Effects of Daily Oral Administration of Quercetin Chalcone and Modified Citrus Pectin on Implanted Colon-25 Tumor Growth in Balb-c Mice

Adam Hayashi, MS, Aric C. Gillen, MS, and James R. Lott, PhD

Abstract

The health benefits of fruits and vegetables have been the subject of numerous investigations over many years. Two natural substances, quercetin (a flavonoid) and citrus pectin (a polysaccharide found in the cell wall of plants) are of particular interest to cancer researchers. Two modified versions of these substances – quercetin chalcone (QC) and a pH-modified citrus pectin (MCP) – are the focus of this study. Previous research has confirmed that quercetin exhibits antitumor properties, likely due to immune stimulation, free radical scavenging, alteration of the mitotic cycle in tumor cells, gene expression modification, anti-angiogenesis activity, or apoptosis induction, or a combination of these effects. MCP has inhibited metastases in animal studies of prostate cancer and melanoma. To date, no study has demonstrated a reduction in solid tumor growth with MCP, and there is no research into the antitumor effect of QC. This study examines the effects of MCP and QC on the size and weight of colon-25 tumors implanted in balb-c mice.

Fifty mice were orally administered either 1 mL distilled water (controls), low-dose QC (0.8 mg/mL), high-dose QC (1.6 mg/mL), low-dose MCP (0.8 mg/mL) or high-dose MCP (1.6 mg/mL) on a daily basis, beginning the first day of tumor palpation (usually eight days post-implantation). A significant reduction in tumor size was noted at day 20 in all groups compared to controls. The groups given low-dose QC and MCP had a 29-percent (NS) and 38-percent ($p<0.02$) decrease in size, respectively. The high-dose groups had an even more impressive reduction in size; 65 percent in the QC group and 70 percent in the mice given MCP (both $p<0.001$).

This is the first evidence that MCP can reduce the growth of solid primary tumors, and the first research showing QC has antitumor activity. Additional research on these substances and their effect on human cancers is warranted.

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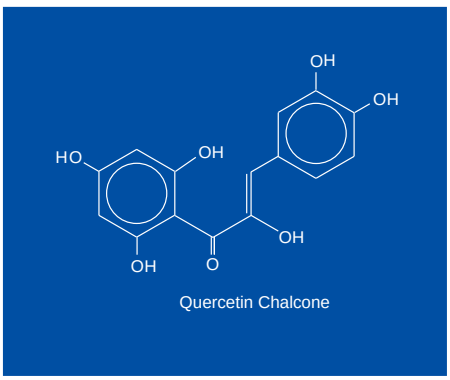
Introduction

The benefits of fruits and vegetables have been studied fairly extensively. Flavonoids, found in many plants, are of particular interest for their anticancer properties. In his text, Boik divides the flavonoids into five categories: anthocyanins, minor flavonoids, flavones or flavonoids, isoflavonoids, and tannins.[1] Quercetin, a member of the flavones group, is thought to be the most widely distributed in nature; approximately 25-50 mg of quercetin is consumed in a normal daily diet.[2] Bioflavonoids have been reported to be involved in several important biological processes including antihistamine effects, immunological modulation, inhibition of platelet aggregation, and antitumor activity.

Early research conducted on the effect of oral administration of quercetin on colon-25 tumors in balb-c mice showed a significant reduction (50%) in size.[3] This research was based on reported antitumor properties of quercetin including: lymphocyte proliferation,[4] neutrophilia,[5] free radical scavenging,[6] anti-angiogenesis, [7] down-regulation of the mitotic cycle in tumor cells,[8] gene expression alteration,[9] and induction of apoptosis (cell suicide).[10] A comprehensive review of quercetin's antitumor effects was conducted recently by Lamson and Brignall and published in this journal.[11]

An important remaining question is how much quercetin is absorbed from an oral dose. Varying estimates have been concluded from clinical studies, ranging from less than one percent to 50 percent (this in ileostomy patients).[12-15]

Figure 1: Quercetin Chalcone



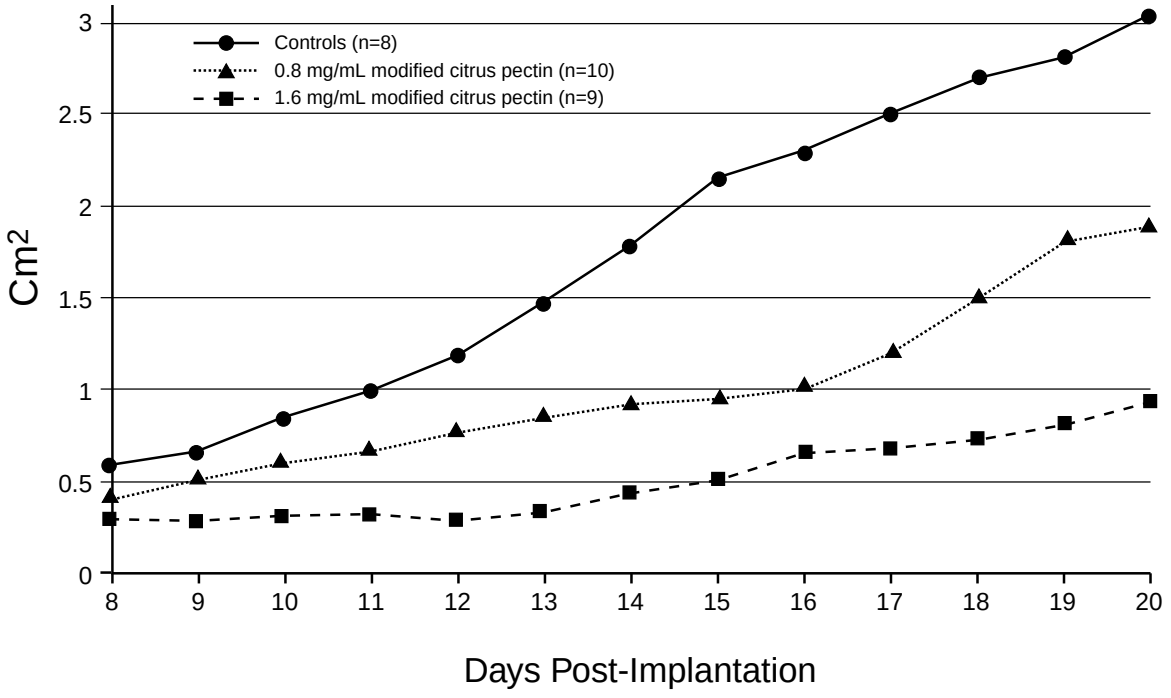
Quercetin Chalcone

A modified version of quercetin – quercetin chalcone (2',3,4,4',6' pentahydroxyflavone, U.S. Patent #5,977,184) – may provide a solution to the potential problem of poor absorption. To convert quercetin to quercetin chalcone (QC), a hydrogen is added to the oxygen at the number 1 position of the center ring, breaking the bond between that oxygen and the number 2 carbon and creating a hydroxyl group (Figure 1). This reduction of quercetin potentially gives QC the same anticancer effects of quercetin with the hydrophilic effect of a chalcone. The hydrophilic effect of QC may allow for greater absorption in the intestine as well as by tumor cells.

Modified Citrus Pectin

A water-soluble polysaccharide extracted from orange peel, citrus pectin is further pH-modified in the laboratory to allow for smaller carbohydrate chains rich in galactose residues.[16] Previous studies have shown a link between administration of modified citrus pectin (MCP) and decreased metastasis of prostate tumors in rats and melanoma in mice.[17,18] With the use of MCP, Pienta et al were able to demonstrate a significant reduction in the number of metastatic MAT-LyLu tumor colonies formed in the lungs of rats.[17] MCP is believed to adhere to tumor cells through cell surface carbohydrate-binding proteins called lectins, preventing aggregation of tumor cells and adhesion to normal cells.[16]

Figure 2: The Effects of Modified Citrus Pectin on the Growth of Colon-25 Tumors Implanted in Balb-c Mice.



Purpose

Although studies have shown MCP to be effective in preventing metastasis, MCP has not been shown previously to inhibit the growth of solid tumors. Furthermore, there have been no studies conducted on the effects of quercetin chalcone as an antitumor agent.

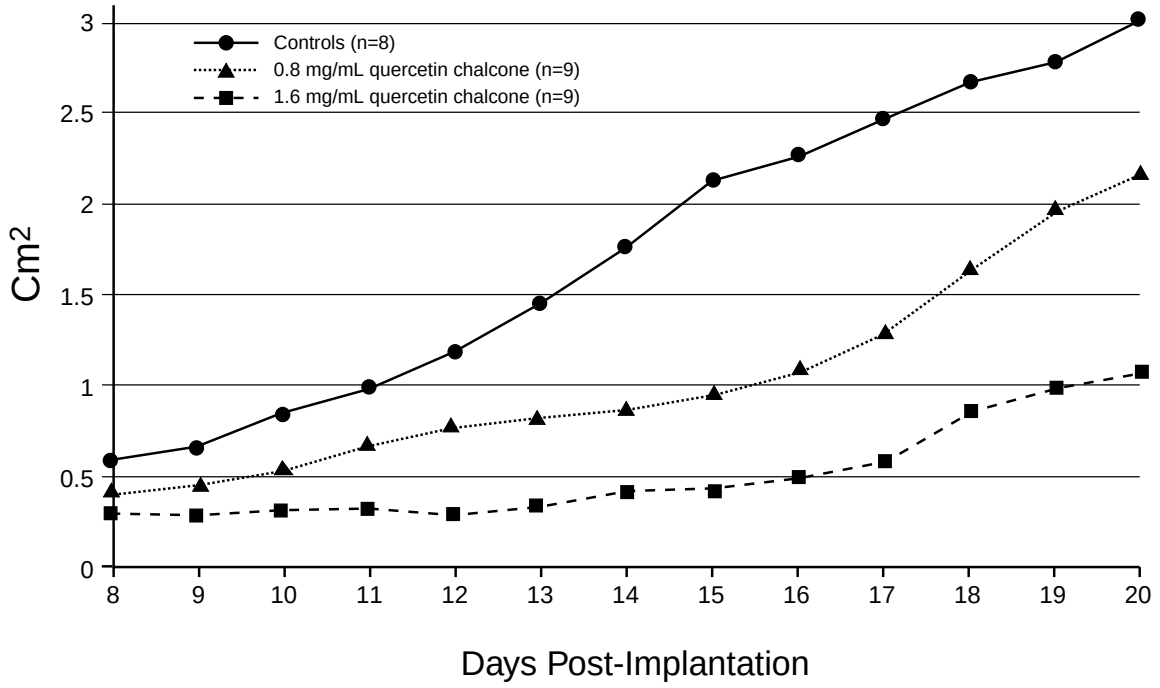
The present study seeks to show the effect of daily oral administration of quercetin chalcone and modified citrus pectin on balb-c mice implanted with colon-25 tumors. The parameters examined were daily changes in tumor size (length x width) and changes in autopsied tumor weights (grams).

Methods

Fifty male and female mice ranging in weight (16-27 g) and age (2-4 months) were bred at the University of North Texas Animal Care Facility. They were implanted with a 2mm x 2mm medullary section (brei) of a human colon-25 tumor from a donor mouse. Implanted sections were measured using a Petri dish with a piece of 1mm x 1mm graph paper attached underneath. Excised tumors were first placed in a Ringers solution to ensure viability after removal from the donor mouse. The moistened 2mm x 2mm section was then injected between the iliac crest and the ribcage posteriorly below the epidermis using a 13-gauge needle. This strain of mice was used because of its weak immune system, thereby facilitating the growth of implanted tumor brei into a solid tumor within days.

The mice were maintained at the University of North Texas Animal Care Facility in a temperature-regulated room and on a 12-hour light-dark cycle. They were given water ad libitum, and fed a diet of Teklad rodent pellets. Quercetin chalcone and modified citrus pectin (U.S. Patent #5,498,702) were donated by Thorne

Figure 3: The Effects of Quercetin Chalcone on the Growth of Colon-25 Tumors Implanted in Balb-c Mice.



Research, Inc. (P.O. Box 25, Dover, Idaho 83825).

Formulation of the Test Solutions

1.6 grams of quercetin chalcone and modified citrus pectin, respectively, were individually combined with one liter of distilled water to form solutions. The 1.6-mg/mL solutions were further diluted with equal parts of distilled water to produce 0.8-mg/mL solutions. These solutions were administered orally using a plastic-tipped pipette on a daily basis at or before noon. Measurements using digital calipers commenced when the tumor could first be palpated (usually eight days post-implantation). Oral dosing was begun on the first day of palpation and ceased on day 20 post-implantation. At this time the tumor was excised and placed in a 2% formalin solution.

The mice were divided into control and test groups as follows:

The mice were divided into control and test groups as follows:

I. Control

Control animals received 1 ml distilled water (n=10).

II. Test Mice (quercetin chalcone)

A. 1 ml 0.8-mg/ml quercetin chalcone solution (n=10)

B. 1 ml 1.6-mg/ml quercetin chalcone solution (n=10)

III. Test Mice (modified citrus pectin)

A. 1 ml 0.8-mg/ml modified citrus pectin solution (n=10)

B. 1 ml 1.6-mg/ml modified citrus pectin solution (n=10)

Students' two-tailed t-test was used to test the statistical significance of the data. This study was approved by the Institutional Animal Care and Use Committee at the University of North Texas.

Table 1: Effects of Modified Citrus Pectin and Quercetin Chalcone on Mean Tumor Weight and Mean Tumor Area at Day 20 Post-implantation.

Modified Citrus Pectin

Tumor Weight						Tumor Size			
n	Dosage	grams		SEM	[%Δ]*	cm^2		SEM	[%Δ]*
8	control	1.57	±	0.45		3.04	±	0.75	
10	0.8 mg	2.183	±	0.54	39%	1.89	±	0.37	-38%*
9	1.6 mg	1.385	±	0.56	-12%	0.93	±	0.20	-69%**

Quercetin Chalcone

Tumor Weight						Tumor Size			
n	Dosage	grams		SEM	[%Δ]*	cm^2		SEM	[%Δ]*
8	control	1.57	±	0.45		3.04	±	0.75	
9	0.8 mg	2.081	±	0.73	33%	2.17	±	0.45	-29%
8	1.6 mg	1.18	±	0.38	-25%	1.06	±	0.22	-65%**

Significance determined using Students' two-tailed t-test.
* $p<0.02$
** $p<0.001$

Results

The groups given MCP showed a significant reduction in tumor size at day 20, compared to controls. A 38-percent ($p<0.02$) reduction in size was seen in the 0.8-mg/mL group, and a 70-percent ($p<0.001$) reduction in size was seen in the 1.6-mg/mL group (Figure 2).

The groups given QC also exhibited reductions in tumor size. The group given 0.8 mg/mL had a statistically non-significant 29-percent reduction in size, while the 1.6-mg/mL group demonstrated a significant reduction of 65 percent ($p<0.001$) (Figure 3).

There were no statistically significant changes in mean tumor weight at day 20 from mice given either MCP or QC. With such dramatic changes in tumor size a similar decrease in mean weight would have been expected; however, this observation might be due to differences in overall tissue constituents of the excised tumors (water vs. organic matrix, or possible necrosis or fibrosis). Histological examination of tumor tissue was not conducted. The data is summarized in Table 1.

Discussion

The purpose of this study was to examine the effects of modified citrus pectin and quercetin chalcone on colon-25 tumors implanted in balb-c mice. The parameters measured were tumor size (length x width) and tumor weight (grams). The changes in mean weight of tumors from mice treated with either MCP or QC were not statistically significant.

The significant decrease in mean tumor size noted in mice fed citrus pectin might

be attributable to MCP's effect on metastasis. In their work with MCP, Platt and Raz found MCP not only reduced the number of experimental metastases, but also reduced the volume of developed metastases.[18] In a related article, Raz points out the importance of tumor emboli (tumor fragments) not only in tumor dissemination, but also in formation of tumors at secondary sites.[19] It seems, according to Pienta, that MCP may inhibit the formation of organized tumor emboli.[17] MCP, because of its small size and galactose-rich side chains, may be able to bind to cell surface galectins (galactose-binding lectins) on tumor cells, preventing the cells from binding to host cell surfaces. This could explain the reduction in tumor size seen with MCP (Figure 2). The tumor brei, a collection of cells extracted from the medulla of a colon tumor, once implanted in the mouse may not be able to undergo adequate implantation and growth due to the presence of MCP. Indeed, when looking at Figure 2, an early departure in growth rates between the experimental and control groups can be seen.

The significance seen in the 1.6-mg/mL QC group may indicate anticancer mechanisms similar to quercetin. Due to its similar structure, similar effects might be expected. Quercetin has been shown to: (1) down-regulate the expression of the mutant p53 gene in breast cancer cell lines; (2) cause G1 phase arrest in several cancer cell lines; (3) inhibit tyrosine kinase responsible for tumor growth; and (4) bind to estrogen II receptor sites, reducing expression of ER negative cells.[11] More research needs to be conducted before such correlations can be made between quercetin and quercetin chalcone. The early results, however, look promising.

Future research might focus on earlier administration of these substances. In the present study, QC and MCP dosing began on day eight, predictably the first day of manual tumor palpation. It is not known what effect might occur if these substances were given on the day of tumor implantation or before. We can infer from these results that tumor growth should be significantly inhibited, but the extent of that inhibition cannot be accurately predicted from the present data. Further animal and human clinical research utilizing these promising substances is suggested.

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