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A Phase I Dose Escalation Study Demonstrates Quercetin Safety and Explores Potential for Bioflavonoid Antivirals in Patients with Chronic Hepatitis C

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The hepatitis C virus (HCV) infects more than 180 million people worldwide, with long-term consequences including liver failure and hepatocellular carcinoma. Quercetin bioflavonoids can decrease HCV production in tissue culture, in part through inhibition of heat shock proteins. If quercetin demonstrates safety and antiviral activity in patients, then it could be developed into an inexpensive HCV treatment for third world countries or other affected populations that lack financial means to cover the cost of mainstream antivirals. A phase 1 dose escalation study was performed to evaluate the safety of quercetin in 30 untreated patients with chronic HCV infection and to preliminarily characterize quercetin's potential in suppressing viral load and/or liver injury. Quercetin displayed safety in all trial participants. Additionally, 8 patients showed a "clinically meaningful" 0.41-log viral load decrease. There was a positive correlation ($r=0.41$, $p=0.03$) indicating a tendency for HCV decrease in patients with a lower ratio of plasma quercetin relative to dose. No significant changes in aspartate transaminase and alanine transaminase were detected. In conclusion, quercetin exhibited safety (up to 5 g daily) and there was a potential for antiviral activity in some hepatitis C patients. Copyright © 2015 John Wiley & Sons, Ltd.

Keywords: bioflavonoids; dose escalation; hepatitis C; phase I; phytomedicine; quercetin.

INTRODUCTION

The hepatitis C virus (HCV) infects 180 million people worldwide and accounts for more than 12 000 deaths in the US each year (Alter, 1997; Armstrong *et al.*, 2006; Wasley *et al.*, 2008). Although some HCV infections clear spontaneously, most patients with HCV become chronic carriers and risk serious complications, including cirrhosis and hepatocellular carcinoma (HCC) (Hoofnagle, 2002).

Curative antivirals are the most effective secondary prevention of HCV-associated chronic liver diseases. Previously, the standard HCV treatment was pegylated interferon combined with ribavirin (RBV). Unfortunately, adverse events or contra-indication excludes 70–80% of HCV patients from interferon-based therapy (Falck-Ytter *et al.*, 2002). Ledipasvir-sofosbuvir (Harvoni; Gilead,

Foster City, CA) was recently introduced as a new interferon-free/RBV-free option that prevents serious complications in patients (Afdhal *et al.*, 2014a, 2014b; Kowdley *et al.*, 2014). But the price of this medication limits access, as cost-benefit analysis estimates a 12 week ledipasvir-sofosbuvir treatment to cost \$94 500 per patient (Younossi *et al.*, 2015). Data suggest that a longer 24-week treatment, costing up to \$189 000, is needed in certain HCV-infected populations, such as patients with liver cirrhosis or previous antiviral exposure (Younossi *et al.*, 2015).

The dietary supplement quercetin has the potential to be developed into an affordable and natural alternative to expensive interferon-free/RBV-free treatments. Quercetin is a bioflavonoid found in many plants, tea, and wine (Romano *et al.*, 2013; Srinivas, 2015). It has several biological benefits including: suppression of respiratory tract infections, decrease in pancreatic cancer risk in male smokers, improvement in early cadaveric renal transplantation outcomes, and prevention of anaphylactic reactions (Fanning *et al.*, 1983; Shoskes *et al.*, 2005; Valentova *et al.*, 2007; Bobe *et al.*, 2008). Previous pharmacokinetic studies have elucidated quercetin's

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metabolism (Harwood *et al.*, 2007). Specifically, extensive first pass metabolism of quercetin in the liver and kidneys results in more conjugated quercetin and a lowered plasma concentration of free unconjugated quercetin. Bioavailability studies have reported quercetin's elimination half-life to range from 31 to 50 h (Walle *et al.*, 2001).

Our previous laboratory efforts determined that quercetin exerts *in vitro* antiviral properties in human HCC tissue culture. We demonstrated that quercetin can indirectly inhibit HCV replication through the suppression of host heat shock proteins (HSP). This effect could be related to the complexes that HSPs form with nonstructural protein 5A (NS5A), an essential HCV protein that is associated with viral production and blocking host antiviral responses (Gonzalez *et al.*, 2009). Additional studies suggest that quercetin can also directly inhibit viral protease nonstructural protein 3 (NS3) (Bachmetov *et al.*, 2012) and NS5A (Bhattacharya *et al.*, 2012). Quercetin's antiviral mechanism is of particular interest in antiviral research because this multi-level approach (direct and indirect) against HCV could maintain selective pressure against viral relapse (Prussia *et al.*, 2011).

Given quercetin's affordability and tolerability, with doses up to 1 g/day demonstrating safety in numerous trials, we sought to determine its safety in patients with HCV at higher doses (up to 5 g daily) (Nieman *et al.*, 2007a, 2007b; McAnulty *et al.*, 2008; Heinz *et al.*, 2010a, 2010b; Shanely *et al.*, 2010). Here we report the results of the first phase 1 clinical trial using quercetin in HCV patients with mild liver dysfunction. In addition, we report preliminary results on viral load to characterize possible antiviral activity. We also investigate quercetin's anti-hepatotoxic effects as measured by the change in levels of aspartate transaminase (AST) and alanine transaminase (ALT), two liver enzymes associated with hepatocyte injury and inflammation (Bala *et al.*, 2012).

METHODS

Patients. We recruited patients from August 2010 to June 2014 with the assistance of hepatologists at the University of California Los Angeles (UCLA) Pflieger Liver Institute. All patients provided written informed consent and the study was approved by the UCLA Office of the Human Research Protection Program. This study was conducted in accordance with the World Medical Association Declaration of Helsinki. Patients were informed of the newly FDA approved antivirals, but chose to enroll in this study because of financial reasons or preference for natural compounds. Eligibility criteria included: 18–65 years of age, either treatment-naïve or not currently taking any antiviral therapy, and contraindications to standard HCV therapies. Additional requirements were the following: detectable HCV RNA in the serum; stable viral load within the previous year (no fluctuation > 2-log scale); compensated liver disease (total serum bilirubin < 1.5 g/dL; INR < 1.5; albumin > 3.4 g/dL, platelet count > 125 000/mm³, no evidence of hepatic encephalopathy or ascites); acceptable liver enzymes (AST/ALT < 1.5 × upper limit of normal); acceptable hematological indices (hemoglobin > 13 g/dL for men and > 12 g/dL for women; neutrophil > 1500/mm³; AFP < 50 ng/mL); and acceptable

renal function (creatinine < 1.5 mg/dL). Exclusion criteria were the following: any other hepatitis etiologies or co-infection with HBV and/or HIV, HCC, a history of non-compliance, current alcohol use, pregnancy, breastfeeding, renal impairment, or renal transplant. As this is the first clinical trial testing quercetin's safety in patients with HCV who are known to have various degrees of liver dysfunction and given the liver is one of quercetin's primary sites of metabolism, we specifically excluded patients with more severe liver damage (AST/ALT > 1.5 × upper limit of normal, total serum bilirubin > 1.5 g/dL, platelet count < 125 000/mm³, abnormal coagulation studies). These eligibility criteria were established to avoid toxicities attributable to impaired clearance of potential quercetin toxic metabolites.

Outcomes. The main objectives were to determine the safety, tolerability, and the maximum tolerated dose (MTD) of quercetin. As secondary outcomes, we examined the change in patient viral load and liver enzyme levels (AST and ALT) between baseline and follow-up. A viral load decrease of 0.41-log (2.6-fold) compared with patient baseline was defined as a “clinically meaningful” decrease (Terrault *et al.*, 2005). A change in AST and ALT exceeding 2 standard deviations (SDs), calculated based on the patient sample at baseline, was considered a notable increase or decrease. The ratio of quercetin plasma concentration (µg/L) to dose level (mg), denoted as pq/d, was calculated as an indicator of the amount of the original dose retained in the plasma.

Follow up. At patient's study entry, we obtained a medical history, complete physical exam, and baseline laboratory assessment. Laboratory measurements (UCLA Laboratory, Los Angeles, CA) included complete blood count with differentials, chemistry (Na, K, Cl, HCO₃, BUN, creatinine, Mg, and Phos), liver function tests (AST, ALT, Alk Phos, Tbilirubin, Dbilirubin, and PT/PTT/INR), hepatitis C viral load, and cholesterol panel (LDL, HDL, Tchol, and triglycerides). These laboratory measures were repeated at clinic visits during weeks 2 and 4 after start of study treatment. At clinic visits, we also monitored patients' vital signs, weight, and adverse events. We defined adverse events according to an adapted NCI-Common Terminology Criteria for Adverse Events (NCI-CTCAE v4.0). Medical compliance was determined by patient attendance to clinic visits and the completion of symptom surveys. An additional follow-up clinic visit with laboratory assessment was obtained 1–2 weeks after dose completion, to assess the duration of viral load change observed while on quercetin (*n* = 22 patients). Plasma quercetin analysis of baseline and week 4 blood samples was performed at Appalachian State University-North Carolina Research Campus, according to protocol as previously described (Nieman *et al.*, 2007a, 2007b).

Dosing. This study used a dose escalation with overdose control (EWOC) design, a Bayesian adaptive Phase I design in which dosing starts at a predetermined level, doses for subsequent patients are chosen using information on adverse effects experienced by previous

patients, and dose escalation is slowed and stopped as the MTD is reached (Rogatko and Xu, 2005; Tighiouart *et al.*, 2005). The EWOC algorithm generates dose trees based on initial dose (250 mg), patient cohort size (3), maximum dose increment (500 mg), and maximum dose. The trial was initially designed with a maximum dose of 2000 mg and then amended for a maximum of 5000 mg. This resulted in the utilization of 11 different dose levels. Patients were instructed to take the quercetin chews orally for 28 days alongside juice or water, preferably at the same time each day.

Statistical analysis. The EWOC method was planned to be used to estimate the MTD (Rogatko and Xu, 2005; Tighiouart *et al.*, 2005); however, the MTD was not reached during the trial. Local regression, implemented with the *lowess* command in Stata 13 (Cleveland, 1979), was used to obtain nonparametric estimates of the relationship between quercetin plasma concentration and dose level and between viral load change and quercetin plasma/dose ratio. Since these relationships were approximately linear, we also summarized the strength of these associations using Pearson correlation coefficients.

RESULTS

Patient characteristics

Baseline clinical and demographic characteristics of the 30 study patients are shown in Table 1. Individuals with HCV genotypes determined through previous sequencing analysis were predominantly genotype 1a or 1b. Most patients had a white ethnic origin and were California residents. Fib-4 scores (a noninvasive index to assess liver scarring) for our patient population (Table 2) ranged from 0.38 to 4.15 with a median of 1.90 (Table 1), a score associated with an 80% chance of minimal to moderate liver fibrosis (Sterling *et al.*, 2006).

Patient adherence

Out of the 34 patients recruited, four withdrew from the study, and a substitute was found for one of the withdrawals, resulting in 30 evaluable patients included in analyses. One patient withdrew because of perceived low urine output but had normal urine and renal studies; one patient who was taking acetaminophen withdrew because of high AST and ALT levels, which came down after acetaminophen and quercetin were discontinued (the AST/ALT levels did not exceed the limit for adverse event reporting), and two patients withdrew because of distance. Compliance was high among the remaining 30 patients, with no adherence issues for any of these remaining patients.

Safety

Quercetin doses ranged from 250 to 5000 mg/day (Table 2). There were three patients per dose group,

Table 1. Patient characteristics (n = 30)

Patient characteristics	
Age, years	
Range	25–65
Sex, N (%)	
Male	20 (66.7)
Female	10 (33.3)
Ethnicity, N (%)	
Hispanic or Latino	3 (10.0)
Black or African American	2 (6.7)
White	25 (83.3)
Geographic region, N (%)	
California	28 (93.3)
Michigan	1 (3.3)
Arizona	1 (3.3)
HCV genotype, N (%)	
1 (a/b)	18 (60.0)
2 (a/b)	2 (6.7)
3 (a/b)	5 (16.7)
Unknown	5 (16.7)
Baseline viral load, IU/mL ^a	$1.0 \times 10^5 - 6.9 \times 10^7$ ($8.3 \times 10^6 \pm 1.2 \times 10^7$)
Baseline AST, U/L ^a	20–243 (67.7 ± 46.6)
Baseline ALT, U/L ^a	24–276 (91.9 ± 58.4)
Baseline Fib-4 ^b	0.38–4.15 (1.90)

Baseline viral load, AST, and ALT values presented as range (Mean ± SD). Fib-4 value presented as range (median).

AST: aspartate transaminase, ALT: alanine transaminase, N: sample size, HCV: hepatitis C virus, IU/mL: international units per milliliter, U/L: units per liter.

^aBaseline viral load, AST, and ALT values presented as range (mean ± SD).

^bBaseline Fib-4 value presented as range (median).

with the exception of three dose levels (2000, 3000, and 4000 mg/day), which had only two patients per group because of withdrawal from the study. High doses were well tolerated, with no adverse events or signs of toxicity. The MTD was not reached during the trial. There was no notable change from baseline measurement to week 2 and 4 measurements for complete blood count, complete metabolic panel, cholesterol panel, and coagulation study.

Liver enzyme response

While we observed several patients with notable AST or ALT decrease, there was no discernible pattern of liver enzyme change among the trial participants. Most patients' AST and ALT remained within 2 SD at week 4. However, there were a few patients with liver enzyme changes that exceeded this threshold (Fig. 1a and 1b). For ALT level changes beyond 2 SD, one patient decreased and one patient increased. For AST level changes beyond 2 SD, two patients decreased and one patient increased.

Viral load response

Fig. 2a and 2b present viral load change at weeks 2 and 4, respectively. There were 8 patients who exhibited a

Table 2. Patient data overview

Dose group (mg)	Patient no.	Fib-4 baseline	Plasma quercetin baseline (µg/L)	Plasma quercetin week 4 (µg/L)	% Change (baseline vs week 4)			
					Plasma quercetin	ALT	AST	Viral load
250	1 ^a	2.36	37.13	136.54	268%	-16%	-26%	-84%
	2 ^a	2.34	123.14	131.76	7%	4%	-5%	-69%
	3	2.52	32.98	232.34	604%	-10%	-29%	-15%
500	4	1.89	121.57	861.78	609%	-13%	-9%	3%
	5	2.65	25.07	966.67	3756%	-4%	-58%	78%
	6	1.56	33.54	1515.81	4419%	-17%	-11%	-8%
1000	7	4.15	62.53	2895.74	4531%	41%	36%	59%
	8	1.31	51.26	405.52	691%	7%	7%	-39%
	9 ^a	1.71	57.79	864.05	1395%	-9%	-5%	-31%
1400	10	1.88	76.16	559.79	635%	-16%	-11%	16%
	11	2.17	68.28	830.56	1116%	7%	4%	16%
	12	1.19	100.66	4216.33	4089%	-12%	4%	24%
2000	13	2.96	17.83	4030.97	22508%	-11%	-14%	-52%
	14	1.92	20.05	1243.43	6102%	-13%	-16%	77%
2500	15	2.38	37.84	2871.57	7488%	90%	7%	645%
	16	2.75	4.3	3769.9	87474%	-35%	-29%	-3%
	17 ^a	1.12	32.72	1778.88	5337%	5%	9%	-85%
3000	18	2.83	31.9	4856.34	15124%	-26%	-9%	32%
	19	0.82	31.09	2505.54	7958%	-15%	-10%	28%
3500	20 ^a	0.38	64.06	822.76	1184%	-19%	-20%	-86%
	21	0.73	179.39	620.32	246%	4%	-5%	-41%
	22	1.32	8.94	4010.93	44785%	2%	-11%	31%
4000	23	1.94	3.79	3875.33	102036%	-8%	-13%	53%
	24	2.61	19.76	3296.24	16581%	-9%	-10%	-35%
4500	25	0.83	2.34	3308.53	141366%	-11%	-15%	-61%
	26 ^{ab}	1.90	22.54	3096.78	13639%	20%	3%	-87%
	27	0.90	20.13	4362.27	21567%	8%	11%	-16%
5000	28	1.89	11.83	5467.32	46101%	-8%	6%	155%
	29 ^b	1.04	9.77	2010.24	20469%	10%	13%	26%
	30 ^b	1.95	5.65	2132.26	37626%	-80%	-73%	-64%

^a0.41-log antiviral response at week 4.

^b0.41-log antiviral response at week 2.

AST: aspartate transaminase, ALT: alanine transaminase, Fib-4: Fibrosis-4 Score, mg: milligram, no.: number, µg/L: microgram per liter.

“clinically meaningful” decrease in HCV (2.6-fold or 0.41-log change) at week 2 and/or week 4. Two individuals had a meaningful decrease only at week 2. Three other patients decreased only at week 4. An additional three patients had decreases at both weeks 2 and 4. Two patients had a viral load increase over threefold. The HCV genotypes of the patients with 0.41-log viral decrease were similar to those among the total patient sample.

Plasma quercetin measurements

The mean plasma quercetin level among patients at baseline was 44 µg/L (SD 42 µg/L) and ranged from 2 to 179 µg/L. The mean plasma level at week 4 was 2256 µg/L (SD 1595 µg/L). There was a positive association between quercetin dose and plasma level at week 4 (Fig. 3), with Pearson correlation coefficient $r=0.60$ ($p<0.05$). Change in plasma quercetin concentration from baseline to week 4 was not associated with age, weight, sex, or HCV genotype (all $p>0.05$).

Liver enzyme (AST and ALT) changes did not have a discernible relationship with week 4 plasma quercetin

across the different doses. The correlations were $r=-0.03$ ($p=0.86$) between AST change and dose and $r=-0.17$ ($p=0.38$) between ALT change and dose. The majority of patients showed changes within 20% of their baseline value.

There was no clear association between plasma quercetin and relative viral load change (Table 2), with Pearson correlation coefficient $r=0.02$ ($p=0.92$). One outlier with a large increase in viral load was observed.

Patients with at least a 0.41-log viral load decrease tended to have the lowest levels of plasma quercetin when compared with other patients in their dose groups (Table 2). Based on this observation, we further investigated the relationship between viral load change and a measure of quercetin retained in the plasma, defined as the ratio of plasma quercetin concentration at week 4 to dose level (pq/d). There was significant correlation ($r=0.41$, $p=0.03$) between percent viral load change and pq/d (Fig. 4), indicating a tendency for viral load decrease in patients with less quercetin from their original dose detected in the plasma (lower pq/d). Conversely, individuals who showed an increase in viral load tended to have more quercetin in the plasma relative to their original dose (higher pq/d).

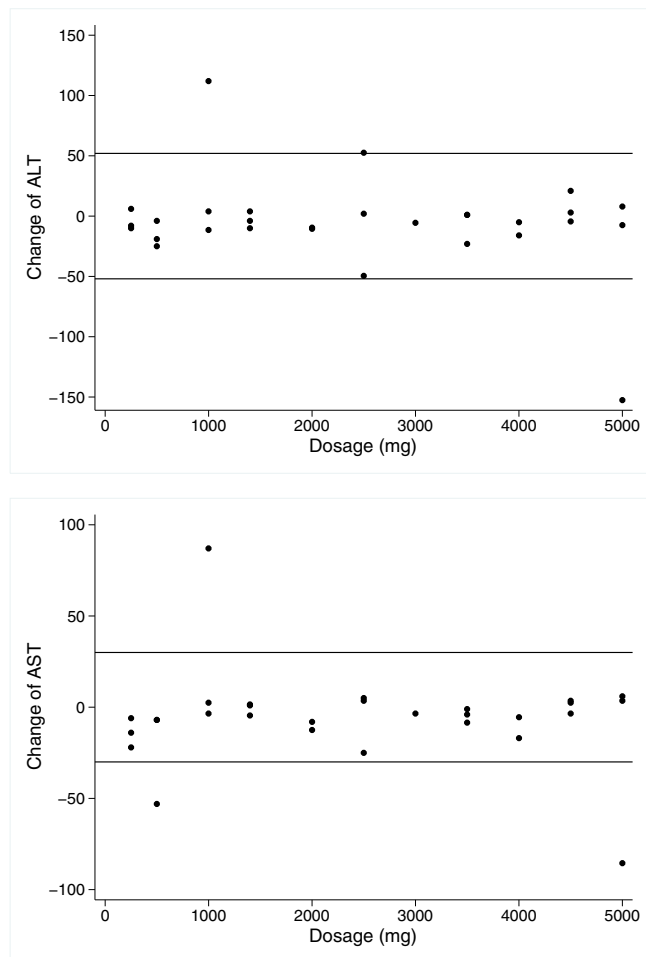


Figure 1. (A) ALT change (week 4). Change in ALT (week 4 minus baseline) by dose. Reference line: ± 2 SD. (B) AST Change (week 4). Change in AST (week 4 minus baseline) by dose. Reference line: ± 2 SD. This figure is available in colour online at wileyonlinelibrary.com/journal/ptr.

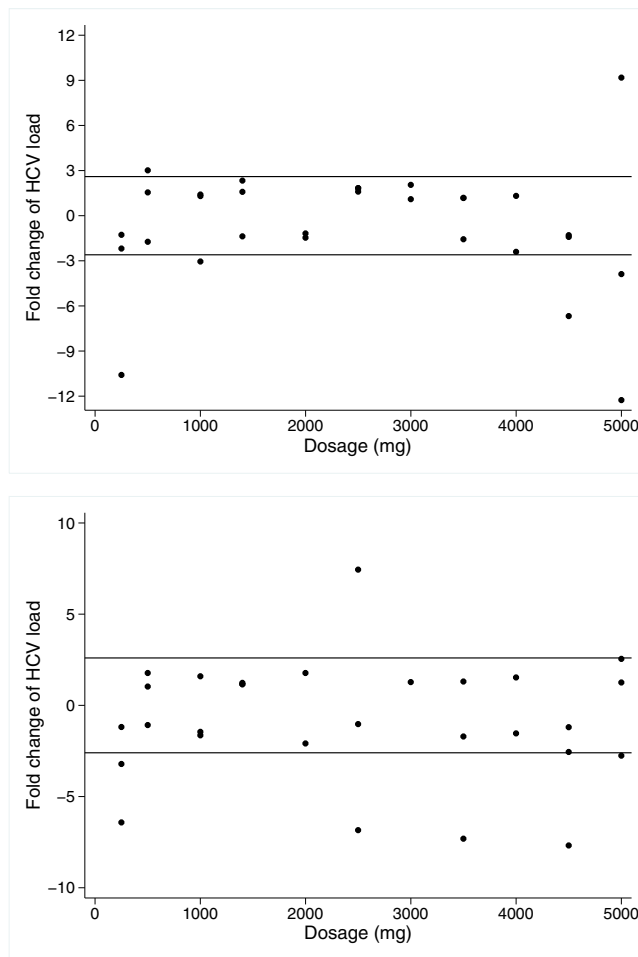


Figure 2. (A) Viral load change (Week 2). Fold change in HCV load (week 2 compared with baseline) by dose. Reference lines: ± 2.6 fold (± 0.41 -log). (B) Viral load change (Week 4). Fold change in HCV load (week 4 compared with baseline) by dose. Reference lines: ± 2.6 fold (± 0.41 -log). This figure is available in colour online at wileyonlinelibrary.com/journal/ptr.

DISCUSSION

We report a clinical trial administering more than 1 g of quercetin daily as an oral supplement, which is the highest dose level examined in clinical trials. Our primary goal of assessing safety was achieved with results indicating that chronically infected hepatitis C patients can tolerate this supplement up to 5000 mg daily for 28 days without adverse events or abnormal laboratory measurements. Due to its excellent tolerability it may prove to be safe for long-term use in other affected populations, such as individuals with HCV-associated liver dysfunction. Furthermore, viral load results suggested that quercetin could potentially have some antiviral activity in some patients. Individuals with a decrease in HCV had a lower level of plasma quercetin in comparison to other patients in the same dose group. This lower plasma quercetin level suggests that the compound may have been removed more efficiently from the bloodstream by the liver (and/or kidneys). However, because of the preliminary nature of this dose escalation clinical trial, we cannot conclude that quercetin has antiviral activity without further investigation. Since this phase I study has affirmed quercetin's safety, future trials may now be conducted to more specifically investigate quercetin's potential as an antiviral compound.

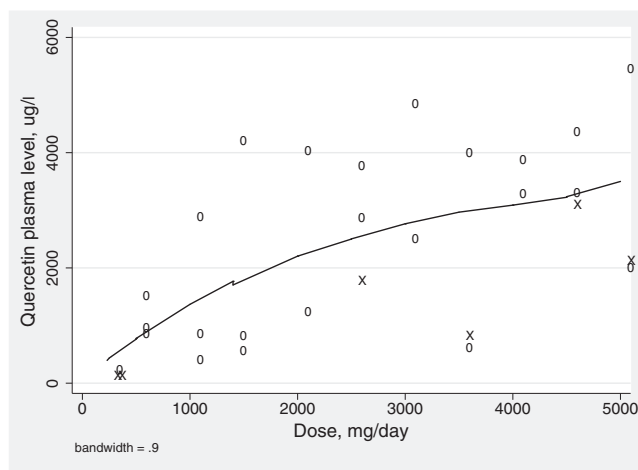


Figure 3. Quercetin dose curve. Scatterplot of quercetin plasma level at week 4 and quercetin dose with local regression line. X = patients at week 4 who displayed at least a 0.41-log (2.6 fold) viral load decrease. This figure is available in colour online at wileyonlinelibrary.com/journal/ptr.

Limitations

One limitation was the strict AST/ALT criteria used to assess patient eligibility. Enrolling HCV patients with

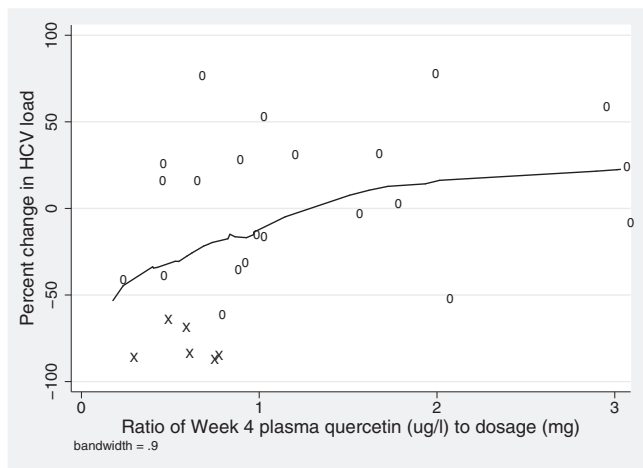


Figure 4. Viral load and plasma quercetin relative to dose. Scatterplot of percent increase in HCV load and quercetin ratio, defined as ratio of week 4 plasma quercetin ($\mu\text{g/L}$) to dose (mg), with local regression line. Two patients with outlying values omitted (Patients 17 and 32). X = patients at week 4 who displayed at least a 0.41-log (2.6 fold) viral load decrease.

less severe liver enzyme dysfunction ($\text{AST/ALT} < 1.5$ of ULN) potentially limits our study towards patients without a large enough AST/ALT baseline elevation to demonstrate a significant decrease in these liver enzyme levels. In addition, there may have been variation in the timing of quercetin ingestion. The time when patients took their daily quercetin may not have been uniform (pre/post meal). Similarly, laboratory tests could have been drawn at different time points in relation to a patient's most recent dose. This could lead to extra variation in AST/ALT , viral load, and plasma quercetin measurements that obscured associations.

The short duration of quercetin administration, 28 days, is another feature of the study that must be considered when interpreting the measurements for viral load and liver enzymes AST/ALT . The 4-week drug phase was selected because the primary objective was to assess safety and the MTD of quercetin in chronic HCV patients. However, antiviral studies are typically designed to be at least 12 weeks in length. Thus, the short duration of this study may not have captured quercetin's potential as an antiviral compound. The shorter time frame also may not have been adequate to detect changes in AST and ALT . Future studies with a primary goal of evaluating quercetin's antiviral capabilities would require a longer drug phase, of at least 12 weeks or even longer (24 weeks) in the case of HCV relapse. These standard trial durations are 3–6 times longer than our study.

Safety and tolerability

All patients tolerated the oral quercetin supplement (250–5000 mg daily) without significant adverse events. Some patients reported that taking quercetin without food resulted in mild stomach discomfort, which was relieved if quercetin was taken after a meal.

Published clinical trials have established the safety and nutritional benefits of up to 1 g of daily quercetin (Nieman *et al.*, 2007a, 2007b; McAnulty *et al.*, 2008; Heinz *et al.*, 2010a, 2010b; Shanely *et al.*, 2010). Our study confirms that quercetin is safe up to 5 g daily. The MTD was not reached during this trial, implying

that the MTD of quercetin is higher than 5 g. If higher bioavailability is required, utilization of quercetin above 5 g is both reasonable and feasible.

Liver enzymes

The absence of a consistent AST/ALT decrease in our patient sample suggests that quercetin lacked a significant effect on these liver enzymes over the short duration of the trial. Nevertheless, our results also indicate that quercetin did not exacerbate liver enzymes (AST/ALT levels below grade 3/4 adverse event reporting) in these patients with chronic HCV. This suggests that quercetin could be well tolerated for future trials in patients with more severe liver dysfunction.

Viral load

The majority of patients with a 0.41-log viral load decrease were in dose groups of 2500–5000 mg, which could be taken as encouragement that quercetin may have some inhibitory effect on HCV. Quercetin's antiviral effect was previously demonstrated *in vitro*, through the inhibition of HSP (Gonzalez *et al.*, 2009). Although quercetin's *in vitro* antiviral activity is stronger than the results seen in our trial, some indications of a viral decrease are encouraging and suggest that quercetin's mechanism of action merits further investigation.

Hypothesized antiviral mechanism

We found a correlation between plasma quercetin/dose (pq/d) ratio and viral load change, such that a smaller ratio was associated with larger viral load decreases. The pattern of patients with lower plasma quercetin/dose ratio experiencing a larger viral decrease may seem counterintuitive at first. However, because the site of quercetin's antiviral activity against HCV is likely in the liver, this ratio may reveal differences in quercetin uptake. Bieger *et al.* describe how quercetin accumulates in the liver and kidneys where it is further metabolized and excreted. In pigs, quercetin can reach levels at least fourfold higher in the liver than in the plasma (Bieger *et al.*, 2008). Research in liver cells (HepG2) also demonstrates that human hepatocytes are capable of concentrating quercetin intracellularly (Boulton *et al.*, 1999). Various studies support that quercetin absorption is mediated through active uptake by sodium-dependent glucose transporters (SGLT1) (Gee *et al.*, 2000; Ader *et al.*, 2001) or the deglycosylation at the cell surface by lactase phlorizin hydrolase and subsequent passive diffusion (Day *et al.*, 2000; Sesink *et al.*, 2003). It is hypothesized that this deglycosylated metabolite is responsible for quercetin's biological activity (Ishisaka *et al.*, 2013).

Clinical trial "responders" with lower pq/d may have higher levels of quercetin in the liver, possibly providing an explanation for decreased viral loads. Specifically, variations in the liver's uptake of quercetin could be responsible for differential levels of plasma quercetin (Fig. 5). In individuals with a low pq/d, more quercetin may accumulate in the liver, resulting in a lower level detected in the plasma. Subsequently, quercetin levels in liver tissue may be high enough to exert antiviral activity.

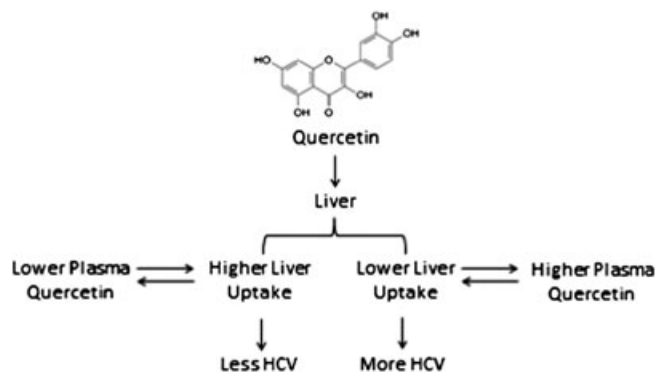


Figure 5. Hypothesized antiviral mechanism.

Alternatively responders may generate higher levels of specific quercetin metabolites that are responsible for antiviral activity, regardless of quercetin liver uptake.

This hypothesis is preliminary and exploratory and further investigation is required to clarify quercetin’s antiviral mechanism. Future clinical trials could better elucidate quercetin antivirals by examining liver tissue in order to directly gauge quercetin liver uptake instead of indirectly measuring these values through plasma concentration. Similarly, additional research on quercetin’s metabolomic profile in patients with and without significant viral change would better characterize the relationship between specific quercetin metabolites and possible antiviral activity.

Clinical applications

Quercetin’s antiviral capabilities have yet to be confirmed. However, accessible natural compounds could have an application in the landscape of current HCV treatment options. Unlike standard HCV drugs that directly target the virus, quercetin may exert antiviral pressure both directly (inhibiting viral proteins) and indirectly (inhibiting host proteins). Potentially, antiviral bioflavonoids could be beneficial in combination with other HCV treatments. An antiviral with multi-level activity could discourage HCV recurrence in patients treated with standard compounds. Although interferon-free direct antiviral agents (DAAs) have not been in clinical practice long enough to fully gauge relapse

rates, viral resistance has continued to be an obstacle throughout the history of HCV treatment. Recent studies show that post-treatment viral relapse is a prominent issue in a subset of patients with HCV treated with a combination of interferon, RBV, and DAAs (McHutchison *et al.*, 2009; Fried *et al.*, 2013; Kowdley *et al.*, 2013). Therefore, HCV treatment strategies could consider applying accessible bioflavonoid compounds to maintain antiviral pressure post-treatment to prevent relapse, preserve DAA efficacy, and optimize the antiviral strategy in lowering the risk of associated liver complications.

The high cost of new FDA approved DAAs creates a need to find alternative antiviral strategies. If quercetin shows anti-HCV activity in future trials, this compound could be an alternative option for patients who cannot access expensive mainstream drugs. This approach has the potential to reach the most affected HCV demographics in a cost-effective manner.

In conclusion, this phase I study demonstrates that quercetin is well tolerated and results suggest that quercetin may have some inhibitory effect on HCV. Future trials should further investigate this natural compound’s mechanism and its potential to become a safe and affordable antiviral for chronic hepatitis C patients.

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Conflict of Interest

All authors have nothing to disclose.

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APPENDICES

HCV: hepatitis C virus
 HCC: hepatocellular carcinoma
 AST: aspartate transaminase
 ALT: alanine transaminase
 PEG-IFN: pegylated interferon
 RBV: ribavirin
 HSP: heat shock protein
 NS3: nonstructural protein 3
 NS5A: nonstructural protein 5A
 UCLA: University of California, Los Angeles
 RNA: ribonucleic acid
 HBV: hepatitis B
 HIV: human immunodeficiency virus
 MTD: maximum tolerated dose
 SD: standard deviation
 Na: sodium
 K: potassium
 Cl: chloride
 HCO₃: bicarbonate
 BUN: blood urea nitrogen
 Mg: magnesium
 Phos: phosphorus
 Alk Phos: alkaline Phosphatase
 Tbilirubin: total bilirubin
 Dbilirubin: direct bilirubin
 PT: prothrombin Time
 PTT: partial thromboplastin Time
 INR: international normalized ratio
 AFP: alpha-fetoprotein
 LDL: low-density lipoprotein
 HDL: high-density lipoprotein
 Tchol: total cholesterol
 NCI-CTCAE v4.0: NCI-Common Terminology Criteria for Adverse Events Version 4.0
 n: sample size
 ASU-NCRC: Appalachian State University-North Carolina Research Campus
 EWOC: escalation with overdose control
 pq/d: ratio of plasma quercetin concentration at week 4 to dose level
 SGLT1: sodium dependent glucose transporters
 LPH: lactase phlorizin hydrolase
 DAAs: direct antiviral agents
 FDA: Food and Drug Administration
 LLC: limited liability company
 TCRF: Tower Cancer Research Foundation
 AACR: American Association for Cancer Research
 NIDDK: National Institution of Diabetes, Digestive and Kidney Disease
 ULN: Upper limit of normal
 dL: deciliter
 mm³: cubic meter
 L: liter
 g: gram
 mg: milligram
 µg: microgram
 g/dL: gram per liter
 mg/dL: milligram per liter
 µg/L: microgram per liter
 p: p-value
 r: Pearson correlation coefficient
 IU/mL: international units per milliliter

U/L: units per liter

no.: number

UCLA-OHRPP: University of California Los Angeles

Office of the Human Research Protection Program

WMA: World Medical Association

Fib-4: Fibrosis-4