Combination Effects of Quercetin, Resveratrol and Curcumin on *In Vitro* Intestinal Absorption

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ABSTRACT

Objective: Quercetin, resveratrol and curcumin are plant derived natural products that are rapidly gaining popularity as supplements for a wide assortment of conditions including cardiovascular disease, cancer, asthma, diabetes, neurodegeneration, aging and stress. Unfortunately the therapeutic potential of these compounds is limited by their poor intestinal and intracellular bioavailability. Therefore this study sought to examine how combinations of quercetin, resveratrol, and curcumin, with and without piperine, 200 nM, affected an *in vitro* permeability model using apical-to-basal permeability across intact caco-2 monolayers. Quercetin, resveratrol and curcumin were applied apically alone or in combination at 50 μM and measured in the basal chamber at 30 min.

Results: Resveratrol received the greatest enhancement in permeability when combined with other agents: quercetin (310%), curcumin (300%), quercetin and curcumin (323%, 350% with piperine). Curcumin also demonstrated increased permeability when combined with quercetin alone (147%) and both quercetin and resveratrol (188%), addition of piperine resulted in a 229% increase in permeability. Quercetin permeability was not significantly affected using any combination, but showed maximal permeability when combined with resveratrol and the lowest permeability when combined with resveratrol, curcumin and piperine together.

Conclusion: Combination of quercetin, resveratrol and curcumin may improve intestinal absorption of resveratrol and curcumin without affecting quercetin absorption. These data highlight the need for further research and suggest that developing combination therapies may improve intestinal absorption of these constituents. Our study also demonstrates that the apical-to-basal permeability across intact caco-2 monolayer model is a viable model to investigate absorption of natural compounds.

Keywords: Quercetin; Resveratrol; Curcumin; caco-2; Permeability; Intestinal absorption

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INTRODUCTION

Quercetin, resveratrol and curcumin are increasingly being researched due to their apparent therapeutic activity in numerous disease states.[1–9] Quercetin, a ubiquitous flavonol found in most land-plants including many foods and dietary supplements, has demonstrated beneficial effects in inflammatory and immune conditions including cardiovascular disease, cancer, asthma/allergies and stress.[1–3] Resveratrol, a naturally produced stilbenoid found in Japanese knotweed (*Fallopia japonica*) and more famously in the skin of red grapes (*Vitis vinifera*), has displayed antioxidant, anti-inflammatory and anticarcinogenic effects and may be beneficial in preventing complications associated with diabetes, atherosclerosis, aging and metabolic disease.[4–6] Resveratrol appears to work through the activation of SIRT1 (silent mating-type information regulation 2 homolog 1) or AMPK (5′-adenosine monophosphate-activated kinase) pathways.[4, 6] Finally, curcumin, a natural phenolic compound (diarylheptanoid) found in turmeric (*Curcuma longa*), is a well-studied inhibitor of the inhibitory kappa B alpha kinase (IκBα kinase), a key activator of nuclear-factor κB (NF-κB).[7] Blocking this central mediator makes curcumin therapeutically active in various inflammatory disease states including arthritis, cardiovascular disease, metabolic disease, neurodegeneration and tumerogenesis.[7–9]

Unfortunately, despite the impressive therapeutic potential of these compounds, they have also demonstrated poor bioavailability when given orally. This feature limits their use as supplements because large doses are required to achieve therapeutic intracellular levels.[3, 10, 11] Curcumin bioavailability has been improved by delivering it in liposomal preparations or by coadministering it with piperine.[12, 13] Piperine is a piperidine alkaloid found in the fruit of the black pepper (*Piper nigrum*), and is responsible for the pungency of black pepper spice. Piperine's effect on curcumin bioavailability is believed to be due to its inhibition of phase II glucuronidation;[14] piperine may also improve intestinal epithelial uptake by inhibiting P-glycoprotein-1 (P-gp; a broad-acting xenobiotic efflux transporter).[15] Interestingly, there is also evidence to suggest quercetin is also a P-gp inhibitor for some compounds; in fact, many herbs/constituents have demonstrated modulation of this efflux pump.[15, 16]

Quercetin, resveratrol and curcumin, despite their various botanical origins, all share similar biosynthesis pathways originating with 4-hydoxycinnamic acid of the shikimate pathway – a major synthesis pathway utilized by plants to synthesize aromatic/phenolic secondary compounds and amino acids. The molecular topography of these three compounds also display similar features which suggests they may share targets for absorption or efflux and may alter each other's uptake (Figure 1). Therefore, we hypothesized that concomitant

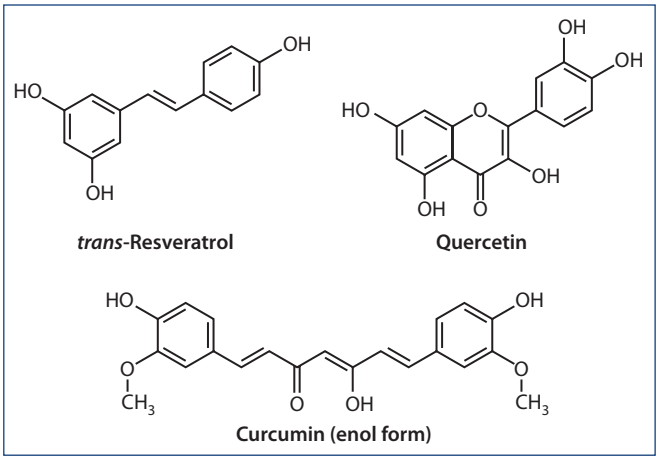

Figure 1: Molecular structures of resveratrol, quercetin and curcumin demonstrating the relative similarities of placement of hydroxyl groups along unsaturated carbon chains and the presence of several phenolic groups.

exposure of all three compounds will increase the apical-to-basal absorption of these constituents.

MATERIALS AND METHODS

REAGENTS

Cell culture reagents: Eagle's minimum essential media (EMEM), Hank's balanced salt solution (HBSS), Pen-strep and trypsin-ethylenediaminetetraacetic acid (EDTA) (0.25%) were purchased from Mediatech (Corning, VA, USA); fetal bovine serum (FBS) was purchased from Atlanta Biologicals (GA, USA). High performance liquid chromatography (HPLC) reagents: acetic acid, sodium acetate, methanol and acetonitrile were purchased from Sigma-Aldrich (MO, USA). Samples of quercetin, resveratrol and curcumin and a blend of curcumin and piperine (PC) were gifts from Gaia Herbs (NC, USA). Additional standards of quercetin, resveratrol, curcumin and piperine were purchased from Sigma-Aldrich.

Stocks of quercetin, resveratrol, curcumin and piperine were prepared in methanol at 1 mg/mL. Stocks were stored at −20°C and monitored regularly for stability. The PC blend was prepared as a 1 mg/mL stock solution and assayed to contain 148 μM curcumin and 0.6 μM piperine.

CELL CULTURE

Caco-2 cells obtained from the American Type Culture Collection (ATCC, Virginia, USA) were cultured in EMEM supplemented with 10% FBS and 1% penicillin-streptomycin. Cells were maintained in 75-cm^2 tissue culture flasks and grown in a 5% CO_2 incubator at 37°C and saturating humidity with media changes every 2–3 days. Cells were passaged by rinsing with PBS and detachment with 0.25% trypsin and 0.5 mM EDTA.

IN VITRO ABSORPTION

For *in vitro* absorption studies cells were harvested and seeded in 24-well plates fitted with polycarbonate Transwell® inserts (6.5 mm i.d., 0.4-m pore size, 0.3 cm^2 of growth area, Corning Costar Co., NY) at a density of 75,000 cells/insert. Cells in the inserts were grown for 21 days and the transepithelial electrical resistance (TEER) measured regularly to monitor the monolayer integrity. Only wells that had a TEER value greater than 400 Ω(cm^2) were used for absorption experiments (TEER = ($R_{monolayer}$ − R_{blank}) × Area).

Inserts with intact monolayers were rinsed with HBSS and transferred to a clean 24-well plate and allowed to equilibrate in HBSS for 1 h. After equilibration TEER was verified and HBSS on the apical side was replaced with HBSS containing the treatments alone or in combination (quercetin, resveratrol and curcumin at 50 μM and piperine at 200 nM). After 30 min 700 μL of HBSS was removed from the basal compartment and immediately combined with an equal volume of 10 mM acetic acid in an HPLC sample vial. Samples were then immediately analyzed by HPLC.

HPLC

Quantitation of quercetin, resveratrol and curcumin was accomplished using an Alliance HPLC system with 2998 photodiode array (Waters Corporation, MA, USA) equipped with an Agilent Eclipse XDB-C18 column (4.6 × 50 mm, 3.5 μm) at 35°C. Mobile phase consisted of A: 0.1 M acetate buffer (pH 4.0) and B: acetonitrile. Flow rate was 0.6 mL/min and gradient conditions were 0 min, 75% A; 8 min, 55% A; 18 min, 55% A followed by 5 min of equilibration at 75% A. Detection was done using the PDA at 306 nm for resveratrol, 364 nm for quercetin and 428 nm for curcumin.

DATA ANALYSIS

The apparent permeability coefficient (P_{app}, cm/s) for each compound/combination was calculated using the following equation:[17, 18]

$$P_{app} = \frac{\frac{dC_b}{dT}}{C_a * A}$$

where (dC_b/dT) is the rate of appearance of the constituent on the basal side (nmol/s), C_a is the concentration of the constituent on the apical side (50 μM) and A is the area of the monolayer (0.3 cm^2). The apparent permeability coefficient is property of individual compounds which describes its ability

to pass through a cell monolayer under the experimental conditions.

Each treatment or treatment combination was repeated three times in separate wells, using new caco-2 monolayers for each experiment. P_{app} values ($n = 3$) between constituent alone and their combinations were compared using the Student's *t*-test (two-tailed, unpaired), statistical significance was assumed at a level of $\alpha = 0.05$.

RESULTS

HPLC ANALYSIS

Quercetin, resveratrol and total curcumanoids were well resolved using this method and quantitation was linear in the range tested (0.15–110 μM) (Figure 2). Quercetin had a retention time of approximately 4.7 min, resveratrol 3.6 min and curcumin displayed the typical three curcuminoids: bisdemethoxycurcumin (11.4 min), demethoxycurcumin (12.1 min) and curcumin (12.9 min) (Figure 2). Only curcumin was detected in the basal chamber in the absorption experiments. Piperine, when included, co-eluted with demethoxycurcumin at 12 min and was detected in the basal chamber, but was not quantitated.

CACO-2 MONOLAYER PERMEABILITY

Each of the three compounds tested showed consistent transport across caco-2 monolayers. When applied singly, quercetin showed the highest apparent permeability ($P_{app} \times 10^{-6}$, cm/s) (10.09 ± 0.77) followed by resveratrol (7.77 ± 1.10) and then curcumin (0.99 ± 0.11) (Figure 3). In combination, both resveratrol and curcumin achieved significantly higher permeability. Quercetin did not receive any significant benefit from combination, but showed the highest permeability when paired with resveratrol (11.90 ± 0.52) and the lowest when combined with resveratrol, curcumin and piperine (8.89 ± 0.40; Figure 3).

Resveratrol, when combined with quercetin and curcumin, showed a significant increase in P_{app} (7.70 ± 1.10 to 25.09 ± 0.48), inclusion of piperine resulted in a further increase to 27.22 ± 0.89. In order to determine if either quercetin or curcumin were responsible for this effect, they were applied singly with resveratrol; however both caused a similar increase in P_{aap} (24.92 ± 0.68 and 24.45 ± 0.69, respectively) (Figure 3). Curcumin demonstrated a similar pattern to resveratrol when applied in combination, though the magnitude was not as great. When paired with quercetin or resveratrol alone, P_{app} increased moderately but not significantly (1.46 ± 0.15 and 1.25 ± 0.07, respectively). When all three were combined there was a significant increase in P_{app} (1.85 ± 0.13), the addition of piperine increased the P_{app} to 2.26 ± 0.21. Interestingly, the greatest effect was observed with curcumin and piperine alone which resulted in a P_{app} of 2.33 ± 0.12 (Figure 3). Together this suggests an additive effect of resveratrol and quercetin on curcumin absorption and that quercetin and resveratrol absorption are also affected by piperine and may compete with curcumin in these pathways.

DISCUSSION

The transepithelial permeability across intact monolayers using caco-2 cells is a well validated and accepted method to model intestinal absorption.[17, 19] It is commonly employed as a preliminary screen to determine transport barriers in the development of pharmaceuticals and recently has been employed to investigate the absorption of natural compounds.[17, 20, 21] The current study demonstrates that this model may also provide a cost-effective method to screen absorption characteristics of naturally derived compounds singly or in combination therapies and to provide preliminary data to support *in vivo* studies.

The main limiting factors of this experiment were 1) poor aqueous solubility of the test substances, and 2) poor aqueous stability of quercetin at physiological pH.[22] In order to prevent precipitation of the test substances alone or in combination, a concentration of 50 μM was chosen. This concentration was stable over the time-frame of the experiment, including analysis time (data not shown). In order to overcome the instability of quercetin in aqueous solutions at pH of 7.4, the treatments were prepared immediately before the experiment and the exposures were limited to 30 min. After 30 min the samples were immediately acidified which

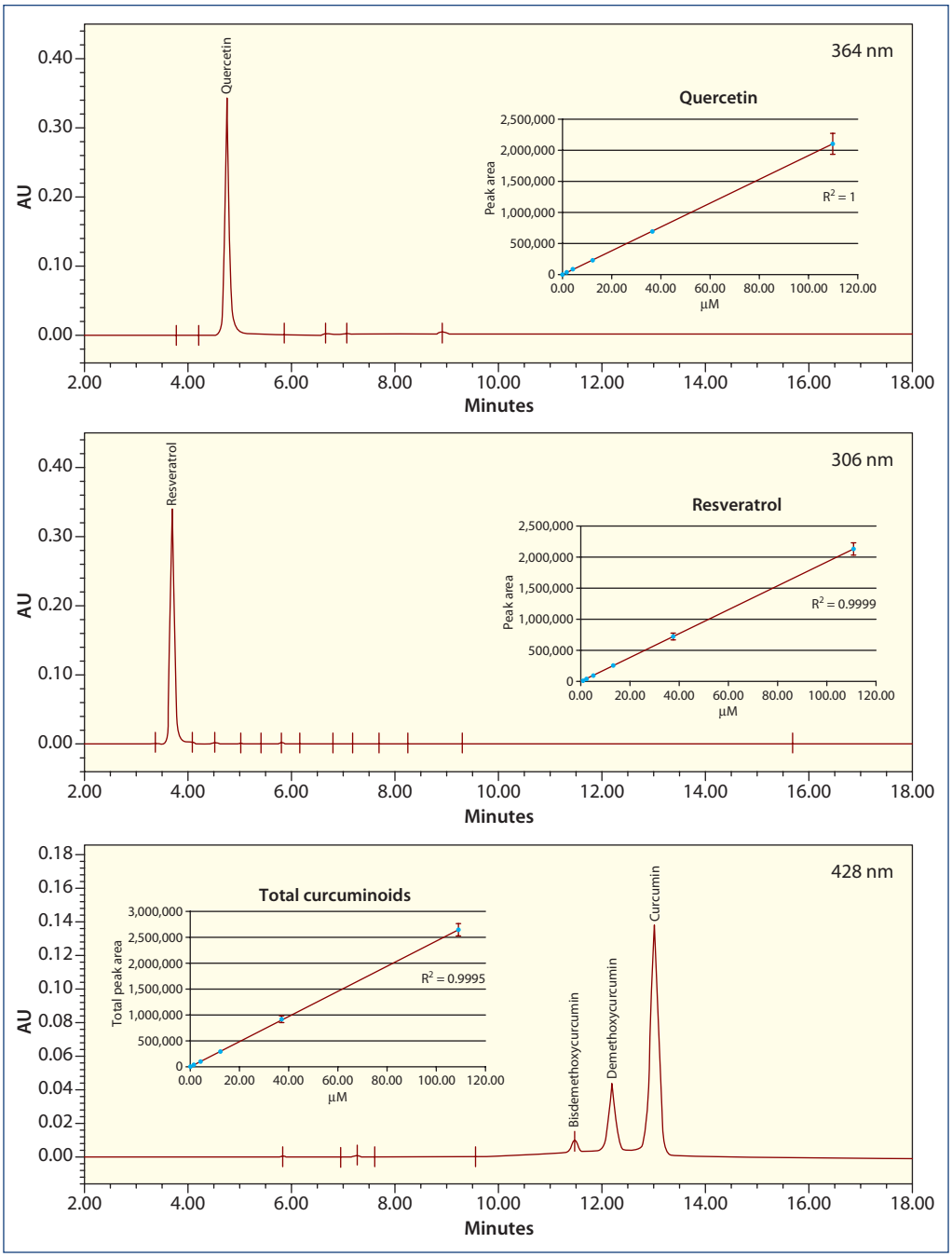

Figure 2: Representative chromatographs and standard curves of quercetin (364 nm), resveratrol (306 nm) and curcumin (428 nm). Seven point standard curves were calculated based on the average peak area ± SD (*n* = 3) at each concentration (0.15–110 µM); curcuminoid standard curve was calculated from the total peak area of the three major curcuminoids.

stabilized the quercetin for analysis (Figure 4). An aqueous concentration (50 µM) was chosen in favor of higher concentrations in non-aqueous solvents (DMSO, dimethylformamide, etc.) in order to better approximate oral ingestion of these supplements (i.e. capsule taken with water).

This study demonstrates that applying quercetin, resveratrol and curcumin in combination can significantly increase apical-to-basal uptake of curcumin and resveratrol. Although quercetin absorption did not significantly increase from these combinations, it did not decrease either – no significant change

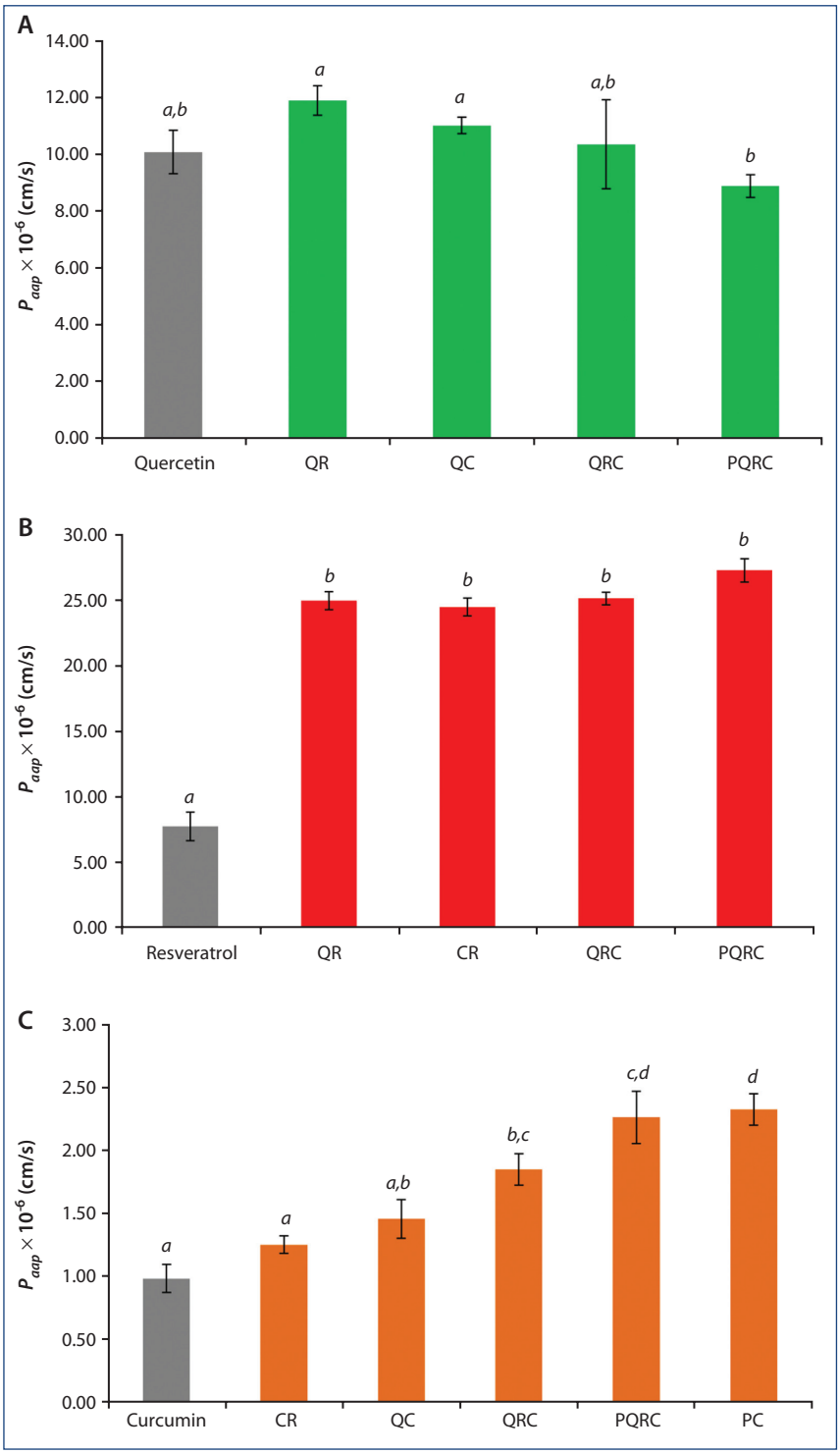

Figure 3: Apical-to-basal permeability of (A) quercetin (Q), (B) resveratrol (R) or (C) curcumin (C), alone or in combination across intact caco-2 monolayers. Compounds were applied apically at 50 µM (piperine (P) at 200 nM) and measured in the basal compartment at 30 min. Data is expressed as the average $P_{app} \times 10^{-6}$ (cm/s) of three separate experiments ± SD. Bars labeled with different letters are significantly different from each other, $P < 0.05$.

from quercetin alone was observed when combined with the other compounds. It is worth mentioning, however, that quercetin showed the lowest absorption in the PQRC combination suggesting piperine may adversely affect quercetin permeability. In contrast, adding piperine appears to have a positive effect on

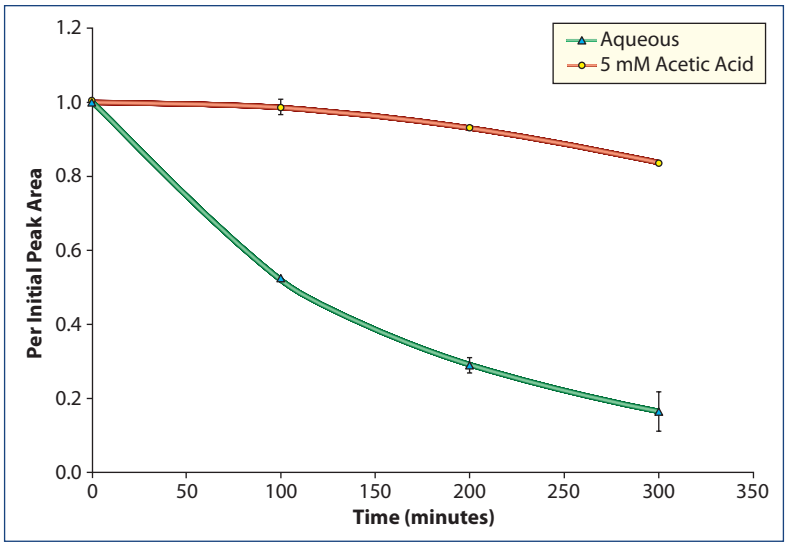

Figure 4: Stabilization of quercetin using acetic acid. Quercetin (25 µM) is rapidly degraded in aqueous solutions at pH 7.4 (green line). In order to preserve quercetin for analysis, samples were acidified using 10 mM acetic acid (final concentration 5 mM, red line). Data is expressed as the ratio of the quercetin peak area at the indicated times over the initial area (t_n/t_0) ± SD; n = 3.

apical-to-basal permeability, with resveratrol and curcumin. This latter observation is consistent with a previous study that showed improved bioavailability of curcumin when applied with piperine.[12]

Resveratrol absorption has been shown to be primarily passive, but suffers from extensive sulfate and glucuronidate conjugation reactions.[23, 24] The increases in resveratrol permeability observed in this study may be due to inhibition of these conjugation reactions and blockade of resveratrol apical export. Regardless of the combination, resveratrol demonstrated a significant increase in permeability and there were no differences observed between combinations; this may suggest that there is overlap in the mechanisms and at the concentrations used the effect was saturated.

Quercetin is known to affect the pharmacokinetics of various compounds by modulating transport and metabolism, some of the observed effects include: inhibition of CYP3A4 (IC_{50} 2 µM),[25, 26] inhibition of phenol sulfotransferase (SULT 1A1) (IC_{50} 13 nM)[24, 27, 28] and inhibition the P-gp efflux pump.[15, 16, 25, 29, 30] These activities could explain increased permeability of curcumin and resveratrol in the presence of quercetin.

Curcumin has also demonstrated inhibition of P-gp and CYP3A4;[15, 31, 32] however, resveratrol does not appear to be exported by P-gp, rather it is exported by the breast cancer resistance protein (BCRP) and multidrug resistance-associated protein 2 (MRP2).[33, 34] Recent evidence also suggests that curcumin inhibits BCRP, which may explain the improved permeability of resveratrol observed with curcumin in the current study.[35]

Similarly, piperine has demonstrated various activities which may contribute to its effect on permeability. Like quercetin and curcumin, piperine inhibits CYP3A4 and P-gp.[15] Piperine also inhibits glucuronidation[14] and may affect MDR1 and BCRP.[36] These activities have been attributed to its effect on curcumin permeability, but this data shows that it may also contribute to absorption of resveratrol since there was a modest rise in P_{aap} when piperine was included.

As mentioned previously, these compounds are products of the same biosynthetic pathway (shikimate) and share several structural elements (Figure 1). It is not surprising therefore that the literature suggests that these compounds utilize and modulate similar pathways for membrane transport and metabolism. Collectively they may cause a substantial effect on P-gp, BCRP, CYP3A4 and other enzymes of xenobiotic metabolism. It is likely that the concerted inhibition, competition or modulation of membrane transporter and biotransformation enzymes of

quercetin, resveratrol, curcumin and piperine results in the improved acute permeability of resveratrol and curcumin observed in the current study.

CONCLUSIONS

Quercetin, resveratrol and curcumin may provide substantial therapeutic benefit especially in aging and chronic inflammatory diseases. This underscores the importance of investigating the bioavailability and efficacy of these compounds in order to maximize their therapeutic potential. These data suggest that delivering these compounds in combination may improve the acute bioavailability of curcumin and resveratrol compared to supplementation with single compounds, allowing for lower overall doses and simpler treatment protocols using combination therapies.

The current study was able to show improved permeability of these compounds when applied in combination; however, future studies are needed to investigate the specific mechanisms including the use of inhibitors for export pathways such as P-gp, MRP2 and BCRP. Additionally, long-term or consistent exposure to these combinations is likely to affect absorption considering that previous studies have demonstrated changes in expression of metabolic and transport enzymes with longer exposures (i.e. UGT1A1, CYP3A4, P-gp).[16, 37] Further studies on the long-term modulation of biotransformation pathways of these combinations are warranted as well as *in vivo* studies using combination therapies.

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DISCLOSURE OF INTERESTS

The authors disclose no conflict of interest in this study and are in no way affiliated with Gaia Herbs.

REFERENCES

1. Boots AW, Haenen GRMM, Bast A. Health effects of quercetin: from antioxidant to nutraceutical. *Eur J Pharmacol.* 2008;585(2–3):325–37.
2. Dajas F. Life or death: neuroprotective and anticancer effects of quercetin. *J Ethnopharmacol.* 2012;143(2):383–96.
3. Russo M, Spagnuolo C, Tedesco I, Bilotto S, Russo GL. The flavonoid quercetin in disease prevention and therapy: facts and fancies. *Biochem Pharmacol.* 2012;83(1):6–15.
4. Prasad K. Resveratrol, wine, and atherosclerosis. *Int J Angiol.* 2012;21(1):7–18.
5. Raederstorff D, Kunz I, Schwager J. Resveratrol, from experimental data to nutritional evidence: the ememrgence of a new food ingredient. *Ann NY Acad Sci.* 2013;1290:136–41.
6. Xu Q, Si L-Y. Resveratrol role in cardiovascular and metabolic health and potential mechanisms of action. *Nutr Res.* 2012;32(9):648–58.
7. Aggarwal BB, Harikumar KB. Potential therapeutic effects of curcumin, the anti-inflammatory agent, against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. *Int J Biochem Cell Biol.* 2009;41(1):40–59.
8. Chandran B, Goel A. A randomized, pilot study to assess the efficacy and safety of curcumin in patients with active rheumatoid arthritis. *Phytother Res.* 2012;26(11):1719–25.
9. DiSilvestro RA, Joseph E, Zhao S, Bomser J. Diverse effects of a low dose supplement of lipidated curcumin in healthy middle aged people. *Nutr J.* 2012;11(1):79–87.
10. Lao CD, Mack I, Ruffin T, Normolle D, Heath DD, Murray SI, Bailey JM, Boggs ME, Crowell J, Rock CL, Brenner DE. Dose escalation of a curcuminoid formulation. *BMC Complement Altern Med.* 2006;6(10):1–4. doi:10.1186/1472-6882-6-10.
11. Porte CL, Voduc N, Zhang G, Seguin I, Tardiff D, Singhal N, Cameron DW. Steady-state pharmacokinetics and tolerability of trans-resveratrol 2000 mg twice daily with food, quercetin and alcohol (ethanol) in healthy human subjects. *Clin Pharmacokinet.* 2010;49(7):449–54.
12. Shoba G, Joy D, Joseph T, Majeed M, Rajendran R, Srinivas PS. Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers. *Planta Med.* 1998;64(4):353–6.
13. Cuomo J, Appendino G, Dern AS, Schneider E, McKinnon TP, Brown MJ, Togni S, Dixon BM. Comparative absorption of a standardized curcuminoid mixture and its lecithin formulation. *J Nat Prod.* 2011;74(4):664–9.

14. Atal CK, Dubey RK, Singh J. Biochemical basis of enhance drug bioavailability by piperine: evidence that piperine is a potent inhibitor of drug metabolism. *J Pharmacol Exp Ther.* 1985;232(1):258–62.

15. Zhou S, Lim LY, Chobay B. Herbal modulation of P-glycoprotein. *Drug Metab Rev.* 2004;36(1):57–104.

16. Okura T, Ibe M, Umegaki K, Shinozuka K, Yamada S. Effects of dietary ingredients on function and expression of P-glycoprotein in human intestinal epithelial cells. *Biol Pharm Bull.* 2010;33(2):255–9.

17. Li Y, Shin YG, Yu C, Kosmeder JW, Hirschelman WH, Pezzuto JM, Breemen RBV. Increasing the throughput and productivity of Caco-2 cell permeability assays using liquic chromatography-mass spectrometry: application to resveratrol absorption and metabolism. *Comb Chem High Throughput Screen.* 2003;6:757–67.

18. Wahlang B, Pawar YB, Bansal AK. Identification of permeability-related hurdles in oral delivery of curcumin using the Caco-2 cell model. *Eur J Pharm Biopharm.* 2011;77:275–82.

19. Center for Drug Evaluation and Research. *Guidance for Industry: Waiver of in vivo Bioavailability and Bioequivalence Studies for Immediate-release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System*. Rockville, MD: Food and Drug Administration; 2000.

20. Zhang L, Lin G, Kovacs B, Jani M, Krajcsi P, Zuo Z. Mechanistic study on the intestinal absorption and disposition of baicalein. *Eur J Pharm Sci.* 2007;31:221–31.

21. Zhang L, Zheng Y, Chow MSS, Zuo Z. Investigation of intestinal absorption and disposition of green tea catechins by Caco-2 monlayer model. *Int J Pharm.* 2004;287:1–12.

22. Olson ER, Melton T, Dong Z, Bowden GT. Stabilization of quercetin paradoxically reduces its proapoptotic effect on UVB-irradiated human keratinocytes. *Cancer Prevent Res.* 2008;1(5):362–8.

23. De Santi C, Pietrabissa A, Spisni R, Mosca F, Pacifici GM. Sulfation of resveratrol, a natural product present in grapes and wine, in the human liver and duodenum. *Xenobiotica.* 2000;30(6):609–17.

24. De Santi C, Pietrabissa A, Spisni R, Mosca F, Pacifici GM. Sulphation of resveratrol, a natural compound present in wine, and its inhibition by natural flavonoids. *Xenobiotica.* 2000;30(9):857–66.

25. Choi JS, Piao YJ, Kang KW. Effects of quercetin on the bioavailability of doxorubicin in rats: role of CYP3A4 and P-gp inhibition by quercetin. *Arch Pharm Res.* 2011;34(4):607–13.

26. Sergent T, Dupont I, Van der Heiden E, Scippo ML, Pussemier L, Larondelle Y, Scheider YJ. CYP1A1 and CYP3A4 modulation by dietary flavonoids in human intestinal Caco-2 cells. *Toxicol Lett.* 2009;191(2–3):216–22.

27. De Santi C, Pietrabissa A, Mosca F, Rane A, Pacifici GM. Inhibition of phenol sulfotransferase (SULT1A1) by quercetin in human adult and foetal livers. *Xenobiotica.* 2002;32(5):363–8.

28. Marchetti F, De Santi C, Vietri M, Pietrabissa A, Spisni R, Mosca F, Pacifici GM. Differential inhibition of human liver and duodenum sulphotransferase activities by quercetin, a flavonoid present in vegetables, fruit and wine. *Xenobiotica.* 2001;31(12):841–7.

29. Kim KA, Park PW, Park JY. Short-term effect of quercetin on the pharmacokinetics of fexofenadine, a substrate of P-glycoprotein, in healthy volunteers. *Eur J Clin Pharmacol.* 2009;65(6):609–14.

30. Bansal T, Awasthi A, Jaggi M, Khar RK, Talegaonkar S. Pre-clinical evidence for altered absorption and biliary excretion of irinotecan (CPT-11) in combination with quercetin: possible ocntribution of P-glycoprotein. *Life Sci.* 2008;83:250–9.

31. Ampasavate C, Sotanaphun U, Phattanawasin P, Piyapolrungroj N. Effects of Curcuma spp. on P-glycoprotein function. *Phytomedicine.* 2010;17:506–12.

32. Cho YA, Lee W, Choi JS. Effects of curcumin on the pharmacokinetics of tamoxifen and its active metabolite, 4-hydroxytamoxifen, in rats: possible role of CYP3A4 and P-glycoprotein inhibition by curcumin. *Pharmazie.* 2012;67(2):124–30.

33. Planas JM, Alfaras I, Colom H, Juan ME. The bioavailability and distribution of trans-resveratrol are constrained by ABC transporters. *Arch Biochem Biophys.* 2012;527(2):67–73.

34. van de Wetering K, Burkon A, Feddema W, Bot A, de Jonge H, Somoza V, Borst P. Intestinal breast cancer resistance protein (BCRP)/Bcrp1 and multidrug resistance protein 3 (MRP3)/Mrp3 are involved in the pharmacokinetics of resveratrol. *Mol Pharmacol.* 2009;75(4):876–85.

35. Kusuhara H, Furuie H, Inano A, Sunagawa A, Yamada S, Wu C, Fukizawa S, Morimoto N, Ieiri I, Morishita M, Sumita K, Mayahara H, Fujita T, Maeda K, Sugiyama Y. Pharmacokinetic interaction study of sulfasalazine in healthy subjects and the impact of curcumin as an in vivo inhibitor or BCRP. *Br J Pharmacol.* 2012;166(6):1793–803.

36. Li S, Lei Y, Jia Y, Li N, Wink M, Ma Y. Piperine, a piperidine alkaloid from Piper nigrum re-sensitizes P-gp, MRP1 and BCRP dependent multidrug resistant cancer cells. *Phytomedicine.* 2011;19(1):83–7.

37. Iwuchukwu OF, Tallarida RJ, Nagar S. Resveratrol in combination with other dietary polyphenols concomitantly enhances antiproliferation and UGT1A1 induction in Caco-2 cells. *Life Sci.* 2011;88(23–24):1047–54.