Comparison of Two Commercial Preparations of Curcumin using the Caco-2 *in vitro* Assay of Human Intestinal Permeability

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ABSTRACT

Objectives: The transport of curcuminoids in Caco-2 cell monolayers, which are widely used as an *in vitro* model of drug permeability in the human small intestine, was investigated. The formulations tested demonstrated two distinct methods for enhancing the intestinal absorption and bioavailability of curcumin: a curcumin–phospholipid complex and a turmerone/piperine-enhanced curcumin product.

Design and outcome measures: Two unique formulations representing distinct approaches to maximizing curcumin bioavailability were compared with each other and with standard control compounds. Outcomes evaluated included apparent permeability, which reflects the ability of drug molecules to penetrate the intestinal tract; percentage recovery of compound through the Caco-2 monolayer; and efflux ratio, which is generally considered to reflect the role of efflux proteins in expelling compounds from the intestinal lumen.

Results: In our widely used Caco-2 cell model experiments, the absorption of curcumin from each unique commercial formulation was equivalent at the single concentration tested. Furthermore, curcumin efflux was substantially reduced in the turmerone/piperine formula, though the difference between formulations was not significant. This reduction of curcumin efflux was expected on the basis of reports of piperine's P-glycoprotein-inhibitory activity.

Conclusion: The results of the present experiment strongly suggest that the incorporation of turmerones and piperine into curcumin preparations may be a simple way to improve the poor bioavailability of curcumin, which has previously been shown to be improved by the formulation of curcumin into curcumin–phospholipid complexes.

Keywords: Curcumin; Absorption; Caco-2; Curcuminoids; Bioavailability

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INTRODUCTION

Curcumin is one of three small molecules known as "curcuminoids," which make up approximately 1–6% of the dried weight of the spice turmeric (*Curcuma longa*). The major curcuminoids are curcumin I (diferuloylmethane), curcumin II (demethoxycurcumin), and curcumin III (bisdemethoxycurcumin).[1] There is a great deal of interest in the potential medicinal properties of curcumin. A recent article referred to over 9000 scientific publications and over 500 patents listed in the Curcumin Resource Database (CRDB), as well as to the more than $150 million of U.S. federal government funding that has been allocated for investigation of curcumin's biomedical applications.[2] Curcumin has been reported to have anti-inflammatory, anticancer, neuroprotective, antiatherosclerotic, and antidiabetic properties. Doses of up to 12 g of curcuminoids have been shown to be safe and well tolerated in humans.[3] Concerns have been raised, however, about the suitability of curcumin as a therapeutic agent. Many plant extracts that demonstrated promising activity *in vitro* have ended up disappointing clinically because of poor bioavailability of active constituents.[1] Molecules with poor bioavailability, such as the curcuminoids, are often multiring large structures with poor water solubility and no mechanism for active uptake. Pharmacokinetic studies have confirmed that curcuminoid uptake into human plasma is poor, typically below 25 nM at oral doses of 3.6–12 g daily taken for 1 week.[1] A recent publication described curcumin as "pharmacodynamically fierce but pharmacokinetically feeble,"[2] meaning it hits many biological targets but does not get there efficiently. A great deal of effort has gone into developing improved delivery methods for curcuminoids, including emulsions, phytosomes, liposomes, nanoparticles, and covalent chemical modifications.[4] Curcumin absorption may also be potentiated by the addition of piperidine[5] or by the turmerone-rich oleoresin component of whole turmeric itself.[6]

CURCUMIN PHYTOSOME

Phytosome and herbosome technology, which creates complexes of plant extracts with phospholipids to facilitate intestinal absorption, emerged in the late 1980s.[1] Since that time, multiple articles and patents have been published detailing the methodology of phytosome preparation.[7] Briefly, these complexes are produced by dissolving, in a roughly stoichiometric ratio, the plant constituents and the phospholipid, often phosphatidylcholine or lecithin, in a nonpolar aprotic solvent. It is believed that the choline head of the phospholipid molecule then binds via hydrogen bonds to the plant polyphenol or related active ingredients.[7] This binding can be confirmed by spectroscopic techniques such as ^{13}C-nuclear magnetic resonance or ^{1}H-nuclear magnetic resonance whereby the signal from the phospholipid obliterates the polyphenol signal as the lipid-soluble phospholipid tail envelops the choline-bound constituents.[8] This is distinct from liposome technology, in which thousands of phospholipid molecules surround each polyphenol molecule.[7] The cited advantages of phytosome technology include improved absorption, protection from destruction by gastric secretions and gut bacteria, decreased liver conjugation (that is, improved metabolic stability), and improved shelf life as compared with raw plant extracts.[1,7] Phytosomes have shown improved antioxidant activity in animal models,[9] as well as an improved plasma uptake of 5.6 times the area under the curve over 120 min when compared with raw curcumin.[10]

CURCUMIN WITH TURMERONE/PIPERINE

A potentially simpler method of absorption enhancement is the use of carrier molecules. Piperine, a component of black and long pepper, has been shown to increase plasma levels of unconjugated curcumin compared with raw curcumin.[11] In addition, the blending of curcuminoids with more volatile turmeric components, such as turmerones, may enhance curcumin adsorption as well as having potential therapeutic properties in their own right. The manufacturing process can be carried out using safe, naturally occurring plant-based lecithins as emulsifying agents.

MATERIALS AND METHODS

In the present study, we investigated the head-to-head absorption of curcuminoids in two distinct curcumin formulations using Caco-2 cell

monolayers, an *in vitro* model commonly used to test drug permeability because it mimics the pharmacokinetics of the human intestinal tract.[12] We compared a curcuminoid formulation that uses both full-spectrum turmeric oleoresin and piperine with a curcuminoid–phospholipid complex (phytosome) formulation. Curcumin–phospholipid complexes have previously been shown to have superior oral bioavailability by a factor of two to six times compared with uncomplexed plant extracts.[1] They have also been studied clinically for conditions such as osteoarthritis[13] and the preservation of small blood vessel health in patients with diabetes.[14] As far as we are aware, this is the first head-to-head absorption study comparing a phytosome formulation with a simple standardized turmerone/piperine-complexed formulation.

We obtained a sample of commercially available curcumin phytosome (batch 32145/M2, manufactured June 2016) from Indena USA (Seattle, WA). The self-reported constituents are shown in Table 1. The curcumin/turmerone/piperine formulation we tested is a stable suspension of standardized turmeric root alcohol extract, standardized turmeric root CO_2 extract, and standardized black pepper extract obtained from Restorative Formulations Inc. (Montpelier, VT). The product is prepared by high-speed blending of turmeric root alcohol extract, turmeric root CO_2 extract, black pepper extract, soy lecithin (approximately 25% phosphatidylcholine), and medium-chain triglycerides. The blend is then homogenized and encapsulated. Table 2 provides specifications and analysis of the curcumin/turmerone/piperine formulation based on the manufacturer's certificate of analysis. The stated total curcuminoids (Tables 1 and 2) were very similar between the two products at 18.3% for curcumin phytosome and 17.0% for curcumin/turmerone/piperine. The main difference in formulations therefore was the method of delivery (phospholipid complexing vs. turmerone/piperine).

CACO-2 ASSAY

The Caco-2 permeability assay was performed bidirectionally using human Caco-2 cells. Caco-2 assay kit (Agilux/Charles River Laboratories, Worcester, MA) cells were cultured for 21 days in standard culture media (Dulbecco's

Table 1: Indena curcumin phytosome analysis as stated by manufacturer's certificate of analysis.

Constituent	Specification (from manufacturer's CofA)	Analysis (of test batch from manufacturer's CofA)
Total curcuminoids (HPLC)	18–22%	18.3%
Water	<7%	1.4%
Heavy metals (total USP)	<40 ppm	Complies
Residual solvents: ethanol	<5000 ppm	910 ppm

CofA, certificate of analysis; HPLC, high-performance liquid chromatography; ppm, parts per million; USP, U.S. Pharmacopeia.

Table 2: Curcumin/turmerone/piperine formula analysis as stated by manufacturer's certificate of analysis.

Constituent	Product specification (from manufacturer's CofA)	Product label claims (of test batch from manufacturer's CofA)
Turmeric root	400 mg	
Turmeric root CO_2 extract	40% turmerones	52.5 mg
Turmeric root alcohol extract	95% curcuminoids	102.5 mg
Black pepper extract	95% piperine	5 mg
Total curcuminoids	>95 mg	95.5 mg
Total turmerones	>20 mg	23.7 mg

CofA, certificate of analysis.

modified Eagle's medium, fetal calf serum 10%, L-glutamine 1%, penicillin-streptomycin 1%) in 24-well plates. On the day of the assay, 5 mL of a 1000-fold dilution of each test compound solution was prepared in transport buffer. The basal assay plate was prepared by adding 750 µL of transport buffer to apical-basolateral (A-to-B) wells and 780 µL of diluted compound solution to B-to-A wells. Caco-2 media (200-µL quantity) was removed from the apical wells and replaced with 200 µL of fresh transport media (repeated twice for a total of three washes). A quantity of 200 µL of the media

Table 3: Analytical conditions.

Sample analysis				
LC Condition 1:	**Curcumin**			
Column ID. & Dimensions:	ACQUITY UPLC BEH C8 1.7 µm, 50×2.1 mm (Waters)	**Time (min)**	**% MPB**	**Flow (mL/min)**
Temperature (°C)	55	Initial	10	1.0
Mobile Phase A:	0.1% Formic Acid in Water	0.20	10	1.0
Mobile Phase B:	0.1% Formic Acid in Acetonitrile	1.65	95	1.0
Weak Needle Rinse:	0.1% Formic Acid in Water	1.90	95	1.0
		1.95	10	1.0
		2.00	10	1.0
LC Condition 2:	**Ranitidine**			
Column ID. & Dimensions:	XSELECT HSS T3 2.5 µm, 30×2.1 mm (Waters)	**Time (min)**	**% MPB**	**Flow (mL/min)**
Temperature (°C)	55	Initial	0	1.0
Mobile Phase A:	0.1% Formic Acid in Water	0.15	0	1.0
Mobile Phase B:	0.1% Formic Acid in Acetonitrile	0.80	95	1.0
Weak Needle Rinse:	0.1% Formic Acid in Water	0.90	95	1.0
		0.95	0	1.0
		1.00	0	1.0
LC Condition 3:	**Talinolol & Warfarin**			
Column ID. & Dimensions:	XSELECT HSS T3 2.5 µm, 30×2.1 mm (Waters)	**Time (min)**	**% MPB**	**Flow (mL/min)**
Temperature (°C)	55	Initial	10	1.0
Mobile Phase A:	0.1% Formic Acid in Water	0.15	10	1.0
Mobile Phase B:	0.1% Formic Acid in Acetonitrile	0.80	95	1.0
Weak Needle Rinse:	0.1% Formic Acid in Water	0.90	95	1.0
		0.95	10	1.0
		1.00	10	1.0
LC Solution ID:				
Mobile Phase A1:	0.1% Formic Acid in Water			
Mobile Phase A2:	NA			
Mobile Phase B1:	0.1% Formic Acid in Acetonitrile			
Mobile Phase B2:	NA			
Weak Needle Rinse:	0.1% Formic Acid in Water			
Strong Needle Rinse:	25:25:25:25 Water:Acetonitrile:Methanol:Isopropanol			
Seal Wash:	0.1% Formic Acid in 95:5 Water:Acetonitrile			
MS Conditions				
Compounds	API5500 with Waters Acquity UPLC			
Ionization Method:	Electrospray			
Positive/Negative Ion:	Positive			
Resolution:	Unit			
Source Temperature (°C):	600			

LC, liquid chromatography; Mobile Phase B Flow, flow rate of mobile phase through column in mL/min; MS, mass spectrometry; %MPB, percent by weight.

from the apical wells was replaced with 200 µL of diluted compound (for A-to-B wells) or 200 µL of fresh transport buffer (for B-to-A wells). Three replicates of 10-µL samples were collected from the apical and basal compartments for t=0 (t0). At t=2 h (T2h), three 10-µL replicate samples were collected from all apical compartments and B-A basal compartments. The 10-µL samples were diluted with 40 µL of transport buffer, then 100 µL of quench solution was added to all T2h samples. The T2h samples were then mixed, followed by 50 µL of all t0 and T2h samples being transferred to sample plates and diluted with 100 µL of Milli-Q water (MilliporeSigma, Burlington, MA) for bioanalysis.

Analyte levels (peak area ratios) were measured on apical and basolateral sides at t0 and T2h. A-to-B and B-to-A fluxes were calculated as the mean of three measurements. Apparent permeability (Papp; in cm/s) was calculated as dQ (flux)/(dt×area×concentration). The efflux ratio (ER) was calculated as (B-to-A)/(A-to-B), that is, Papp(B-A)/Papp(B-A), where typically a ratio greater than 2 is evidence of efflux. P-glycoprotein (P-gp) efflux was challenged by testing with or without P-gp inhibitor (dosing solutions prepared with and without verapamil at a final assay concentration of 25 µM). Two formulations as described previously were tested. Curcumin phytosome was dissolved in the assay buffer to achieve a test concentration of 10 µM curcumin I (diferuloylmethane). Similarly, the liquid formulation Triple Turmeric Px (TTPx; Restorative Formulations Inc.) was diluted directly into the assay buffer at the same 10 µM curcumin I concentration. The assay was performed at a single time point of T2h, and the temperature was held constant at 37°C. Four control compounds were also used to compare permeability and reflux, namely ranitidine (low permeability), talinolol (highly effluxed), talinolol with 25 µM verapamil (inhibition of efflux), and warfarin (high permeability). Analytical conditions are summarized in Table 3.

RESULTS

In Figure 1, the mean Papp (A→B) and (B→A) is plotted for curcumin I (diferuloylmethane), as well as for the control drug ranitidine, which is considered a low-permeability compound. As expected, curcumin absorption was comparable to the low-permeability control, because as reported, curcumin is considered to have low intestinal permeability. Mean Papp (A→B) was 0.487 (±0.181 SEM) (10^{-6} cm/s) for curcumin phytosome compared with 0.390 (±0.130 SEM) (10^{-6} cm/s) for TTPx curcumin, a nonsignificant difference (P>0.05). Although studies of curcumin permeability in the Caco-2 model are rare, these values are in the same range as the mean Papp (A→B) value of 1.23 (±0.21) (10^{-6} cm/s) for 10 µg/mL curcumin reported by Xue *et al*.[15] In the present study, mean

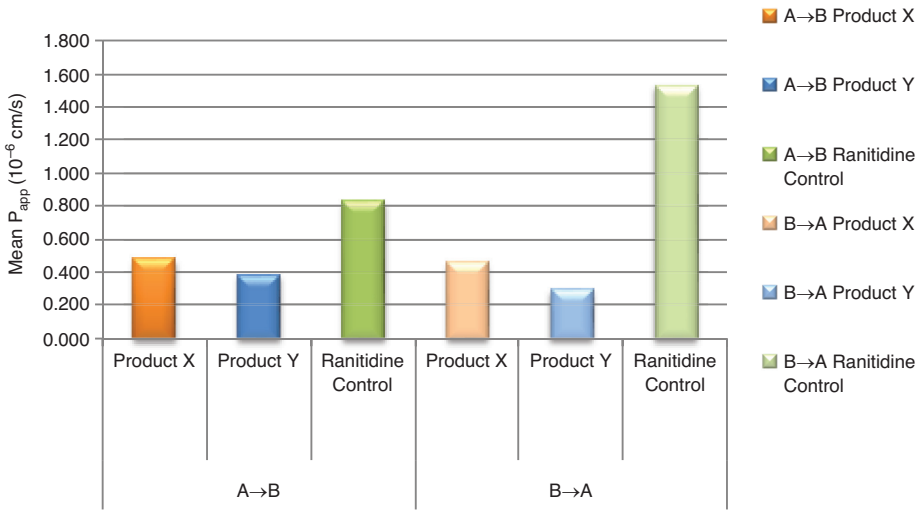

Figure 1: Curcumin absorption and efflux.

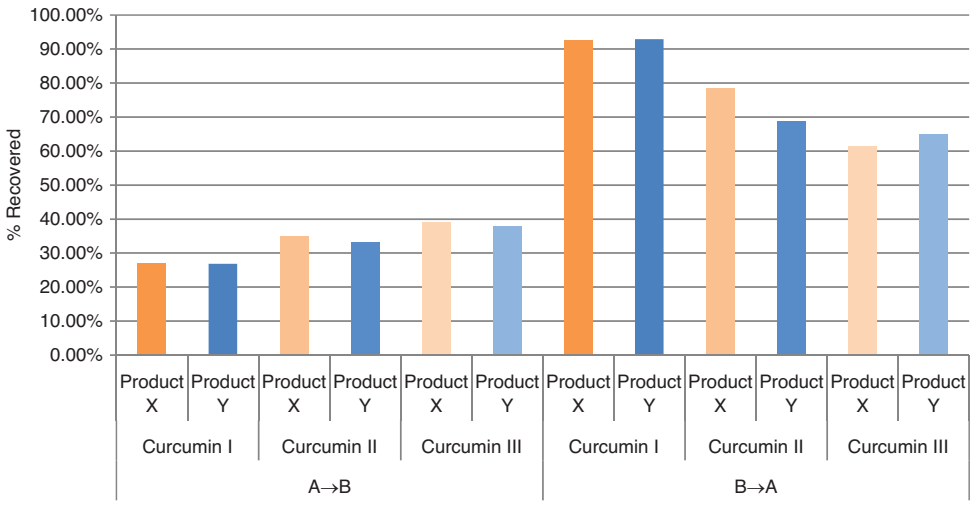

Figure 2: Recovery of individual curcuminoids.
Product X, phospholipid complex; Product Y, curcumin/turmerone/piperine.

Papp (B→A) absolute values were 0.464 (±0.071) (10^{-6} cm/s) for curcumin from the phytosome compared with 0.303 (±0.046) (10^{-6} cm/s) for TTPx curcumin, also a nonsignificant difference (P>0.05), although there is a measured lower efflux in the TTPx formulation. Again, these results are similar to the value of mean Papp (B→A) of 1.44 (±0.09) for curcumin reported by Xue *et al*.[15]

Curcuminoid recovery for both directions is shown in Figure 2: 27.1% of phytosome curcumin I recovered vs. 26.8% for TTPx, 34.8% of phytosome curcumin II recovered vs. 33.0% for TTPx, and 39.0% of phytosome curcumin III recovered vs. 37.9% for TTPx. These low recovery rates may be indicative of metabolism by the Caco-2 cells or accumulation of the compound in the cell monolayer. In contrast, A→B recovery was greater than 90% for all control substances (ranitidine, talinolol, warfarin). In the B→A direction, recoveries were much higher, and again both formulations were nearly equivalent with 92.5% of phytosome curcumin I recovered vs. 92.98% for TTPx, 78.3% of phytosome curcumin II recovered vs. 68.7% for TTPx, and 61.5% of phytosome curcumin III recovered vs. 64.8% for TTPx.

The net ER (i.e. mean Papp[B→A]/Papp[B→A]) was calculated as 0.95 (±0.58) for phytosome curcumin I vs. 0.78 (±0.42) for TTPx curcumin I. This compares to an ER of 7.83 (±0.51) for the highly effluxed control compound talinolol, a highly statistically significant difference vs. curcumin I in both test formulations (P<0.005). Additional ER values are reported in Table 4, including a talinolol plus verapamil control, which, as expected, greatly reduced the ER for talinolol by inhibition of P-gp, a membrane transport protein known to efflux compounds from the intestinal lumen.

Table 4: Net Efflux Ratio.

	Net efflux ratio	
	Mean (B–A)/(A–B)	SEM
Product Y curcumin I	0.78	0.42
Warfarin control	0.91	0.09
Product X curcumin I	0.95	0.58
Ranitidine control	1.84	0.36
Talinolol control	7.83	0.51
Talinolol+25 µM verapamil	1.50	0.11

SEM, standard error of the mean.

Typically, ERs of 2 or greater are considered to represent significant active transport, whereas values less than 2 indicate a predominance of passive diffusion. Xue *et al*. reported that for curcumin, the relative activity of active transport is concentration-dependent in a monolayer of Caco-2 cells, with passive diffusion dominating at curcumin concentrations less than 10 µg/mL and active transport involved at concentrations greater than 10 µg/mL.[15] Piperine, a naturally occurring alkaloid, has been

shown to enhance curcumin absorption in rodents and humans at a dose of 25 mg piperine per 2 g of curcumin.[8] One important mechanism established for the bioavailability-enhancing property of piperine is the capacity to reduce efflux via inhibition of P-gp. This may explain the trend for the lower ER seen in TTPx, which contains standardized piperine extract, vs. the phytosome formulation. Because piperine also improves bioavailability in plasma via inhibition of curcuminoid liver conjugation, its effects on improving bioavailability may be multiplied *in vivo*.[16]

DISCUSSION

In the present study, the transport of curcuminoids was investigated in Caco-2 cell monolayers, which are widely used as an *in vitro* method modeling drug permeability in the human small intestine. Two unique formulations representing distinct approaches to maximizing curcumin bioavailability were tested and compared with each other and with standard control compounds. Parameters evaluated included Papp, which reflects the ability of drug molecules to penetrate the intestinal tract; percent recovery of compound through the Caco-2 monolayer; and ER, which is generally accepted to reflect the role of the efflux proteins in expelling compounds from the intestinal lumen.

CONCLUSION

The purpose of this study was to compare two distinct methods for enhancing intestinal absorption and bioavailability of curcumin: a curcumin–phospholipid complex and a tumerone/piperine-enhanced curcumin product. In the Caco-2 cell model, curcumin absorption was shown to be statistically equivalent ($P>0.05$ used for all comparisons) at the single concentration tested. There was a measured reduced efflux of curcumin in the turmerone/piperine formula, which was expected on the basis of reports of piperine's P-gp-inhibitory activity. This trend may have proven to be more pronounced at higher concentrations of curcumin, where it has previously been shown that efflux plays a more significant role in poor curcumin uptake in the small intestine.

The results of the present experiment strongly suggest that the incorporation of turmerones and piperine into curcumin preparations may be a simple way to improve curcumin bioavailability, which has been shown to be improved by the formulation of curcumin into curcumin–phospholipid complexes. To confirm this, it is recommended that these tumerone/piperine formulations (or similar absorption enhancers/P-gp inhibitors) be investigated further in animal and human pharmacokinetic studies. Future studies should also endeavor to assess bioavailability for both curcumin and its metabolites, because it remains unclear which compounds are responsible for the range of therapeutic benefits reported.

STUDY LIMITATIONS

Given the variance between the two replications of the experiment, future trials should consider a higher number of replications. Also, in this experiment, only a single curcumin concentration was tested. It is recommended that future experiments include a range of concentrations. In addition, optimization of the tumerone/piperine concentrations and ratio was not part of this investigation but should be considered for future studies.

COMPETING INTERESTS

The author is a scientific consultant with Restorative Formulations Inc., manufacturer of curcumin/turmerone/piperine products.

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