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Quercetin Suppresses Early Colon Carcinogenesis Partly through Inhibition of Inflammatory Mediators

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Abstract

We have demonstrated that 0.45% quercetin added to a diet containing corn oil (15% w/w), as the lipid source, and cellulose (6% w/w), as the fiber source, was able to suppress the formation of high multiplicity aberrant crypt foci (ACF > 4 AC/focus), to lower proliferation and enhance apoptosis in a rat model of colon cancer. This experiment determined whether quercetin was acting as an antiinflammatory molecule in an in vivo model of colon cancer. We used weanling (21 d old) Sprague Dawley rats (n = 40) in a 2×2 factorial experiment to determine the influence of quercetin on iNOS, COX-1 and COX-2 expressions, all of which are elevated in colon cancer. Half of the rats received a diet containing either 0 or 0.45% quercetin, and within each diet group, half of the rats were injected with saline or azoxymethane (AOM, 15 mg/kg BW, sc, 2× during wk 3 and 4). The colon was resected 4 wk after the last AOM injection, and the mucosa scraped and processed for RNA isolation. Data from this experiment were analyzed using a mixed model in SAS for main effects and their interaction. AOM injection stimulated ($P < 0.0001$) iNOS expression. However there was an interaction such that, relative to rats injected with saline, AOM-injected rats consuming diets without quercetin had significantly elevated iNOS expression (5.29-fold), but the expression in AOM-injected rats consuming the diet with quercetin was not significantly elevated (1.68-fold). COX-1 expression was 20.2% lower ($P < 0.06$) in rats consuming diets containing quercetin. COX-2 expression was 24.3% higher ($P < 0.058$) in rats consuming diets without quercetin. These data suggest inflammatory processes are elevated in this early stage of colon carcinogenesis, yet quercetin may protect against colon carcinogenesis by down-regulating the expressions of COX-1 and COX-2.

Keywords

iNOS; COX-1; COX-2; aberrant crypt foci

INTRODUCTION

Experimental data suggest that diets rich in fruits and vegetables protect against chronic diseases, such as cancer (Wargovich, 1997). However, human cohort (Koushik et al., 2007) or prospective (McCullough et al., 2003) studies have demonstrated limited benefits of fruit and vegetable consumption for colon cancer prevention. Yet, in each of these human trials, a similar conclusion was made that those individuals consuming the lowest quantities of fruits and vegetables were at the greatest risk for colon cancer. Within the prospective study by McCullough et al. (2003), data suggest that plants within certain phytochemical subgroups may provide more protection than others.

As we continue to document the health benefits of consuming diets containing higher quantities of fruits and vegetables, it is imperative that we address which of these foods are protective, and thus, what compounds present in them are helping to maintain health and through which mechanisms they confer the protection. Hallmarks of dietary compounds that effectively suppress colon cancer include an ability to alter cell cytokinetics, preferably by both inhibiting proliferation and decreasing apoptosis (Chang et al., 1997; Hong et al., 2001). In addition, the current literature suggests that suppression of inflammation in the colon is a key pathway whereby the risk of cancer development can be suppressed (Karin, 2006).

Quercetin is a flavonoid present in many plant sources at levels sufficient so that it is considered to be the major flavonol present in the human diet (Yang et al., 2001). We (Warren et al., 2003) and others (Volate et al., 2005) have demonstrated the ability of quercetin to suppress the formation of early neoplastic lesions of colon cancer (aberrant crypt foci, ACF) through an inhibition of proliferation and apoptosis. However, the mechanism whereby colon epithelial cell cytokinetics is modified by quercetin remains to be completely defined. The question addressed by the current research was whether quercetin may alter colonocyte proliferation and apoptosis through its ability to suppress the expression of inflammatory mediators, including COX-1, COX-2, and iNOS. The level and activity of these enzymes are typically upregulated in colon cancer (Kawanishi et al., 2006; Corona et al., 2007) and has been documented to be suppressed by other dietary bioactive (e.g., naringin) molecules (Vanamala et al., 2006).

MATERIALS AND METHODS

Sprague-Dawley rats (male, weanling, n = 40) were individually housed in a temperature and humidity controlled room, which provided a 12h light/dark cycle. The experiment used a 2×2 experimental design of treatments including 0 or 0.45% quercetin in the diet and either an injection of saline (control) or azoxymethane (AOM, 15 mg/kg body weight, sc, 2×, wk 3 and 4). Control diets contained 6 g/100 g cellulose, 15 g/100 g corn oil, 22 g/100 g casein, 51 g/100 g dextrose and a complete vitamin-mineral mix (Harlan Teklad, Madison, WI). Quercetin diets were prepared by replacing an equivalent mass of dextrose for quercetin dihydrate (Sigma, St. Louis, MO). Quercetin levels were confirmed by HPLC analysis (Patil et al., 1995). Rats were assigned to the treatment groups such that the initial weights were similar.

Four weeks after the second AOM injection, rats were terminated, and samples for PCR analyses were collected in which a glass slide was used to scrape the mucosal layer and samples were placed into a denaturation solution (Totally RNA kit; Ambion, Austin, TX). The samples were then homogenized on ice using a Teflon-in-glass homogenizer prior to being frozen at -80°C until the RNA was isolated using a Totally RNA kit followed by treatment with DNase (DNA Free; Ambion). Prior to performing PCR analysis, all samples were tested for quality using an Agilent 2100 Bioanalyzer (Agilent, Palo Alto, CA). PCR reactions for COX-1, COX-2 and iNOS were performed using commercially available primer sets (Applied Biosystems, Foster City, CA) or with designed primers and SYBR green master mix. Expression was normalized to 18S levels (Davidson et al., 2004).

Data were analyzed using the Proc Mixed procedure of SAS to determine the effect of diet, carcinogen treatment and their interaction on gene expression. Data are reported as Least Squares Means with differences detected using Fisher's LSD.

RESULTS

Diets contained 0.435% quercetin, which was close to the desired level (0.45%). There were no differences in intake or weight gain among any of the treatments.

To determine whether signaling through the pro-inflammatory pathways was enhanced by carcinogen injection and whether dietary quercetin is able to blunt that response, we examined the expression of COX-1, COX-2, and iNOS. We found no enhancement in COX-1 expression in response to the carcinogen. However, there was a 20.2% reduction ($P < 0.06$) in COX-1 expression in rats consuming diets containing quercetin. We next determined the effect of carcinogen and dietary quercetin on COX-2 expression. COX-2 is a pro-inflammatory enzyme found greatly upregulated in colon tumors (Takeda et al., 2003). At this early time point in the rat model of colon cancer (prior to tumor formation), we found no effect of carcinogen on COX-2 expression. However, similar to COX-1 expression, there was a 24.3% elevation ($P < 0.06$) in COX-2 expression in colonocytes from rats consuming diets without quercetin. The expression of iNOS is considered to promote carcinogenesis, as it is routinely noted in colon tumors (Ambs et al., 1998). AOM strongly upregulated iNOS expression, which was observed within four weeks of the last injection. Unlike COX-1 and COX-2 expression, quercetin had no effect on the iNOS mRNA levels in scraped mucosa from the colon.

DISCUSSION

A diet rich in plant-based foods that contains a variety of biologically active molecules, such as quercetin, may suppress carcinogenesis through a variety of mechanisms (Terry et al., 2001; Yang et al., 2001). It is because of the multistep nature of colon carcinogenesis that there are opportunities to influence tumor progression through diet modification (Bruce et al., 2000). We have previously demonstrated that including 0.45% quercetin in the diet of rats will suppress formation of high multiplicity ACF, suppress proliferation and enhance apoptosis when cancer has been induced by injection of azoxymethane (Warren et al., 2003). In this study, we evaluated whether these effects were observed because of quercetin's ability to influence the increases in pro-inflammatory molecules that are known to occur in colon tumors. Takeda et al. (2003) demonstrated early polyp growth that leads to subsequent increases in COX-2 expression is dependent upon COX-1 expression. Our results suggest that dietary quercetin is able to suppress the levels of both COX-1 and COX-2 expression at this very early stage of colon carcinogenesis. Although quercetin had no effect on expression of iNOS, it is possible that quercetin may suppress the promotion of colon carcinogenesis through its ability to lower pro-inflammatory cyclooxygenase gene expression in vivo.

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Fig. 1. Dietary quercetin reduced ($P < 0.06$) expression of COX-1 by 20.2%. Values are means of saline and AOM-injected rats ($n = 20/\text{diet}$).

Fig. 2.

Rats consuming diets without quercetin had elevated ($P < 0.058$) COX-2 expression (24.3%). Values are means of saline and AOM-injected rats ($n = 20/\text{diet}$).

Fig. 3. Carcinogen (azoxymethane, AOM) injection increased ($P < 0.01$) iNOS expression by 5.29-fold and 1.68-fold for the control and quercetin diets, respectively, compared to the saline injected rats consuming the same diets. Values are fold changes in AOM-injected rats ($n = 10/\text{diet}$), relative to the saline-injected rats.