since the results of this study differ from those of other studies, which have shown that quercetin is an effective monofunctional inducer of Phase 1 and Phase 2 enzymes in the colon,

we must consider the reasons for this disparity. One possible reason is the model used for this study, a colon carcinoma cell line; previous research has indicated that flavonoids are classified as “blocking agents” and are more effective in chemoprevention prior to the initiation stage of carcinogenesis. In this model, the cells are already heavily mutated and poorly differentiated, so quercetin may not be as effective as in other models without a carcinogen present or other more well-differentiated tumor cell lines (i.e. Caco-2, HT-29). In addition, it is possible that the SW-480 cell line lacks part of the signaling cascade required for induction of these xenobiotic-metabolizing enzymes, such as NF- κ B or the Antioxidant Response Element. These mechanisms may be found readily in other cells and especially whole animals, which may be more appropriate models for studying the induction of these particular enzymes.

Another explanation for the outcome of this study is the use of isolated quercetin outside the context of whole foods. Epidemiological studies that have linked increased flavonoid intake with decreased risk of cancer have measured whole food intake, and one cannot expect to get the same results by extracting one compound from the food and testing that compound alone. Although the use of pure compounds is preferable for mechanistic studies, they may not be appropriate if one wishes to study the effect of regulating cancer prevention mechanisms in humans. This study also used lower doses of quercetin than were used in past research, and these doses might not be effective to produce the changes in enzyme activity and cell proliferation. The concentrations of quercetin used in this study (0-40 μ M) are more physiological than those used in previous research and could feasibly be attained in the colonic lumen through dietary means, but they might not be able to produce the magnitude of change needed to prevent colon cancer.

Limitations

This study used the SW-480 human colon carcinoma cell line as a model for the human colon, but the results from any *in vitro* study cannot be extrapolated directly to humans. Cell culture studies are a much simpler model and are appropriate for studying mechanisms, but animal and human systems are so complex that all the interactions that would occur in those models cannot be predicted from a cell culture study. In addition, we are using an isolated flavonoid, which one would never consume outside the context of a whole food. The synergistic effects that occur between flavonoids and other components of a food (proteins, fats, starches, fiber, vitamins, and minerals) may be partially responsible for the chemopreventive properties attributed to quercetin. In this simple mechanistic study, we could not observe any of these synergistic effects – only the actions of isolated quercetin. The form of quercetin used in this study also differs from that found in whole foods: we used quercetin aglycone for this study, while the quercetin in foods is generally in the glycosylated form. Although pure compounds are necessary to study mechanisms, they are not representative of the whole food and cannot be used to study dietary intake in humans. Finally, the small sample size utilized in the enzyme activity assays does not provide the statistical power to show significant differences between treatment means. We can show the trends in the data, but we had insufficient sample volume to be able to run enough replicates to show significance.

Future Research

As mentioned earlier, this study is the first *in vitro* study to use the SW-480 cell line to test the effect of physiological concentrations of quercetin on the activity of these Phase 1 and Phase 2 enzymes – GST, QR, and CYP1A1. More studies utilizing these lower concentrations of

quercetin (1-10 μM) are needed to determine if these levels are effective for chemoprevention in the colon, and if a dose-response effect on enzyme activity results. Future studies could also measure the effect of quercetin on other Phase 1 and Phase 2 enzymes, as well as make use of other colon carcinoma cell lines (i.e. Caco-2, HT-29), which are more differentiated and may be more appropriate models for this type of study. Further investigation could also focus on the metabolism and absorption of the different forms of quercetin by the colonic epithelium, to determine exactly which enzymes and transporters are involved at specific locations in the intestinal tract. It would also be interesting to conduct similar studies on enzyme activity in the colon using extracts of the whole foods, such as apples or onions, to compare to the isolated quercetin. In addition, one could study the effect of quercetin on protein and gene expression to further understand the relationship between nutrition and risk for chronic disease. The field of phytochemicals and cancer prevention is still relatively new, and much research still needs to be done before we fully understand the effects and mechanisms of these compounds.

In conclusion, the results of this study failed to confirm the hypothesis that the flavonoid quercetin acts as a monofunctional inducer of Phase 1 and Phase 2 enzymes in the SW-480 cell line. These results are contradictory to other studies that have been conducted in this area, and this may be due to the use of an in vitro model, the particular cell line used, or the doses of quercetin used. The possibility exists that the SW-480 cell line may not be the optimal model to study the effect of dietary compounds on xenobiotic-metabolizing enzymes, and it may be beneficial to compare these results with future studies utilizing other cell culture models.

REFERENCES

- Abraham, E. (2000) NF-kappaB activation. *Critical Care in Medicine* 28: N100-104.
- ACS (2002a) *Cancer Facts & Figures 2002*: 10.
- ACS (2002b) *Colorectal Cancer*: 1-4.
- ACS (2002c) *Nutrition and Physical Activity Guidelines for Cancer Prevention*.
- ACS (2003) *Cancer Facts & Figures 2003*: 10.
- Allen, S., Mueller, L., Williams, S., Quattrochi, L., & Raucy, J. (2001) The use of a high-volume screening procedure to assess the effects of dietary flavonoids on human cyp1a1 expression. *Drug Metabolism and Disposition* 29: 1074-1079.
- Anzenbacher, P., & Anzenbacherova, E. (2001) Cytochromes P450 and metabolism of xenobiotics. *Cellular and Molecular Life Sciences* 58: 737-747.
- Armstrong, F., & Mathers, J. C. (2000) Kill and cure: dietary augmentation of immune defences against colon cancer. *Proceedings of the Nutrition Society* 59: 215-220.
- Beecher, G. (2003) Overview of dietary flavonoids: nomenclature, occurrence and intake. *Journal of Nutrition* 133: 3248S-3254S.
- Belinsky, M., & Jaiswal, A. (1993) NAD(P)H:quinone oxidoreductase 1 (DT-diaphorase) expression in normal and tumor tissues. *Cancer Metastasis Reviews* 12: 103-117.
- Benson, A., Hunkeler, M., & Talalay, P. (1980) Increase of NAD(P)H:quinone reductase by dietary antioxidants: possible role in protection against carcinogenesis and toxicity. *Proceedings of the National Academy of Sciences* 77: 5216-5220.

- Bingham, S. (2000) Diet and colorectal cancer prevention. *Biochemical Society Transactions* 28: 12-16.
- Birt, D. F., Hendrich, S., & Wang, W. (2001) Dietary agents in cancer prevention: flavonoids and isoflavonoids. *Pharmacology & Therapeutics* 90: 157-177.
- Bloch, A. (1995) Position of the American Dietetic Association: Phytochemicals and Functional Foods. *The Journal of the American Dietetic Association* 95: 493-496.
- Brattain, M. G., Willson, J., Koterba, A., Patil, S., & Venkateswarlu, S. (1999) Colorectal Cancer. In J.R. Masters, & B. Palsson, eds. *Human Cell Culture: Cancer Cell Lines Part 2* Kluwer Academic Publishers, Norwell, MA.
- Bravo, L. (1998) Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition Reviews* 56: 317-333.
- Breinholt, V., Lauridsen, S., & Dragsted, L. (1999) Differential effects of dietary flavonoids on drug metabolizing and antioxidant enzymes in female rat. *Xenobiotica* 29: 1227-1240.
- Breinholt, V., Molck, A., Svendsen, G., Daneshvar, B., Vinggaard, A., Poulsen, M., & Dragsted, L. (2003) Effects of dietary antioxidants and 2-amino-3-methylimidazo[4,5-f]-quinoline (IQ) on preneoplastic lesions and on oxidative damage, hormone status, and detoxification capacity in the rat. *Food and Chemical Toxicology* 41: 1315-1323.
- Brouard, C., Siess, M., Vernevaut, M., & Suschetet, M. (1988) Comparison of the effects of feeding quercetin or flavone on hepatic and intestinal drug-metabolizing enzymes of the rat. *Food and Chemical Toxicology* 26: 99-103.
- Canivenc-Lavier, M.-C., Vernevaut, M.-F., Totis, M., Siess, M.-H., Magdalou, J., & Suschetet, M. (1996) Comparative effects of flavonoids and model inducers on drug-metabolizing enzymes in rat liver. *Toxicology* 114: 19-27.

- Carriere, V., Chambaz, M., & Rousset, M. (2001) Intestinal responses to xenobiotics. *Toxicology in Vitro* 15: 373-378.
- CDC (2003) 2003 Cancer Burden Data Fact Sheet: Georgia. Centers for Disease Control.
- Choi, J., Kim, J., Lee, J., Kang, C., Kwon, H., Yoo, Y., TW, K., Lee, Y., & Lee, S. (2001) Induction of cell cycle arrest and apoptosis in human breast cancer cells by quercetin. *International Journal of Oncology* 19: 837-844.
- Ciolino, H., Daschner, P., & Yeh, G. (1999) Dietary flavonols quercetin and kaempferol are ligands of the aryl hydrocarbon receptor that affect CYP1A1 transcription differentially. *Biochemical Journal* 340: 715-722.
- Clapper, M. L., & Szarka, C. E. (1998) Glutathione S-transferases - biomarkers of cancer risk and chemopreventive response. *Chemico-Biological Interactions* 111-112: 377-388.
- Cook, N., & Samman, S. (1996) Flavonoids - Chemistry, metabolism, cardioprotective effects, and dietary sources. *Journal of Nutritional Biochemistry* 7: 66-76.
- Day, A., Gee, J., DuPont, M., Johnson, I., & Williamson, G. (2003) Absorption of quercetin-3-glucoside and quercetin-4'-glucoside in the rat small intestine: the role of lactase phlorizin hydrolase and the sodium-dependent glucose transporter. *Biochemical Pharmacology* 65: 1199-1206.
- de Vries, J. H., Janssen, P. K., Hollman, P. C., van Staveren, W. A., & Katan, M. B. (1997) Consumption of quercetin and kaempferol in free-living subjects eating a variety of diets. *Cancer Letters* 114: 141-144.
- Denda, A., Endoh, T., Tang, Q., Tsujiuchi, T., Nakae, D., & Konishi, Y. (1998) Prevention by inhibitors of arachidonic acid cascade of liver carcinogenesis, cirrhosis and oxidative

- DNA damage caused by a choline-deficient, L-amino acid-defined diet in rats. *Mutation Research* 402: 279-288.
- Dwyer, J., Picciano, M., & Raiten, D. (2003) Food and dietary supplement databases for What We Eat in America - NHANES. *Journal of Nutrition* 133: 624S-634S.
- Engman, H. A., Lennernas, H., Taipalensuu, J., Otter, C., Leidvik, B., & Artursson, P. (2001) CYP3A4, CYP3A5, and MDR1 in human small and large intestinal cell lines suitable for drug transport studies. *Journal of Pharmaceutical Sciences* 90: 1736-1751.
- Fernandez, E., Negri, E., La Vecchia, C., & Franceschi, S. (2000) Diet diversity and colorectal cancer. *Preventive Medicine* 31: 11-14.
- Fischer, J., & Fisher, H. (2000) Supplementation with the flavonoid quercetin alters the activity of some antioxidant enzymes. *Journal of the American Dietetic Association* 100: A-11.
- Flood, A., Velie, E., Chatterjee, N., Subar, A. F., Thompson, F. E., Lacey Jr, J. V., Schairer, C., Troisi, R., & Schatzkin, A. (2002) Fruit and vegetable intakes and the risk of colorectal cancer in the Breast Cancer Detection Demonstration Project follow-up cohort. *American Journal of Clinical Nutrition* 75: 936-943.
- Fund, W. C. R. (1997) Food, nutrition, and the prevention of cancer: a global perspective. American Institute for Cancer Research, Washington, DC.
- Ganey, P., & Roth, R. (2001) Concurrent inflammation as a determinant of susceptibility to toxicity from xenobiotic agents. *Toxicology* 169: 195-208.
- Gee, J., Hara, H., & Johnson, I. (2002) Suppression of intestinal crypt cell proliferation and aberrant crypt foci by dietary quercetin in rats. *Nutrition and Cancer* 43: 193-201.

- Gee, J. M., DuPont, M. S., Day, A. J., Plumb, G. W., Williamson, G., & Johnson, I. T. (2000) Intestinal transport of quercetin glycosides in rats involves both deglycosylation and interaction with the hexose transport pathway. *Journal of Nutrition* 130: 2765-2771.
- Giovannucci, E. (2003) Diet, body weight, and colorectal cancer: a summary of the epidemiological evidence. *Journal of Womens Health* 12: 173-182.
- Giovannucci, E., & Willett, W. C. (1994) Dietary factors and risk of colon cancer. *Annals of Medicine* 26: 443-452.
- Go, V. L. W., Wong, D. A., & Butrum, R. (2001) Diet, nutrition, and cancer prevention: where are we going from here? *Journal of Nutrition* 131: 3121S-3126S.
- Gomez, S., Le, G., Clarke, C., Glaser, S., France, A., & West, D. (2003) Cancer incidence patterns in Koreans in the US and in Kangwha, South Korea. *Cancer Causes Control* 14: 167-174.
- Guengerich, F. P. (1992) Characterization of human cytochrome P450 enzymes. *The FASEB Journal* 6: 745-748.
- Guengerich, F. P. (1993) Cytochrome P450 enzymes. *American Scientist* 81: 440-448.
- Habig, W., Pabst, M., & Jakoby, W. (1974) Glutathione-s-transferase: the first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry* 249: 7130-7139.
- Hayes, J. D., & Pulford, D. (1995) The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprevention and drug resistance. *Critical Reviews in Biochemistry and Molecular Biology* 30: 445-600.
- He, Y., Friesen, M., Ruch, R., & Schut, H. (2000) Indole-3-carbinol as a chemopreventive agent in 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) carcinogenesis: inhibition of

- PhIP-DNA adduct formation, acceleration of PhIP metabolism, and induction of cytochrome P450 in female F344 rats. *Food and Chemical Toxicology* 38: 15-23.
- Henderson, C. J., Sahraoui, A., & Wolf, C. R. (2000) Cytochrome P450s and chemoprevention. *Biochemical Society Transactions* 28: 42-46.
- Hertog, M., Hollman, P. C., Katan, M. B., & Kromhout, D. (1993) Intake of potentially anticarcinogenic flavonoids and their determinants by adults in The Netherlands. *Nutrition and Cancer* 20: 21-29.
- Hollman, P. C., de Vries, J. H., van Leeuwen, S., Mengelers, M. J., & Katan, M. B. (1995) Absorption of dietary quercetin glycosides and quercetin in healthy ileostomy volunteers. *American Journal of Clinical Nutrition* 62: 1276-1282.
- Hollman, P. C., van Trijp, J. M., Mengelers, M. J., de Vries, J. H., & Katan, M. B. (1997) Bioavailability of the dietary antioxidant flavonol quercetin in man. *Cancer Letters* 114: 139-140.
- Hosoi, T., Hirose, R., Saegusa, S., Ametani, A., Kiuchi, K., & Kaminogawa, S. (2003) Cytokine responses of human intestinal epithelial-like Caco-2 cells to the nonpathogenic bacterium *Bacillus subtilis* (natto). *International Journal of Food Microbiology* 82: 255-264.
- Huber, W., McDaniel, L., Kaderlik, K., Teitel, C., Lang, N., & Kadlubar, F. (1997) Chemoprotection against the formation of colon DNA adducts from the food-borne carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) in the rat. *Mutation Research* 376: 115-122.
- Izuishi, K., Kato, K., Ogura, T., Kinoshita, T., & Esumi, H. (2000) Remarkable tolerance of tumor cells to nutrient deprivation: possible new biochemical target for cancer therapy. *Cancer Research* 60: 6201-6207.

- Jaiswal, A. (2000) Regulation of genes encoding NAD(P)H: Quinone oxidoreductases. *Free Radical Biology and Medicine* 29: 254-262.
- Jones, D. P., & DeLong, M. J. (2000) Detoxification and Protective Functions of Nutrients. In M.H. Stipanuk, ed. *Biochemical and Physiological Aspects of Human Nutrition* W.B. Saunders Company, Philadelphia, PA.
- Kantola, T., Kivisto, K., & Neuvonen, P. (1998) Grapefruit juice greatly increases serum concentrations of lovastatin and lovastatin acid. *Clinical Pharmacology and Therapeutics* 63: 397-402.
- Katske, F., Shoskes, D., Sender, M., Poliakin, R., Gagliano, K., & Rajfer, J. (2001) Treatment of interstitial cystitis with a quercetin supplement. *Techniques in Urology* 7: 44-46.
- Khanduja, K., Gandhi, R., Pathania, V., & Syal, N. (1999) Prevention of N-nitrosodiethylamine-induced lung tumorigenesis by ellagic acid and quercetin in mice. *Food and Chemical Toxicology* 37: 313-318.
- Kiss, I., Sandor, J., Pajkos, G., Bogner, B., Hegedus, G., & Ember, I. (2000) Colorectal cancer risk in relation to genetic polymorphism of cytochrome P450 1A1, 2E1, and glutathione S-transferase M1 enzymes. *Anticancer Research* 20: 519-522.
- Knekt, P., Jarvinen, R., Seppanen, R., Helleovaara, M., Teppo, L., Pukkala, E., & Aromaa, A. (1997) Dietary flavonoids and the risk of lung cancer and other malignant neoplasms. *American Journal of Epidemiology* 146: 223-230.
- Kore, A., Jeffery, E., & Wallig, M. (1993) Effects of 1-isothiocyanato-3-(methylsulfinyl)propane on xenobiotic metabolizing enzymes in rats. *Food and Chemical Toxicology* 31: 721-729.

- Krishnan, K., Ruffin IV, M. T., & Brenner, D. E. (2000) Chemoprevention for colorectal cancer. *Critical Reviews in Oncology/Hematology* 33: 199-219.
- Kuo, S. (1996) Antiproliferative potency of structurally distinct dietary flavonoids on human colon cancer cells. *Cancer Letters* 110: 41-48.
- Leibovitz, A., Stinson, J., McCombs, W. r., McCoy, C., Mazur, K., & Mabry, N. (1976) Classification of human colorectal adenocarcinoma cell lines. *Cancer Research* 36: 4562-4569.
- Lowry, O., Rosebrough, N., Farr, A., & Randall, R. (1951) Protein measurement with the Folin phenol reagent. *Journal of Biochemistry* 193: 265-275.
- Luceri, C., Caderni, G., Sanna, A., & Dolara, P. (2002) Red wine and black tea polyphenols modulate the expression of cyclooxygenase-2, inducible nitric oxide synthase and glutathione-related enzymes in azoxymethane-induced F344 rat colon tumors. *Journal of Nutrition* 132: 1376-1379.
- Manach, C., Morand, C., Demigne, C., Texier, O., Regerat, F., & Remesy, C. (1997) Bioavailability of rutin and quercetin in rats. *FEBS Letters* 409: 12-16.
- Manach, C., Morand, C., Texier, O., Favier, M.-L., Agullo, G., Demigne, C., Regerat, F., & Remesy, C. (1995) Quercetin metabolites in plasma of rats fed diets containing rutin or quercetin. *Journal of Nutrition* 125: 1911-1922.
- Manson, M. M., Ball, H. W., Barrett, M. C., Clark, H. L., Judah, D. J., Williamson, G., & Neal, G. E. (1997) Mechanism of action of dietary chemoprotective agents in rat liver: induction of phase I and II drug metabolizing enzymes and aflatoxin B1 metabolism. *Carcinogenesis* 18: 1729-1738.

- Masimirembwa, C., Otter, C., Berg, M., Jonsson, M., Leidvik, B., Jonsson, E., Johansson, T., Backman, A., Edlund, A., & Andersson, T. (1999) Heterologous expression and kinetic characterization of human cytochromes P-450: validation of a pharmaceutical tool for drug metabolism research. *Drug Metabolism and Disposition* 27: 1117-1122.
- Mayer Jr, W. B. (2001) The Effects of Iron and Quercetin on Apoptosis in a Human Hepatoma Cell Line. *Foods and Nutrition*. University of Georgia, Athens, GA.
- McKinnon, R. A., Burgess, W. M., Hall, P. M., Roberts-Thomson, S. J., Gonzalez, F. J., & McManus, M. E. (1995) Characterization of CYP3A gene subfamily expression in human gastrointestinal tissues. *Gut* 36: 259-267.
- Meunier, V., Bourrie, M., Berger, Y., & Fabre, G. (1995) The human intestinal epithelial cell line Caco-2; pharmacological and pharmacokinetic applications. *Cell Biology and Toxicology* 11: 187-194.
- Michels, K. B., Giovannucci, E., Joshipura, K. J., Rosner, B. A., Stampfer, M. J., Fuchs, C. S., Colditz, G. A., Speizer, F. E., & Willett, W. C. (2000) Prospective Study of Fruit and Vegetable Consumption and Incidence of Colon and Rectal Cancers. *Journal of the National Cancer Institute* 92: 1740-1752.
- Mikami, K., Naito, M., Ishiguro, T., Yano, H., Tomida, A., Yamada, T., Tanaka, N., Shirakusa, T., & Tsuruo, T. (1998) Immunological quantitation of DT-diaphorase in carcinoma cell lines and clinical colon cancers: advanced tumors express greater levels of DT-diaphorase. *Japanese Journal of Cancer Research* 89: 910-915.
- Mikulcik, E., & Fischer, J. (2001) Possible mechanism for protective effect of the flavonoid quercetin. *Journal of the American Dietetic Association* 101: A-35.

- Moon, S., Cho, G., Jung, S., Gai, S., Kwon, T., Lee, Y., Madamanchi, N., & Kim, C. (2003) Quercetin exerts multiple inhibitory effects on vascular smooth muscle cells: role of ERK1/2, cell cycle regulation, and matrix metalloproteinases. *Biochemical and Biophysical Research Communications* 301: 1069-1078.
- Murota, K., Shimizu, S., Chujo, H., Moon, J., & Terao, J. (2000) Efficiency of absorption and metabolic conversion of quercetin and its glucosides in human intestinal cell line Caco-2. *Archives of Biochemistry and Biophysics* 384: 391-397.
- Murota, K., Shimizu, S., Miyamoto, S., Izumi, T., Obata, A., Kikuchi, M., & Terao, J. (2002) Unique uptake and transport of isoflavone aglycones by human intestinal Caco-2 cells: comparison of isoflavonoids and flavonoids. *Journal of Nutrition* 132: 1956-1961.
- Musonda, C., Helsby, N., & Chipman, J. (1997) Effects of quercetin on drug metabolizing enzymes and oxidation of 2',7-dichlorofluorescein in HepG2 cells. *Human Experimental Toxicology* 16: 700-708.
- Nijveldt, R. J., van Nood, E., van Hoorn, D. E., Boelens, P. G., van Norren, K., & van Leeuwen, P. A. (2001) Flavonoids: a review of probable mechanisms of action and potential applications. *American Journal of Clinical Nutrition* 74.
- Olthof, M. R., Hollman, P. C., Buijsman, M. N., van Amelsvoort, J. M., & Katan, M. B. (2003) Chlorogenic acid, quercetin-3-rutinoside, and black tea phenols are extensively metabolized in humans. *Journal of Nutrition* 133: 1806-1814.
- Olthof, M. R., Hollman, P. C., Vree, T. B., & Katan, M. B. (2000) Bioavailabilites of quercetin-3-glucoside and quercetin-4'-glucoside do not differ in humans. *Journal of Nutrition* 130: 1200-1203.

- Park, J.-G., Yang, H.-K., Hay, R. J., & Gazdar, A. (1994) Colorectal Cancer Cell Lines. In R.J. Hay, J.-G. Park, & A. Gazdar, eds. Atlas of Human Tumor Cell Lines Academic Press, Inc., San Diego, CA.
- Penn, D. (2003) Effects of the phytochemicals quercetin and genistein on the phase 1 and phase 2 enzyme activities. Department of Foods and Nutrition. The University of Georgia, Athens, GA.
- Petri, N., Tannergren, C., Holst, B., Mellon, F., Bao, Y., Plumb, G., Bacon, J., O'Leary, K., Kroon, P., Knutson, L., Forsell, P., Eriksson, T., Lennernas, H., & Williamson, G. (2003) Absorption/metabolism of sulforaphane and quercetin, and regulation of phase ii enzymes, in human jejunum in vivo. *Drug Metabolism and Disposition* 31: 805-813.
- Piskula, M., & Terao, J. (1998) Quercetin's solubility affects its accumulation in rat plasma after oral administration. *Journal of Agricultural and Food Chemistry* 46: 4313-4317.
- Rushmore, T., & Kong, A. (2002) Pharmacogenomics, regulation and signaling pathways of phase I and II drug metabolizing enzymes. *Current Drug Metabolism* 3: 481-490.
- Salucci, M., Stivala, L., Maiani, G., Bugianesi, R., & Vannini, V. (2002) Flavonoids uptake and their effect on cell cycle of human colon adenocarcinoma cells (Caco2). *British Journal of Cancer* 86: 1645-1651.
- Sampson, L., Rimm, E., Hollman, P. C., de Vries, J. H., & Katan, M. B. (2002) Flavonol and flavone intakes in US health professionals. *Journal of the American Dietetic Association* 102: 1414-1420.
- Scalbert, A., & Williamson, G. (2000) Dietary intake and bioavailability of polyphenols. *Journal of Nutrition* 130: 2073S-2085S.

- Scambia, G. (1994) Quercetin enhances transforming growth factor beta 1 secretion by human ovarian cancer cells. *International Journal of Cancer* 57: 211-215.
- Siess, M., Leclerc, J., Canivenc-Lavier, M., Rat, P., & Suschetet, M. (1995) Heterogeneous effects of natural flavonoids on monooxygenase activities in human and rat liver microsomes. *Toxicology and Applied Pharmacology* 130: 73-78.
- Siess, M.-H., Le Bon, A.-M., Canivenc-Lavier, M.-C., & Suschetet, M. (2000) Mechanisms involved in the chemoprevention of flavonoids. *BioFactors* 12: 193-199.
- Slattery, M. L., Boucher, K., Caan, B. J., Potter, J. D., & Ma, K.-N. (1998) Eating patterns and risk of colon cancer. *American Journal of Epidemiology* 148: 4-16.
- Slattery, M. L., & Caan, B. J. (2001) Nutrition and Colon Cancer - Chapter 23. In A.M. Coulston, C.L. Rock, & E.R. Mosen, eds. *Nutrition in the Prevention and Treatment of Disease* Academic Press, Inc., San Diego, CA.
- Sousa, R., & Marletta, M. (1985) Inhibition of cytochrome P-450 activity in rat liver microsomes by the naturally occurring flavonoid, quercetin. *Archives of Biochemistry and Biophysics* 240: 345-357.
- Steinmetz, K., & Potter, J. D. (1996) Vegetables, fruit, and cancer prevention: a review. *Journal of the American Dietetic Association* 96: 1027-1039.
- Sutoh, I., Kohno, H., Nakashima, Y., Hishikawa, Y., Tabara, H., Tachibana, M., Kubota, H., & Nagasue, N. (2000) Concurrent expressions of metallothionein, glutathione S-transferase-pi, and P-glycoprotein in colorectal cancers. *Diseases of the Colon and Rectum* 43: 221-232.
- Talalay, P., Fahey, J. W., Holtzclaw, W. D., Prester, T., & Zhang, Y. (1995) Chemoprotection against cancer by Phase 2 enzyme induction. *Toxicology Letters* 82/83: 173-179.

- Tan, W., Lin, D., Xiao, Y., Kadlubar, F., & Chen, J. (1999) Chemoprevention of 2-amino-1-methyl-6-phenyl-midazo 4,5-b pyridine-induced carcinogen-DNA adducts by Chinese Cabbage in rats. *World Journal of Gastroenterology* 5: 138-142.
- Thomas, K., Waxman, C., & Waxman, D. (1998) Enzymatic analysis of cDNA-expressed human CYP1A1, CYP1A2, and CYP 1B1 with 7-ethoxyresorufin as substrate. In I. Phillips, & E. Shepard, eds. *Cytochrome P450 Protocols. (Methods in Molecular Biology.)* Humana Press, Clifton, NJ.
- Uda, Y., Price, K. R., Williamson, G., & Rhodes, M. J. C. (1997) Induction of the anticarcinogenic marker enzyme, quinone reductase, in murine hepatoma cells in vitro by flavonoids. *Cancer Letters* 120: 213-216.
- Valerio, L., Kepa, J., Pickwell, G., & Quattrochi, L. (2001) Induction of human NAD(P)H:quinone oxidoreductase (NQO1) gene expression by the flavonol quercetin. *Toxicology Letters* 119: 49-57.
- Voorrips, L. E., Goldbohm, R. A., van Poppel, G., Sturmans, F., Hermus, R. J. J., & van den Brandt, P. A. (2000) Vegetable and Fruit Consumption and Risks of Colon and Rectal Cancer in a Prospective Cohort Study. *American Journal of Epidemiology* 152: 1081-1092.
- Wadsworth, T., McDonald, T., & Koop, D. (2001) Effects of Gingko biloba extract (EGb 761) and quercetin on lipopolysaccharide-induced signaling pathways involved in the release of tumor necrosis factor-alpha. *Biochemical Pharmacology* 62: 963-974.
- Walgren, R., Lin, J., Kinne, R., & Walle, T. (2000) Cellular uptake of dietary flavonoid quercetin 4'-beta-glucoside by sodium dependent glucose transporter SGLT1. *Pharmacology and Experimental Therapeutics* 55: 1721-1727.

- Walgren, R., Walle, U. K., & Walle, T. (1998) Transport of quercetin and its glucosides across human intestinal epithelial Caco-2 cells. *Biochemical Pharmacology* 55: 1721-1727.
- Walle, T., Otake, Y., Walle, U. K., & Wilson, F. A. (2000) Quercetin glycosides are completely hydrolyzed in ileostomy patients before absorption. *Journal of Nutrition* 130.
- Wang, W., Heideman, L., Chung, C., Pelling, J., Koehler, K., & Birt, D. (2000) Cell-cycle arrest at G2/M and growth inhibition by apigenin in human carcinoma cell lines. *Molecular Carcinogenesis* 28: 102-110.
- Wang, W., & Higuchi, C. M. (1995) Induction of NAD(P)H:quinone reductase by vitamins A, E, and C in Colo205 colon cancer cells. *Cancer Letters* 98: 63-69.
- Wattenberg, L. (1997) An overview of chemoprevention: current status and future prospects. *Proceedings of the Society of Experimental Biology in Medicine* 216: 133-141.
- Williamson, G., Plumb, G. W., Uda, Y., Price, K. R., & Rhodes, M. J. (1996) Dietary quercetin glycosides: antioxidant activity and induction of the anticarcinogenic phase II marker enzyme quinone reductase in Hepalclc7 cells. *Carcinogenesis* 17: 2385-2387.
- Wolffram, S., Block, M., & Ader, P. (2002) Quercetin-3-glucoside is transported by the glucose carrier SGLT1 across the brush border membrane of rat small intestine. *Journal of Nutrition* 132: 630-635.
- Yang, C. S., Brady, J. F., & Hong, J.-Y. (1992) Dietary effects on cytochrome P450, xenobiotic metabolism, and toxicity. *The FASEB Journal* 6: 737-744.
- Yang, C. S., Landau, Janelle M., Huang, Mou-Tuan, & Newmark, Harold L. (2001) Inhibition of carcinogenesis by dietary polyphenolic compounds. *Annual Reviews of Nutrition* 21: 381-406.

- Yang, K., Lamprecht, S., Liu, Y., Shinozaki, H., Fan, K., Leung, D., Newmark, H., Steele, V., Kelloff, G., & Lipkin, M. (2000) Chemoprevention studies of the flavonoids quercetin and rutin in normal and azoxymethane-treated mouse colon. *Carcinogenesis* 21: 1655-1660.
- Yannai, S., Day, A., Williamson, G., & Rhodes, M. (1998) Characterization of flavonoids as monofunctional or bifunctional inducers of quinone reductase in murine hepatoma cell lines. *Food and Chemical Toxicology* 36: 623-630.
- Zhang, H., Ahmadi, A., Arbman, G., Zdolsek, J., Carstensen, J., Nordenskjold, B., Soderkvist, K., & Sun, X. (1999) Glutathione S-transferase T1 and M1 genotypes in normal mucosa, transitional mucosa, and colorectal adenocarcinoma. *International Journal of Cancer* 84: 135-138.
- Zhang, K., & Wong, K. P. (1997) Glutathione conjugation of chlorambucil: measurement and modulation by plant polyphenols. *The Biochemical Journal* 325: 417-422.
- Zhang, K., Wong, K. P., & Chow, P. (2003) Conjugation of chlorambucil with GSH by GST purified from human colon adenocarcinoma cells and its inhibition by plant polyphenols. *Life Science* 72: 2629-2640.