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ORIGINAL ARTICLE

Comparison of systemic availability of curcumin with that of curcumin formulated with phosphatidylcholine

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Abstract
Purpose Curcumin, a major constituent of the spice turmeric, suppresses expression of the enzyme cyclooxygenase 2 (Cox-2) and has cancer chemopreventive properties in rodents. It possesses poor systemic availability. We explored whether formulation with phosphatidylcholine increases the oral bioavailability or affects the metabolite profile of curcumin.
Methods Male Wistar rats received 340 mg/kg of either unformulated curcumin or curcumin formulated with phosphatidylcholine (Meriva) by oral gavage. Rats were killed at 15, 30, 60 and 120 min post administration. Plasma, intestinal mucosa and liver were analysed for the presence of curcumin and metabolites using HPLC with UV detection. Identity of curcumin and metabolites was verified by negative ion electrospray liquid chromatography/tandem mass spectrometry.
Results Curcumin, the accompanying curcuminoids desmethoxycurcumin and bisdesmethoxycurcumin, and the metabolites tetrahydrocurcumin, hexahydrocurcumin, curcumin glucuronide and curcumin sulfate were identified in plasma, intestinal mucosa and liver of rats which had received Meriva. Peak plasma levels and area under the plasma concentration time curve (AUC) values for parent curcumin after administration of Meriva were fivefold higher than the equivalent values seen after unformulated curcumin. Similarly, liver levels of curcumin were higher after administration of Meriva as compared to unformulated curcumin. In contrast, curcumin concentrations in the gastrointestinal mucosa after ingestion of Meriva were somewhat lower than those observed after administration of unformulated curcumin. Similar observations were made for curcumin metabolites as for parent compound.
Conclusion The results suggest that curcumin formulated with phosphatidylcholine furnishes higher systemic levels of parent agent than unformulated curcumin.

Keywords Curcumin · Phosphatidylcholine · Bioavailability · Rat · Cancer Chemoprevention · Metabolism

Introduction

The incidence of cancer continues to rise as a consequence of an increasingly aging population. Improved identification of individuals at risk of developing the disease has increased the feasibility of employing long-term chemoprevention strategies in cancer management. Constituents of the diet are considered to be a promising source of novel efficacious and safe cancer chemopreventive agents [23]. One dietary polyphenol which has been the focus of considerable preclinical and early clinical chemoprevention studies is curcumin

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[1,7-bis(4-hydroxyl-3-methoxyphenyl)-1,6-heptadiene-3,5-dione]. Curcumin is the major constituent of the spice turmeric extracted from the root of *Curcuma longa* Linn. Curcumin is a powerful antioxidant and inhibits the expression of the enzyme cyclooxygenase 2 (Cox 2) at least in part via interference with activation of the transcription factor NFkB [1, 4, 18]. In vitro, curcumin inhibits the growth of cancer cells with an IC_{50} value of 20–75 μM [8, 20]. In rodent models, curcumin has been shown to prevent cancer in the colon, skin, stomach, duodenum, soft palate, tongue, sebaceous glands and breast [11, 12, 19]. Curcumin undergoes avid metabolism by conjugation (glucuronidation and sulfation) and reduction pathways [9, 10]. Clinical pilot studies have associated curcumin consumption with regression of pre-malignant lesions of bladder, soft palate, stomach, cervix and skin [3, 13]. Preclinical and clinical pilot studies suggest that concentrations of curcumin achieved in plasma and target tissues are low, probably caused, at least in part, by its extensive metabolism [6, 7, 9, 10]. In a phase I trial, plasma and urine concentrations of curcumin in patients, who had ingested 3,600 mg curcumin orally, were 11.1 nmol/l and 1.3 μmol/l, respectively [21]. Curcumin concentrations in colorectal tissues of patients on this dose were 7.7–12.7 nmol/g, whilst levels in the liver were below the limits of detection [6, 7]. In another study, peak plasma concentrations 1–2 h after oral dosing, reached 0.41–1.75 μM in patients receiving 4–8 g curcumin [3]. It is neither practical nor desirable to increase the oral dose of curcumin above that already investigated. Formulating poorly absorbed drugs with phosphatidylcholine has previously been shown to increase their plasma bioavailability. For example, the anti-schistosomal activity of praziquantel was increased by incorporation into phosphatidylcholine-containing liposomes [15] and the intestinal permeability of hexarelin was increased 20-fold by such a formulation strategy [5]. Mindful of these facts, we tested the hypothesis that a formulation of curcumin with soy phosphatidylcholine (curcumin phospholipid complex "Meriva", Indena SpA, Milan) might improve the systemic availability of curcumin in plasma and tissues in rat.

Materials and methods

Chemicals

Curcumin (CAS 458-37-7) and curcumin phospholipid complex (Meriva) were supplied by Indena SpA (Milan, Italy). The preparation of Meriva using EpiKuron 130 P, a de-oiled, powdered soybean lecithin enriched with 30% phosphatidylcholine is subject of a European patent application (EP 06004820), which was filed March 2006. Meriva contained 16.89% curcuminoids, of which 93.82% was curcumin, the ratio of curcumin to Epikuron 130 P was 1:4. Commercially available curcumin obtained by extraction of *Curcuma* spp. contains 94% curcuminoids of which 77% was curcumin, 17% desmethoxycurcumin and 6% bisdesmethoxycurcumin as determined by HPLC, (Fig. 2a, for structures see Fig. 1). Tetrahydrocurcumin and hexahydrocurcumin provided by Phytopharm plc (Cambridge, UK) had been synthesized as described previously [24]. Dosing suspensions were prepared in 1% methylcellulose at 17 mg curcumin/ml. Experiments were carried out under animal project license PPL 40/2496, granted to Leicester University by the UK Home Office. The experimental design was vetted by the Leicester University Local Ethical Committee for Animal Experimentation and met the standards required by the UKCCCR for animal welfare [25]. Male Wistar albino rats (250 g) were purchased from Harlan UK Ltd (Bicester, UK) and kept under a 12 h light/dark cycle on standard lab chow. Animals were fasted overnight and received unformulated or formulated curcumin at 340 mg/kg (in terms of curcumin) by oral gavage. At 15, 30, 60 and 120 min animals were exsanguinated under terminal anaesthesia. Group size

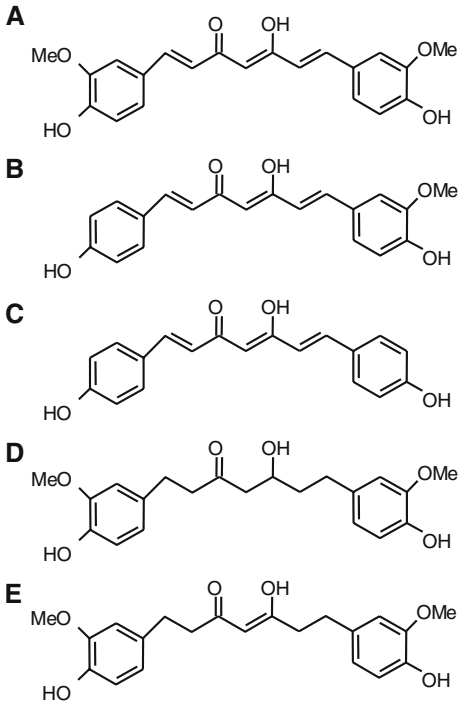

Fig. 1 Structures of curcumin (**a**), desmethoxycurcumin (**b**), bisdesmethoxycurcumin (**c**), hexahydrocurcumin (**d**) and tetrahydrocurcumin (**e**)

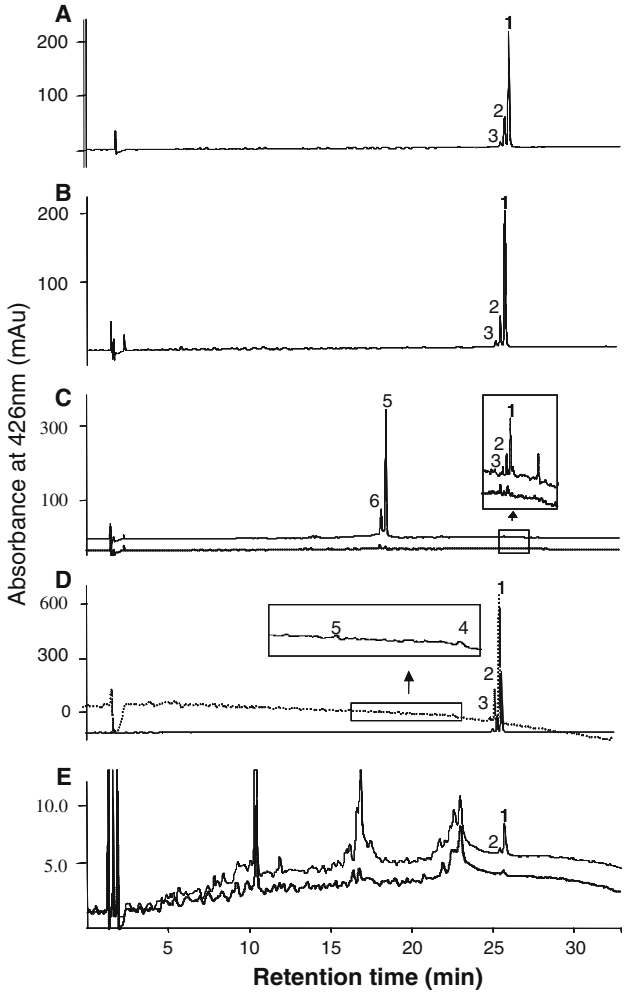

Fig. 2 HPLC chromatograms of curcumin in 50% aqueous acetonitrile (**a**), an extract of rat plasma spiked with curcumin (**b**), or extracts of plasma (**c**), mucosa (**d**) or liver (**e**) from rats that received either curcumin (*dotted line*) or Meriva (*solid line*). Bio-matrices were obtained 30 min post curcumin administration. In **c**, **d** and **e** *solid lines* and *dotted lines* represent bio-matrices from rats given Meriva and non-formulated curcumin, respectively. Peaks 1–6 correspond to curcumin, desmethoxycurcumin, bisdesmethoxycurcumin, curcumin sulfate, curcumin glucuronide and desmethoxycurcumin glucuronide, respectively. For details of administration and HPLC analysis see Materials and methods

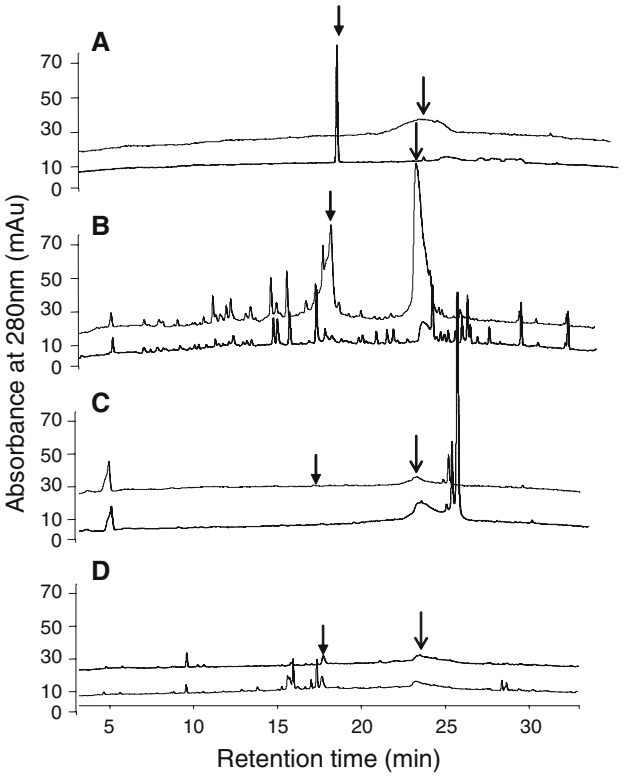

Fig. 3 HPLC chromatograms of reduced curcumin metabolites tetrahydrocurcumin (*open arrow*) and hexahydrocurcumin (*solid arrow*) in 50% aqueous acetonitrile (**a**), extracts of rat plasma (**b**), mucosa (**c**) and liver (**d**). Bio-matrices were obtained 60 min post curcumin administration. In **b**, **c** and **d** *solid lines* and *dotted lines* represent bio-matrices from rats given Meriva and non-formulated curcumin, respectively. For details of administration and HPLC analysis see Materials and methods

was three rats per time point. Whole blood was collected by cardiac puncture into heparinised tubes, centrifuged immediately at 7,000×*g* for 15 min, plasma was then decanted and stored at −80°C until analysis. Liver and gastrointestinal tract from stomach to anus were removed. The intestinal tract was flushed with phosphate buffered saline, dissected longitudinally and then washed a second time to remove residual content. Mucosa was collected from small intestine and colon by scraping gently with a spatula. Liver and mucosa were flash-frozen in liquid nitrogen and stored at −80°C.

Sample preparation

Curcumin and curcumin metabolites were extracted from plasma by solid phase extraction. Plasma (1 ml) was loaded onto a 1 cc Oasis HLB cartridge (Waters, Elstree, UK), washed with 25:25:1 methanol:water:glacial acetic acid (1 ml), and eluted with 1 ml of methanol containing 2% glacial acetic acid. Eluant was evaporated to dryness at 45°C under a stream of nitrogen, and the residue was re-suspended in 75 µl of 50% aqueous acetonitrile. Standard solutions of curcumin (5–1,000 ng/ml) were prepared in 1 ml human plasma (obtained from the National Blood Transfusion Centre, Sheffield, UK) and extracted as described above. Extraction efficiency was 59% with 2.5 and 4.5% intra and inter day variability, 99% accuracy and response was linear over the range 5–1,000 ng/ml with an R^2 value consistently of 0.999.

Mucosa (100 mg) was suspended in 1.15% KCl (1 ml) and centrifuged (16,000×*g*, 60 s). The pellet

was re-suspended in 1.15% KCl (1 ml) and homogenized using a blade homogeniser set at top speed for 2 × 20 s. Aliquots (0.1 ml) of homogenate were mixed with an equal volume of acetone:formic acid (9:1), the mixture was vortexed and kept at −20°C for 30 min prior to centrifugation (16,000×*g*, 5 min). The supernatant was decanted and evaporated to dryness at 45°C under a stream of nitrogen. The residue was re-suspended in 75 µl of 50% aqueous acetonitrile prior to analysis. Liver was homogenized 1:4 in isotonic KCl. An aliquot (0.5 ml) of liver homogenate was mixed with 2 ml of acetone:formic acid (9:1), and the mixture was immediately vortexed. Samples were kept at −20°C for 30 min prior to centrifugation (16,000×*g*, 10 min). Supernatant was evaporated to dryness at 45°C under a stream of nitrogen, and the residue was re-suspended in 75 µl of 50% aqueous acetonitrile prior to analysis.

HPLC analysis

Analysis of samples was performed using a Varian Prostar series HPLC instrument comprising a model 230 pump and a model 410 autosampler. Separation was achieved with an Atlantis dC18 column (4.6 × 150 mm, 3 µm, Waters, Elstree, UK) with a guard (4.6 × 20 mm, 3 µm), kept at 35°C. The mobile phase consisted of two components: A: 10 mM ammonium acetate pH 4.5, B: acetonitrile. Initial conditions were 95% A progressing to 55% A at 20 min and 5% A at 33 min. The flow rate was 1.5 ml/min. Curcumin and conjugated metabolites were detected at 426 nm and reduced curcumin metabolites at 280 nm using a Varian 325 UV-vis detector.

LC/MS/MS analysis

The identity of curcuminoids was verified by negative ion electrospray tandem mass spectrometry employing multiple reaction monitoring (MRM). Analysis was performed using an API 2000 LC/MS/MS (Applied Biosystems MDS Sciex, Warringon, UK) equipped with an Agilent 1100 series sample delivery system. Separation of curcumin and metabolites was achieved as described above, except that mobile phase A consisted of 5 mM ammonium acetate pH 4.5, the flow rate was 0.31 ml/min and the column size was 3.1 × 50 mm, 3 µm. MS/MS conditions consisted of declustering potential −26 V, focusing potential −350 V, electrode potential −12 V, cell entrance potential −16 V, cell exit potential −20 V and a temperature of 500°C. Identification of curcuminoids was by MRM using suitable transitions.

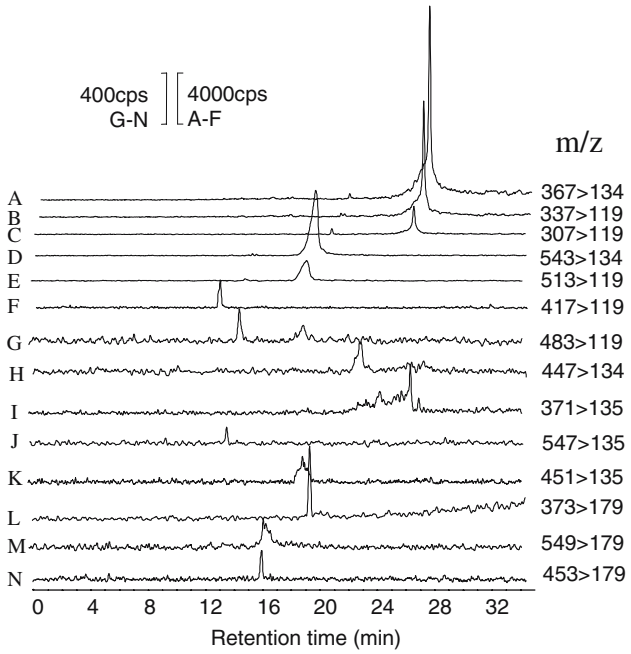

Fig. 4 Representative LC/MS/MS chromatograms of extracts of plasma (**a**–**j**, **l**, and **m**) and mucosa (**k** and **n**) from rats, which had received Meriva at 340 mg/kg by oral gavage, demonstrating multiple reaction monitoring transitions indicative of curcumin (**a**), desmethoxycurcumin (**b**), bis-desmethoxycurcumin (**c**), curcumin mono-glucuronide (**d**), desmethoxycurcumin mono-glucuronide (**e**), desmethoxycurcumin mono-sulfate (**f**), bis-desmethoxycurcumin mono-sulfate (**g**), curcumin mono-sulfate (**h**), tetrahydrocurcumin (**i**), tetrahydrocurcumin mono-glucuronide (**j**), tetrahydrocurcumin mono-sulfate (**k**), hexahydrocurcumin (**l**), hexahydrocurcumin mono-glucuronide (**m**), hexahydrocurcumin mono-sulfate (**n**). Tissues were obtained 30 min after Meriva administration. For details of administration and HPLC analysis see Materials and methods

Pharmacokinetic analysis

Estimations of area under the plasma concentration curve (AUC) were obtained using a non-compartmental, extra-vascular plasma model with WinNonLin version 2.1.

Results and discussion

Rats received curcumin in unformulated or Meriva form at a dose of 340 mg/kg (in terms of curcumin) by oral gavage. Plasma, liver tissue and intestinal mucosa were obtained at several time points up to 2 h post administration. Curcumin and species tentatively characterized as curcumin glucuronide, curcumin sulfate, tetrahydrocurcumin and hexahydrocurcumin were detected in plasma, intestinal mucosa and liver of rats after administration of either intervention (Figs. 2, 3).

As Meriva the curcumin phospholipid complex used here has never been employed before, we wished initially to confirm the identity of detectable drug-derived species in animals, which had received Meriva. To that end, extracts of bio-matrices were subjected to HPLC-mass spectrometric analysis. The analysis furnished incontrovertible proof of presence, based upon specific MRM transitions (in brackets), in the gut mucosa, plasma and liver of curcumin (367 > 134, Fig. 4a) and the following species: desmethoxycurcumin and bisdesmethoxycurcumin (337 > 119, Fig 4b and 307 > 119, Fig. 4c), two curcuminoids co-extracted with curcumin from the *curcuma* plant, hexahydrocurcumin (373 > 179), curcumin sulfate (447 > 134) and desmethoxycurcumin sulfate (417 > 119, Fig. 4l, h, f). The curcumin metabolites curcumin glucuronide (543 > 134) and tetrahydrocurcumin (371 > 135) were unambiguously identified only in gut mucosa and in plasma (Fig. 4d, i). Additional peaks were detected only in plasma, albeit at low abundance: glucuronides of desmethoxycurcumin (513 > 119), bisdesmethoxycurcumin (483 > 119), hexahydrocurcumin (549 > 179) and tetrahydrocurcumin (547 > 135, Fig. 4e, g, m, j, respectively). Sulfate metabolites of tetrahydrocurcumin (451 > 135) and hexahydrocurcumin (453 > 179) were detected in mucosa and in mucosa and liver, respectively (Fig. 4k, n). The occurrence of these species has previously been suggested in blood or tissues of rodents, which received unformulated curcumin [9, 10]. Therefore the results suggest that formulation with phosphatidylcholine does not confound the qualitative pattern of curcumin metabolism in vivo.

Next, we compared plasma and tissue levels of curcumin in animals that had received either unformulated or Meriva. Formulation dramatically and significantly increased curcumin levels in plasma (Fig. 5a) and liver (Fig. 6a) as compared to concentrations measured in animals that received unformulated curcumin. The lipophilic character of the curcumin–phosphatidylcholine complex may facilitate diffusion of curcumin across biological membranes in the gastro-intestinal tract via formation of a phospholipid monolayer on the mucosal surface, thus supporting the transition of curcumin from the hydrophilic gut content across lipophilic membranes into cells. This facilitated diffusion then results in improved absorption. Improved absorption facilitated by phosphatidylcholine has been demonstrated for an analogous complex of another polyphenol, silibinin [2]. An alternative explanation implicating interference of the phospholipid with curcumin metabolism is unlikely, since there is no evidence whatsoever for an inhibitory effect of phosphatitylcho-

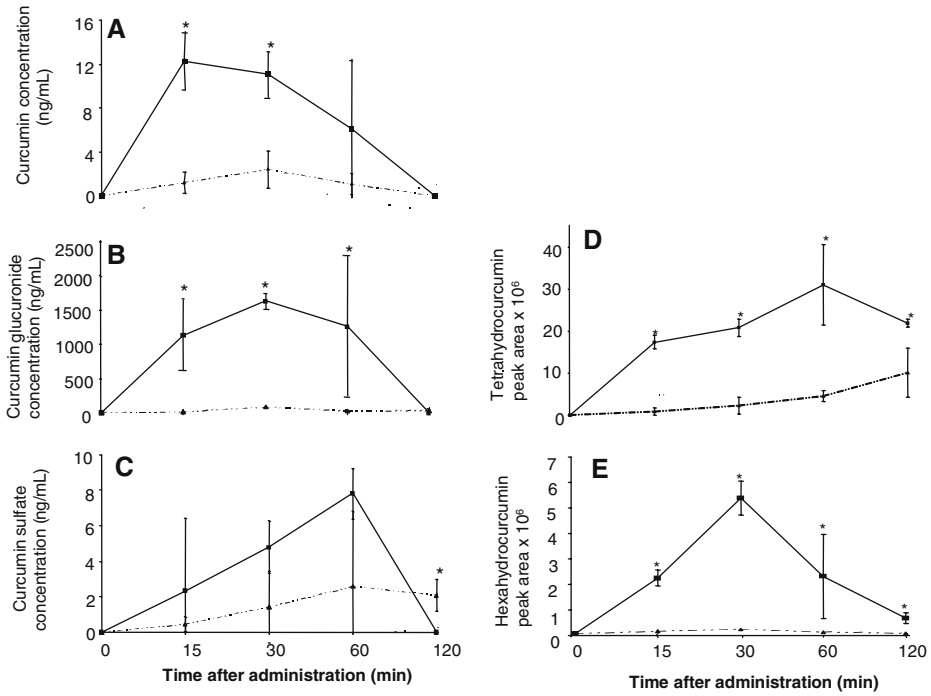

Fig. 5 Plasma levels of curcumin (**a**), curcumin glucuronide (**b**), curcumin sulfate (**c**), tetrahydrocurcumin (**d**) and hexahydrocurcumin (**e**) in rats, which had received curcumin (*broken line*) or Meriva (*solid line*) at 340 mg/kg by oral gavage. Values are the mean ± SD ($n = 3$). Curcumin conjugated metabolite concentrations were estimated using the curcumin calibration curve. *Star* indicates that values at that time point were significantly different from each other ($P < 0.01$). For details of administration and HPLC analysis see Materials and methods

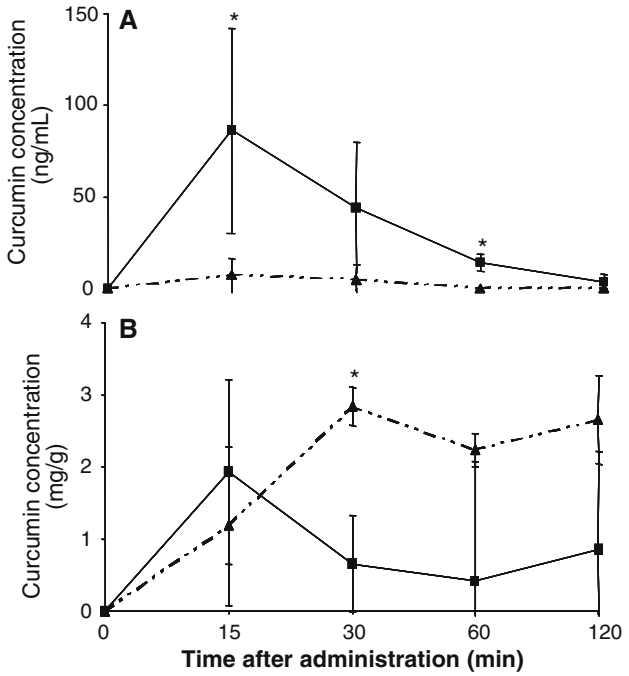

Fig. 6 Levels of curcumin in liver (**a**) and gastrointestinal mucosa (**b**) of rats, which had received curcumin (*broken line*) or Meriva (*solid line*) at 340 mg/kg by oral gavage. Values are the mean ± standard deviation ($n = 3$). *Star* indicates that values at that time point were significantly different from each other ($P < 0.01$). For details of administration and HPLC analysis see Materials and methods

line, a normal diet constituent, on drug metabolism. Curcumin levels in the gut mucosa, observed after administration of Meriva, were moderately lower than those after unformulated curcumin (Fig. 6b). The results presented here suggest that the administration of curcumin as Meriva is superior to that of unformulated curcumin if tissues other than the gastrointestinal tract are targeted, whilst maximal levels in the gastrointestinal tract can be achieved with unformulated curcumin. Both formulated and unformulated curcumin were completely removed from plasma within 2 h. Peak plasma levels of curcumin were approximately fivefold higher for Meriva than for the unformulated agent (Table 1), although maximal systemic concentrations of curcumin achieved by administration of the curcumin phospholipid complex were still considerably below the values (>10–20 μM), which have been shown to elicit pharmacological effects in cells or cell free systems. Plasma levels of curcumin sulfate, curcumin glucuronide, tetrahydrocurcumin and hexahydrocurcumin observed after administration of Meriva were 3- to 20-fold higher than those seen after unformulated curcumin (Fig. 5b–e; Table 1). Though evidence of chemopreventive efficacy for metabolites of curcumin is not available, one cannot exclude that they possess some pharmacological activity. Tetrahydrocurcumin, hexahydrocurcumin and curcumin sulphate have been shown to be weak inhibitors of phorbol ester-induced prostaglandin E2 activity [9]. Tetrahydrocurcumin and hexahydrocurcumin were weak inhibitors of lipopolysaccharide-induced nitric oxide synthase activity and mRNA expression [17], and tetrahydrocurcumin was a stronger antioxidant [16] and inhibitor of lipid peroxidation than its parent curcumin [22]. It is therefore conceivable that the considerable levels of curcumin metabolites observed after administration of Meriva may contribute to efficacy.

Table 1 Estimated plasma peak levels (C_{max}), time of peak levels (T_{max}) and AUC values for unformulated and curcumin phospholipid complex (Meriva)

	C_{max} (nM)	T_{max} (min)	AUC (μg min/ml)[a]
Unformulated			
Curcumin	6.5 ± 4.5	30	4.8
Curcumin glucuronide	225 ± 0.6	30	200.7
Curcumin sulfate	7.0 ± 11.5	60	15.5
Meriva			
Curcumin	33.4 ± 7.1	15	26.7
Curcumin glucuronide	4,420 ± 292	30	4,764.7
Curcumin sulfate	21.2 ± 3.9	60	24.8

[a] AUC was calculated using WinNonLin and employing a non-compartmental model

Although too few data points were collected for robust pharmacokinetic analysis, tentative area under the curve (AUC) values were calculated from curcumin plasma concentrations. The plasma $AUC_{0–120\ min}$ for curcumin after administration of Meriva was fivefold higher than that for unformulated curcumin (Table 1). It is conceivable that the improved bioavailability of curcumin, when administered as a complex with phospholipid increases the potential scope of medical applications for curcumin. Interestingly, in a recent study, intravascular administration of liposomally encapsulated curcumin using phosphocholine and phosphoglycerol technology was shown to suppress the growth of pancreatic tumours in nude mice [14]. These authors neither compared their formulation with unformulated curcumin, nor did they measure curcumin in the bio-matrix.

In conclusion, administration of curcumin as phospholipid complex greatly increased plasma and hepatic bioavailability of parent compound and metabolites as compared to unformulated curcumin. Further investigation will show whether prolonged daily or multiple daily dosing of Meriva is safe and

can furnish tissue levels superior to those achieved with unformulated curcumin. The results presented here suggest that for chemoprevention intervention studies targeting sites other than the gastrointestinal tract, curcumin formulated with phosphatidylcholine may well be more advantageous than unformulated curcumin.

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References

1. Aggarwal BB, Shishioda S (2006) Molecular targets of dietary agents for prevention and therapy of cancer. Biochem Pharmacol 71:1397–1421
2. Barzaghi N, Crema F, Gatti G, Pifferi G, Perucca E (1990) Pharmacokinetic studies of IdB 1016, a silybin-phosphatidylcholine complex, in healthy human subjects. Eur J Drug Metab Pharmacokinet 15:333–338
3. Cheng AL, Hsu CH, Lin JK, Hsu MM, Ho YF, Shen TS, Ko JY, Lin JT, Lin BR, Wu MS, Yu HS, Jee SH, Chen GS, Chen TM, Chen CA, Lai MK, Pu YS, Pan MH, Wang YJ, Tsai CC, Hsieh CY (2001) Phase 1 clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions. Anticancer Res 21:2895–2900
4. Duvoix A, Blasius R, Delhalle S, Schenkenburger M, Morceau F, Henry E, Dicato M, Diederich M (2005) Chemopreventive and therapeutic effects of curcumin. Cancer Lett 223:181–190
5. Fagerholm U, Sjostrom B, Sroka-Markovic J, Wijk A, Svensson M, Lennernas H (1998) The effect of a drug-delivery system consisting of soybean phosphatidyl choline and medium chain monoacylglycerol on the intestinal permeability of hexarelin in the rat. J Pharm Pharmacol 50:467–473
6. Garcea G, Jones DJL, Singh R, Dennison AR, Farmer PB, Sharma RA, Steward WP, Gescher AJ, Berry DP 2004) Detection of curcumin and its metabolites in hepatic tissue and portal blood of patients following oral administration. Br J Cancer 90:1011–1015
7. Garcea G, Berry DP, Jones DJL, Singh R, Dennison AR, Farmer PB, Sharma RA, Steward WP, Gescher AJ (2005) Consumption of the putative chemopreventive agent curcumin by cancer patients: assessment of curcumin levels in the colorectum and their pharmacodynamic consequences. Cancer Epidemiol Biomarkers Prev 14:120–125
8. Goel A, Boland CR, Chauhan DP (2001) Specific inhibition of cyclooxygenase-2 (Cox-2) expression by dietary curcumin in HT-29 human colon cancer cells. Cancer Lett 172:111–118
9. Ireson C, Orr S, Jones DJL, Verschoyle R, Lim CK, Luo JL, Howells L, Plummer S, Jukes R, Williams M, Steward WP, Gescher A (2001) Characterization of metabolites of the chemopreventive agent curcumin in human and rat hepatocytes and in the rat in vivo, and evaluation of their ability to inhibit phorbol ester-induced prostaglandin E-2 production. Cancer Res 61:1058–1064
10. Ireson C, Jones DJL, Orr S, Coughtrie MWH, Boocock DJ, Williams ML, Farmer PB, Steward WP, Gescher AJ (2002). Metabolism of the cancer chemopreventive agent curcumin in human and rat intestine. Cancer Epidemiol Biomarkers Prev 11:105–111
11. Kawamori T, Lubet R, Steele VE, Kelloff GJ, Kaskey RB, Rao CV, Reddy BS (1999) Chemopreventive effect of curcumin, a naturally occurring anti-inflammatory agent, during the promotion/progression stages of colon cancer Cancer Res 59:597–601
12. Kelloff GJ, Crowell JA, Hawk ET, Steele VE, Lubet RA, Boone CW, Covey JM, Doody LA, Omenn GS, Greenwald P, Hong WK, Parkinson DR, Bagheri D, Baxter GT, Blunden M, Doeltz MK, Eisenhauer KM, Johnson K, Knapp GG, Longfellow DG, Malone WF, Nayfield SG, Seifried HE, Swall LM, Sigman CC (1996) Strategy and planning for chemopreventive drug development: clinical development plans II. J Cell Biochem 63(suppl 26):54–71
13. Kuttan R, Sudheeran PC, Joseph CD (1987) Turmeric and curcumin as topical agents in cancer therapy. Tumori 73:29–31
14. Li L, Braiteh FS, Kurzrock R (2005) Liposome-encapsulated curcumin—in vitro and in vivo effects on proliferation, apoptosis, signaling, and angiogenesis. Cancer 104:1322–1331
15. Mourao SC, Costa PI, Salgado HRN, Gremiao MPD (2005) Improvement of antischistosomal activity of praziquantel by incorporation into phosphatidylcholine-containing liposomes. Int J Pharm 295:157–162
16. Osawa T, Sugiyama Y, Inayoshi M, Kawakishi S (1995) Antioxidative activity of tetrahydrocurcuminoids. Biosci Biotechnol Biochem 59:1609–1612
17. Pan MH, Lin-Shiau SY, Lin JK (2000) Comparative studies on the suppression of nitric oxide synthase by curcumin and its hydrogenated metabolites through down-regulation of IκB kinase and NFκB activation in macrophages. Biochem Pharmacol 60:1665–1676
18. Plummer SM., Holloway KA, Manson MM, Munks RJL, Kaptein A, Farrow S, Howells L (1999) Inhibition of cyclooxygenase 2 expression in colon cells by the chemopreventive agent curcumin involves inhibition of NF- kappa B activation via the NIK/IKK signalling complex. Oncogene 18:6013–6020
19. Rao CV, Rivenson A, Simi B, Reddy BS (1995) Chemoprevention of colon carcinogenesis by dietary curcumin, a naturally occurring plant phenolics compound. Cancer Res 55:259–266
20. Shao Z-M, Shen Z-Z, Liu C-H, Sartippour MR, Go VL, Heber D, Nguyen M (2002) Curcumin exerts multiple suppressive effects on human breast carcinoma cells. Int J Cancer 98:234–240
21. Sharma RA, Euden SA, Platton SL, Cooke DN, Shafayat A, Hewitt HR, Marczylo TH, Morgan B, Hemingway D, Plummer SM, Pirmohamed M, Gescher AJ, Steward WP (2004) Phase I clinical trial of oral curcumin: biomarkers of systemic activity and compliance. Clin Cancer Res 10:6847–6854
22. Sugiyama Y, Kawakishi S, Osawa T(1996) Involvement of the beta-diketone moiety in the antioxidative mechanism of tetrahydrocurcumin. Biochem Pharmacol 52:519–525
23. Surh YJ (2003) Cancer chemoprevention with dietary phytochemicals. Nat Rev Cancer 3:768–780
24. Uehara SI, Yasuda I, Akiyama K, Morita H, Takeya K, Itokawa H (1987) Diarylheptanoids from the rhizomes of *curcuma xanthorrhiza* and alponia officinarum. Chem Pharm Bull 35:3298–3304
25. Workman P, Twentyman P, Balkwill F, Balmain A, Chaplin D, Double J, Embleton J, Newell D, Raymond R, Stables J, Stephens T, Wallace J (1998) United Kingdom Co-ordinating Committee on Cancer Research (UKCCCR) guidelines for the welfare of animals in experimental neoplasia (second edition). Br J Cancer 77:1–10