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ARTICLE

Quercetin Aglycone Is Bioavailable in Murine Pancreas and Pancreatic Xenografts

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Quercetin is a potential chemopreventive and chemotherapeutic agent for pancreatic and other cancers. This study examined the distribution of quercetin in plasma, lung, liver, pancreas, and pancreatic cancer xenografts in a murine in vivo model and the uptake of quercetin in pancreatic cancer MiaPaCa-2 cells in a cellular in vitro model. Mice were randomly allocated to control or 0.2 and 1% quercetin diet groups utilizing the AIN93G-based diet (n = 12 per group) for 6 weeks. In addition, 6 mice from each group were injected weekly with the chemotherapeutic drug gemcitabine (120 mg/kg mouse, ip). MiaPaCa cells were collected from culture medium after cells were exposed to 30 μM quercetin for 0.5, 1, 2, 4, 8, and 24 h. Levels of quercetin and 3-O'-methylquercetin in mouse tissues and MiaPaCa-2 cells were measured by high-pressure liquid chromatography following enzymatic hydrolysis and then extraction. The study showed that quercetin is accumulated in pancreatic cancer cells and is absorbed in the circulating system, tumors, and tissues of pancreas, liver, and lung in vivo. A higher proportion of total quercetin found in tumors and pancreas is aglycones. Gemcitabine cotreatment with quercetin reduced absorption of quercetin in the mouse circulatory system and liver. Results from the study provide important information on the interpretation of the chemotherapeutic efficacy of quercetin.

KEYWORDS: Quercetin; bioavailability; pancreas; pancreatic cancer; in vivo; HPLC

INTRODUCTION

Pancreatic cancer is the fourth leading cause of cancer-related death for both men and women in the United States (*1*). The 5-year relative survival rate for all stages is approximately 5%. These statistics underscored the urgent need to identify new approaches and new agents for the treatment and prevention of pancreatic cancer. Recent epidemiological studies have suggested that dietary flavonoids, such as kaempferol, quercetin, and myricetin reduce pancreatic cancer risks (*2*−*4*). Extended research by our team and others has supported epidemiological findings and provided some evidence that quercetin has many biological activities, such as antitumor and antiproliferative effects on a wide range of human cancers (see review in ref *5*) including pancreatic (*6*, *7*). However, most studies investigated biological activities in cultured cells by using the free form of quercetin or aglycone and did not take absorption and metabolism into consideration for the interpretation of results. Dietary flavonoids were present in the human circulating system predominantly as conjugates of glucuronides and sulfates that are likely to have differential biological activities and distribution patterns in tissues and cells compared with flavonoid aglycones (*8*−*10*). More importantly, bioactive food components must be sufficiently absorbed in the gastrointestinal tract and reach pharmacological levels in target tissues to have the potential to exert biological activity.

Quercetin (3,3′,4′,5,7-pentahydroxyflavone) is a naturally occurring flavone found in many fruits and vegetables in the form of quercetin glucosides. The mechanism of quercetin glucoside absorption involves the luminal hydrolysis of the glucosides by lactase phlorizin hydrolase followed by diffusion of released aglycone and/or the transport by sodium-dependent glucose transporter with subsequent deglycosylation within the enterocyte by cytosolic β-glucosidase (*11*). Quercetin aglycone is metabolized by phase II drug-metabolizing enzymes, uridine-5′-diphosphate-glucuronosyl-transferase, sulfotransferase, and catechol-*O*-methyltransferase, which leads to the formation of quercetin glucuronide and sulfate conjugates with or without methylation on the catechol functional group of quercetin. Hydroxyl groups of quercetin may be multiply conjugated (*12*, *13*). In blood the predominant forms of quercetin are conjugates (sulfate and glucuronidates with optional methylation), whereas organs such as lung, liver, and kidney contain a higher proportion of free quercetin and 3′-*O*-methylated quercetin isorhamnetin. Quercetin has a relatively long plasma half-life of 12−28 h (*14*).

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Although the bioavailability of quercetin taken in by rats (*15*, *16*), pigs (*16*, *17*), mice (*18*), and humans (*19*, *20*) has been reported, information on quercetin distribution in pancreatic cancer cells, pancreatic tissue, and pancreatic tumor in xenograft is not available at present. In this study we have investigated the uptake of quercetin in pancreatic cancer MiaPaCa-2 cells in cellular in vitro. Furthermore, we have determined the distribution of quercetin in plasma, lung, liver, pancreas, and tumor xenograft in a murine in vivo model fed long-term with diets containing either 0.2 or 1% quercetin.

MATERIALS AND METHODS

Materials. All solvents used were of HPLC grade (Fisher Scientific, Fair Lawn, NJ). Quercetin dihydrate, isorhamnetin, and β-glucuronidase/sulfatase (type H-5 from *Helix pomatia*) were purchased from Sigma-Aldrich (St. Louis, MO). Quercetin and tamarixetin were purchased from Chromadex (Irvine, CA). Internal standard 3,3′,4′-trihydroxyflavone was purchased from Indofine (Hillsborough, NJ).

Cell Culture. The human pancreatic cancer cell line MiaPaCa-2 (American Type Culture Collection, Manassas, VA) was cultured in DMEM supplemented with 10% heat-inactivated fetal bovine serum, 100 units/mL penicillin, 100 μg/mL streptomycin, and 292 μg/mL glutamine and incubated at 37 °C in a humidified atmosphere with 5% CO_2. For the experimental procedures cells were plated in full media. After 24 h, they were starved overnight before the addition of 30 μM quercetin (diluted from 100 mM stock solution in DMSO with cell culture medium) for the indicated time. Final DMSO concentration was $<0.1\%$. One milliliter aliquots of the media were frozen after the incubation time. Cells were washed three times with ice-cold PBS, harvested by scraping, and spun at 14000 rpm for 1 min. The supernatant was aspirated, and the cell pellets were frozen in liquid nitrogen and kept at −80 °C until further analysis. Protein concentration was measured with a Pierce Protein Assay kit (Pierce, Rockford, IL).

Cell Quercetin Extraction. Frozen cell pellets (3×10^6 cells) were broken by a pellet pestle and then extracted three times with 80 μL of MeOH. In each extraction the mixture was vortexed for 1 min and then centrifuged at 13000*g* for 5 min. Combined MeOH was dried with a SpeedVac at room temperature and the residue reconstituted in 200 μL of H_2O/MeOH (1:3). Aliquots of culture medium were acidified with an equal volume of aqueous methanol (methanol/water/acetic acid 45:50:5) and then centrifuged. For both cell pellet and culture medium, aliquots of 50 μL were injected into the HPLC.

Animals and Diets. Animal studies were approved by the Chancellor's Animal Research Committee of the University of California, Los Angeles, in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Nude mice (Charles River Laboratories, San Diego, CA) were housed four per cage in a room with controlled temperature (20−22 °C). The mouse diet was AIN-93G purified rodent diet (Dyets, Bethlehem, PA) supplemented or not with quercetin (5% w/w) for 8 weeks. Diet was replaced every week, and the stability of quercetin was analyzed on days 0 (as control), 1, 2, 4, and 7 by HPLC following extraction. Mouse blood was drawn by retro-orbital bleeding, and plasma was collected in weeks 2, 4, and 6 and pooled from four mice for each sample. After 8 weeks, the animals were anesthetized, and whole blood was taken by cardiac puncture. The lung and liver were preserved as freshly frozen and in 10% formalin before processing. The pancreas was preserved as freshly frozen. Tissues were processed at the Tissue Procurement and Histology Core Laboratory for hematoxylin and eosin staining. They were evaluated by a pathologist.

The orthotopic xenograft model was performed as described earlier (*21*). Briefly, 3×10^6 MiaPaCa-2 cells were injected subcutaneously into the flank of nude mice. After 4 weeks, the donor tumor was harvested and minced to fragments of approximately 1 mm^3. One tumor fragment was placed into the tail of the pancreas of recipient nude mice. Starting 2 days prior to the surgery, the mice were fed the AIN-93G diet. In the second test, the animals were divided in two groups of eight animals each: control and quercetin 5% for 8 weeks. In the third test, animals were divided in six groups of six animals each: control, gemcitabine, quercetin 0.2%, quercetin 0.2% with gemcitabine, quercetin 1%, and quercetin 1% with gemcitabine. Quercetin was administered orally mixed with the powder diet in the respective percentage starting day 1 after surgery. Gemcitabine (120 mg/kg) was given ip on days 10, 17, 24, 31, and 38. After 6 weeks, the animals were anesthetized, and blood and tissues were obtained similarly as described.

Quercetin Extraction in Mice Plasma and Tissues. Plasma samples were acidified with 0.1 volume of 0.58 M acetic acid to limit flavonoid losses (*22*). To the acidified mouse plasma samples (110 μL, equivalent to 100 μL of plasma) in a 2 mL microcentrifuge tube was added 29 μL of β-glucuronidase in 0.2 M sodium acetate buffer (pH 5.0) containing 500 U of β-glucuronidase and 12.5 U of sulfatase. An aliquot of 71 μL of 0.2 M sodium acetate buffer (pH 5.0) containing 1% ascorbic acid was added. The mixture was vortexed and then incubated at 37 °C for 2 h. After the incubation period, 600 μL of ethyl acetate was added followed by the addition of 20 μL of internal standard 3,3′,4′-trihydroxyflavone in EtOH (25 μM). Quercetin and its metabolites were extracted with 600 μL of ethyl acetate three times. Each extraction mixture was vortexed for 1 min and then centrifuged at 600 rpm for 5 min. Supernatant was transferred to a glass test tube and combined. Solvent was removed in a SpeedVac at room temperature until complete dryness. Residue was reconstituted in 150 μL of acetone, vortexed, and then sonicated for 1 min, followed by the addition of 50 μL of H_2O. Supernatant was then transferred to a glass insert, and an aliquot of 25 μL of mixture was injected to HPLC. For mouse tissue analysis, frozen tissue was weighed and homogenized in buffer with a tissue grinder, and internal standard was added. The mixture was then hydrolyzed and extracted similarly as plasma samples.

High-Pressure Liquid Chromatography (HPLC). HPLC analysis was performed with an RP-18 Luna column (150 × 4.6 mm, 3 μm, Phenomenex, Torrance, CA) on an Agilent 1100 HPLC system (Santa Clara, CA) comprising an autosampler and a quaternary pump coupled to a photodiode array detector. The mobile phase consisted of a binary gradient of 0.1% (v/v) orthophosphoric acid in water (eluent A) and acetonitrile (eluent B), used with a flow rate of 0.6 mL/min in the following conditions: 20−35% B (0−12 min); 35−70% B (12−18 min); and 70−20% B (18−23 min). Column temperature was held at 30 °C. The chromatograms were recorded at 370 and 258 nm for quercetin and its conjugates and metabolites. Data were analyzed with Hewlett-Packard Chemstation software. Concentrations of quercetin in cell pellets and culture media were determined by HPLC using external calibration. Mouse plasma and tissue concentrations were determined by internal calibration. Concentration of the stock solutions was determined spectrophotometrically using Beer's law. Calibration standards were prepared from the stock solutions by series dilution. The calibration curves generated from standard solutions of quercetin showed a linear relationship between peak area and concentration in the range from 12.5 ng/mL to10 μg/mL. The detection limit for quercetin is 6 ng/mL with a 25 μL injection.

Statistics. Descriptive statistics, such as mean and standard deviation, were used to summarize the results. Statistical comparisons were made using a paired two-tailed Student's *t* test with a confidence level of 95%. Statistical significance was defined by a *P* value of 0.05.

RESULTS

Quercetin Degradation in Aqueous Cell Culture Media and Penetration into Cells in Culture. Quercetin is highly labile in cell culture conditions and undergoes degradation and oxidation along with metabolism (*23*, *24*). To examine quercetin taken up into MiaPaCa-2 cells and its changes following exposure to cell culture condition, we collected samples of cells and medium after cells were treated with 30 μM quercetin and incubated for 0.5, 1, 2, 4, 8, and 24 h. Control samples were collected without quercetin treatment. HPLC profile of quercetin uptake by cells showed that quercetin is eluted at 16.7 min. Quercetin concentration plateaus at 1 h and then decreases steadily. At 24 h, its level fell from 15.54 (±2.29) nmol of peak concentration to 0.29 (±0.30) nmol (**Figure 1A**). Meanwhile, small but detectable amounts of quercetin metabolites isorhamnetin (3′-methylquercetin, Rt = 19.2 min) and tamarixetin (4′-*O*-methylquercetin, Rt = 18.9 min) were observed starting 0.5 h, both of which increase slightly with time and were present until 24 h. A peak at Rt = 14.4 min was also observed in cells exposed

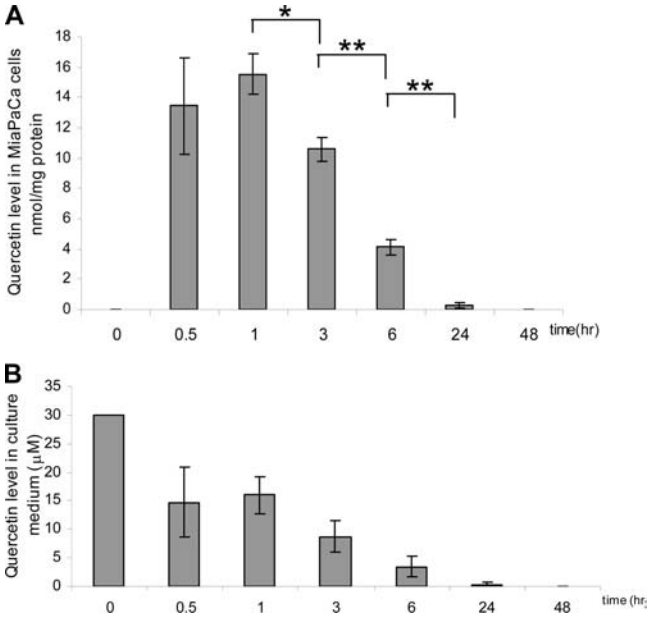

Figure 1. (**A**) Uptake of quercetin in MiaPaCa-2 cells at different time points as indicated. Values are means ± SEM of three separate measurements (∗, $P < 0.05$; ∗∗, $P < 0.01$). (**B**) Change of quercetin level in cell culture medium with incubation. Preparation of cells and quantitative measurement of quercetin in culture medium at different time points were described under Materials and Methods. Values are means ± SEM of three separate measurements.

to quercetin. The area of the peak appears to increase with exposure time up to 6 h and then disappears at 24 h. The online UV−vis absorption spectrum of the peak is identical to that of quinone as published by Spencer (*25*). Quercetin, with an intrinsic catechol moiety in its structure, is known to form quinone and quinone methides in a cellular in vitro model that is the subject of research interests due to its pro-oxidant chemistry (*23*, *25*). Subsequently, we examined the stability of quercetin in cell culture medium following incubation. The quercetin level declined to half of its initial concentration in 1 h and fell to 0.34 (±0.59) μM in 24 h (**Figure 1B**). Also, trace but detectable amounts of quercetin metabolites isorhamnetin and tamarixetin were observed starting from 0.5 h but disappeared at 24 h of exposure. To examine if MiaPaCa-2 cells were responsible for the rapid disappearance of quercetin and the production of two O-methylated quercetins, we have performed similar experiments in the absence of MiaPaCa-2 cells. We found that the HPLC profile and the change of quercetin level in cell culture medium were parallel to those of experiments in the presence of cells, with 30.18 ± 0.91, 14.44 ± 5.60, 15.53 ± 3.41, 5.01 ± 2.05, 0.63 ± 1.09, and 0.16 ± 0.16 μM at 0, 0.5, 1, 3, 6, and 24 h, respectively. A quinone species is also formed in culture medium in the presence or absence of cells. These experiments demonstrated approximately 15-fold of quercetin ratio between medium and cells (3×10^6). Thus, the MiaPaCa-2 cells are capable of accumulating quercetin.

Plasma Absorption of Quercetin in Mice after Oral Administration of Quercetin Diets. In the first experiment, a group of four mice was treated with a 5% quercetin diet for 8 weeks (calculated as 5.3 g/(kg of body wt/day) quercetin intake). The dosage and length of feeding were selected to determine the quercetin bioavailability and toxicity in mice. During the course of study, the stability of quercetin in the diet was measured at days 1, 2, 3, 4, and 7. Mouse plasma was collected in weeks 2, 4, and 6 and pooled from four mice for each sample. Data showed that quercetin was absorbed into the mouse blood time-dependently and gave total concentrations (quercetin plus isorhamnetin) of

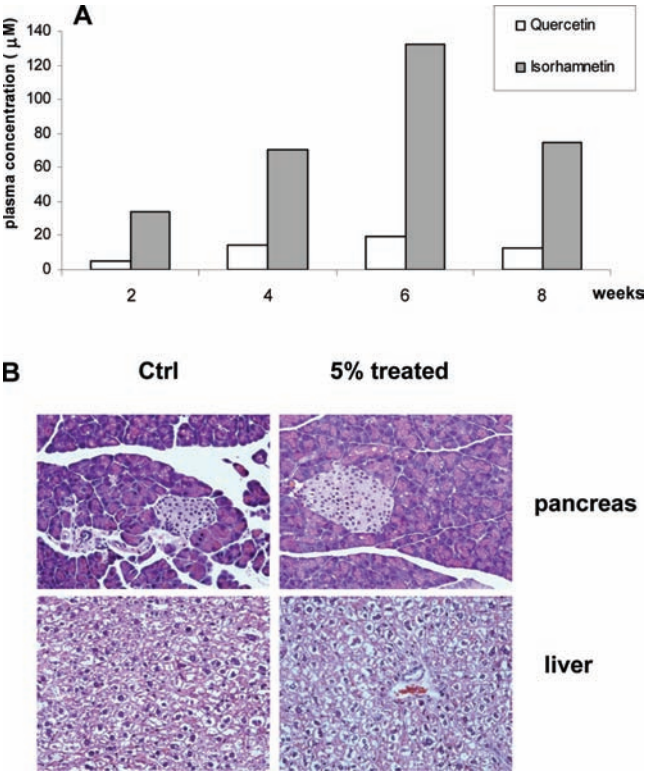

Figure 2. Change of quercetin and isorhamnetin concentrations in plasma of mice fed a 5% quercetin diet for 8 weeks: (**A**) plasma absorption of quercetin and isorhamnetin at different time points; (**B**) histology of liver and pancreas of nude mice fed a 5% quercetin diet for 8 weeks. Mice were fed a diet containing 5% quercetin for 8 weeks, and blood was collected and pooled at different times as indicated. Concentrations of quercetin and isorhamnetin in plasma were measured by HPLC as described under Materials and Methods.

Table 1. Blood Chemistry Panel of Mice Fed a 5% Quercetin Diet for 8 Weeks

group[a]	control	5% quercetin diet
ALT, 7.0−227.0 U/L	54.0 ± 25.5	35.0 ± 3.6
AST, 37.0−329.0 U/L	262.5 ± 227.0	126.3 ± 28.0
BUN, 2.0−71.0 mg/dL	23.5 ± 2.1	18.0 ± 1.7
creatinin, 0.1−2.1 mg/dL	0.3 ± 0.0	0.3 ± 0.1
total bilirubin, 0.1−1.1 mg/dL	0.6 ± 0.1	0.4 ± 0.1

[a] ALT, alanine aminotransferase; AST, alanine transaminase; BUN, blood urea nitrogen.

39.4, 84.5, and 152.0 μM at 2, 4, and 6 weeks, respectively. However, at the time mice were sacrificed after 8 weeks feeding, plasma concentration had decreased to 87.8 ± 17.9 μM (**Figure 2A**). Mice also exhibited slightly increased body weight but did not have signs of other adverse effects as revealed by measurements of blood chemistry panel (**Table 1**) and histology (**Figure 2B**). We observed large variation in blood alanine aminotransferase (ALT) and alanine transaminase (AST) levels for control mice. This was likely due to the small sample size. Despite this variation, all tests were normal for all of the animals and organs except one animal in the control group having an AST of 423 U/L (normal = 37−329 U/L). Clinically, this mouse had normal liver function and its other liver chemistries (ALT and bilirubin) were normal.

The HPLC profile of mouse plasma shows two peaks with retention times at 16.7 and 19.1 min as standard quercetin and methylated 3′-*O*-methylquercetin after samples were hydrolyzed with β-glucuronidase/sulfatase. Comparison with the retention

times and online UV−vis maxima of the DAD responses of the authentic samples facilitated the confirmation of these peaks. The majority of the quercetin (approximately 83%) is metabolized to isorhamnetin, which is in accordance with a previous report that about 79% of the metabolites in the free access rat plasma were methylated (*26*).

In the second test, 16 tumor-bearing mice were administered 5% quercetin or control diets for 8 weeks. The average plasma concentration is 50.23 (±15.37) μM for quercetin and 58.02 (±11.07) μM for isorhamnetin as measured after enzymatic hydrolysis. Because 5% quercetin intake did not produce tumor growth inhibition, further data were not collected and lower doses were adopted.

In the third study, analysis of the blood of tumor-bearing mice administered lower doses of 1.0 and 0.2% quercetin diets for 6 weeks (calculated as 1.5 and 0.3 g/(kg of body wt/day) quercetin intake, respectively) revealed a dose-dependent plasma total concentration after enzyme hydrolysis (**Table 2**). Plasma samples in the 1% quercetin intake group showed free quercetin and isorhamnetin, albeit in very low levels (about 4−15% of total levels), as measured without enzymatic hydrolysis. They also showed about 3 times higher concentrations of total quercetin compared to the 0.2% quercetin intake group. In both intake groups the level of methylated quercetin was consistently about half of the total quercetin and was noticeably less than that from the plasma of the nude mice with 5% quercetin intake. Furthermore, we observed the group of mice fed the quercetin diet and injected with gemcitabine at the same time showed lower blood levels of quercetin and methylated quercetin (**Table 3**) compared with the group fed the quercetin diet only. Generally, plasma levels of quercetin and isorhamnetin are lower in both quercetin plus gemcitabine groups. Statistical significance was observed in quercetin level in the 0.2% quercetin intake group and in isorhamnetin level in the 1% intake group.

Table 2. Quercetin and Isorhamnetin Levels in Plasma of Xenograft Mice Fed 0.2 or 1% Quercetin Diet for 6 Weeks[a]

	μM	
	0.2% quercetin diet	1% quercetin diet
quercetin	3.29 ± 0.40	8.68 ± 1.21 (0.19 ± 0.09[b])
isorhamnetin	3.27 ± 0.91	13.97 ± 2.11 (0.07 ± 0.04[b])

[a] Values are mean ± SEM. [b] Aglycone.

Table 3. Quercetin and Isorhamnetin Levels in Plasma of Xenograft Mice Fed 0.2 or 1% Quercetin Diet with and without Gemcitabine[a]

	μM			
	0.2% quercetin diet		1% quercetin diet	
	Q	Q + G	Q	Q + G
quercetin	4.42 ± 0.51[b]	2.35 ± 0.10	9.43 ± 1.49	7.94 ± 2.00
isorhamnetin	4.46 ± 1.96	2.27 ± 0.19	18.19 ± 3.31[b]	9.95 ± 1.24

[a] Values are mean ± SEM. Q, quercetin diet; Q + G, quercetin diet with gemcitabine injection every week. [b] Different from Q + G cotreatment, $P < 0.05$.

Tissue Levels of Quercetin in Xenograft Mice after Oral Administration of 0.2 and 1% Quercetin Diet. To investigate the levels of quercetin in pancreas and tumor as target tissues, we also incorporated tissues of lung and liver as metastases to the liver and lung are common findings, especially with tumors of the pancreas. Generally, all tissues from mice fed the 1% quercetin diet accumulated 4−10 times higher concentrations of quercetin and methylated quercetin compared to those from mice fed the 0.2% quercetin diet (**Table 4**). The level of total quercetin is highest in the liver, followed by the pancreas, lung, and tumor, whereas in the lower intake group, the highest concentration was observed in the pancreas, followed by the liver and tumor. In addition, the majority of the quercetin and methylated quercetin was found in their aglycone forms in these tissues including tumor. Among them, pancreas accumulated 84% quercetin aglycone and tumor 65%. Gemcitabine treatment resulted in significant reduction in quercetin only in the liver of mice fed the 0.2% quercetin diet (0.30 ± 0.07 vs 1.07 ± 0.1, $p < 0.05$). Qinone species, present in cell extract as well as in culture medium, was not found in any tissue or plasma of mice.

DISCUSSION

This study shows for the first time that quercetin and its metabolites are distributed in pancreas and xenograft tumors in mice after administration of 0.2 and 1% quercetin diets. Our data demonstrated that quercetin is distributed in the organs of liver, lung, pancreas, and tumor tissue, and its levels were within the same order of magnitude. Tissues including liver and lung in the 1% intake group accumulated about 4−10 times higher concentrations of total quercetin than in the 0.2% intake group. In the 1% quercetin intake group, the level of total quercetin is highest in the liver, followed by the pancreas, lung, and tumor, whereas in the lower intake group, the highest concentration was observed in the pancreas, followed by the liver, tumor, and lung. These results are in agreement with a previous study in that quercetin is widely distributed in SC tumor, liver, lung, spleen, heart, etc., in tumor-bearing mice (*27*). Furthermore, we found that all investigated organs contained a considerable proportion of deconjugated quercetin and isorhamnetin, ranging from 40 to 100% of the total quercetin concentration. In tumor, 64% of quercetin and 75% of isorhamnetin are aglycone forms. A similar metabolism pattern has been found in rats and pigs after oral intake of a quercetin diet. Rat tissues of lung, liver, and kidney were shown to possess a high deconjugation activity due to the presence of enzymes with β-glucuronidase activity (*16*, *17*). This high enzyme activity also resulted in an ex vivo conversion of 3-*O*-glucuronide to free aglycone during the extraction, which may also cause in vivo conversion in the tissues (*23*). Enzyme with β-glucuronidase activity can also be released under certain physiological conditions

Table 4. Quercetin and Isorhamnetin Levels in Tissues of Xenograft Mice Treated with 1 and 0.2% Quercetin for 6 Weeks[a]

	nmol/g of wet tissue			
	0.2% quercetin diet		1% quercetin diet	
	Q	I	Q (aglycone)	I (aglycone)
liver	0.65 ± 0.13	0.37 ± 0.09	5.12 ± 1.24 (5.45 ± 1.28)	3.69 ± 0.88 (3.56 ± 0.92)
lung	0.67 ± 0.18	ND[b]	3.04 ± 0.41 (2.17 ± 0.45)	3.44 ± 0.51 (1.36 ± 0.38)
pancreas	1.16 ± 0.47	0.69 ± 0.21	4.72 ± 1.60 (3.98 ± 1.48)	3.46 ± 0.32 (3.14 ± 1.27)
tumor	0.51 ± 0.12[c]	0.30 ± 0.09[c]	2.64 ± 1.15[d] (1.69[e])	2.16 ± 0.35[d] (1.61[e])

[a] Values are mean ± SEM. Q, quercetin; I, isorhamnetin. [b] ND, not detected. [c] $n = 5$. [d] $n = 6$. [e] $n = 2$.

such as inflammation (*28*), carcinogenic tissue (*29*), and tumor; the tissues of the latter contain large amounts of β-glucuronidase (*30*, *31*).

The glucuronidation of quercetin could take place at the 3-, 7-, 3′-, and 4′-positions when quercetin was incubated with human liver cell-free extract (*10*). When incubated with mouse liver microsomes, four quercetin metabolites were observed (*18*). Many of the glucuronide metabolites retain or decrease the biological activity, but mostly the antioxidant activities were examined (*10*, *32*, *33*). Quercetin aglycone is generally considered to be the more active form. In our study all pancreas, liver, lung, and tumor tissues accumulate significant amounts of free quercetin. The high and selective accumulation of chemopreventive agents at the tumor site is essential for the success of disease treatment in vivo. Studies show that the efficiency of quercetin glucosides absorption is higher than that for the aglycone itself in humans (*14*) as well as in animals (*34*). To this end, our animals fed quercetin aglycone may provide a disadvantageous condition for its absorption into various tissues including the tumor. The high level of quercetin, along with the high ratio of aglycone/conjugates found in the pancreas and tumor, may contribute to the tumor inhibitory activity of quercetin.

We demonstrated that quercetin is absorbed into mouse blood in a dose-responsive manner. The plasma of tumor-bearing mice fed 0.2 and 1% quercetin diets produced dose-dependent concentration increases in total quercetin and its metabolites. Plasma samples in the 1% quercetin treated group showed quercetin and isorhamnetin aglycones in very low levels, which is in line with the reported studies in that aglycones are generally either absent in blood or present in low concentrations. During the course of absorption, quercetin is conjugated in the small intestine and later in the liver. This process includes methylation, sulfation, and glucuronidation, which is a metabolic detoxication process common to many xenobiotics that restricts their potential toxic effects and facilitates biliary and urinary elimination by increasing their hydrophilicity. Eleven quercetin metabolites have been reported and their structures identified in plasma, and therefore the conjugation mechanisms are considered to be highly efficient (*19*). We found >80% of the absorbed quercetin is methylated to isorhamnetin. The high methylation of quercetin was observed starting in week 2 and remained the same until week 8 in the blood of nude mice.

We observed that the ratio of methylated/nonmethylated quercetin was lower in plasma of both 0.2 and 1% quercetin intake groups by comparison to that of nude mice with high quercetin intake. Although comparison needs to be made with caution due to the difference in dosages, a lower dose has been reported to favor the higher proportion of methylated quercetin in human plasma (*35*). To this end, our data may suggest tumor implantation and bearing may affect metabolism in mice. In addition, chemotherapeutic drug gemcitabine cotreatment with quercetin reduced the absorption of quercetin in the circulatory system and liver. The absorption of quercetin into blood and the subsequent metabolic profile were reported to be influenced by various factors including the route of administration (*26*), dosage (*33*), food matrix (*36*, *37*), etc. Chronic quercetin exposure has been reported to affect fatty acid catabolism in rat lung (*38*). It is possible that gemcitabine alone alters liver metabolism or that frequent ip injections change the eating habit and therefore the blood absorption.

In summary, this study showed that quercetin can be accumulated in pancreatic cancer MiaPaCa-2 cells in vitro and absorbed effectively in the circulating system, tumors, and tissues of pancreas, liver, and lung in vivo. Whereas higher proportions of quercetin and methylated quercetin found in organs of pancreas, lung, and liver, as well as in tumors, are aglycones, conjugates were found almost exclusively in plasma (>98%). A lower ratio of methylated/nonmethylated quercetin was found in plasma of both 0.2 and 1% quercetin intake groups compared to that of nude mice. Chemotherapeutic drug gemcitabine cotreatment with quercetin reduced the absorption of quercetin in the circulatory system and liver but not in the other tissues investigated. As quercetin is eliminated mainly in bile, its levels in the pancreas and liver generate high concentration variations. It is important to note that quercetin (1%) cotreatment with gemcitabine significantly inhibited the growth of tumor in orthotopic murine model(*39*). Our data provide important information on the therapeutic efficacy of quercetin.

ABBREVIATIONS USED

ALT, alanine aminotransferase; AST, alanine transaminase; BUN, blood urea nitrogen; DAD, diode array detector; G, gemcitabine; HPLC, high-pressure liquid chromatography; I, isorhamnetin; Q, quercetin; Rt, retention time.

Supporting Information Available: AACR submission. This material is available free of charge via the Internet at http://pubs.acs.org.

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